The Role of Candidate Gene x Environment Interactions in Eating Pathology:

An investigation of the 5-HTTLPR polymorphism

Vanja Rozenblat

OCRID Number: 0000-0001-9846-525X

September, 2017

Master of Psychology (Clinical)/Doctor of Philosophy

Melbourne School of Psychological Sciences

The University of Melbourne

Thesis submitted in partial fulfilment of the degree Master of Psychology (Clinical)/Doctor of Philosophy
DECLARATION

I, Vanja Rozenblat, declare that my thesis is my own work, except where acknowledged in the preface, towards the Master of Psychology (Clinical)/Doctor of Philosophy degree, and that due acknowledgment has been made in text to all other materials used. The research reported in this thesis was conducted in accordance with the principles for the ethical treatment of human participants, as approved for this research by the University of Melbourne Human Research Ethics Committee. The word count for this thesis is below the maximum length allowable (100,000 words), exclusive of tables, references, and appendices, as specified by the Research Higher Degrees Committee.

Vanja Rozenblat

Vanja Rozenblat
ABSTRACT

The present thesis with publication aimed to clarify current scientific understanding of candidate gene x environment (GxE) interactions in the eating disorder (ED) field, and to build upon existing findings through specific investigation of adolescent disordered eating and the 5-HTTLPR polymorphism. Study 1 involved a systematic review of published studies of GxE interactions involving 5-HTTLPR in the ED field, followed by four meta-analyses involving the combination of raw data from the original studies. From the four analyses undertaken (investigating the environmental variables traumatic life events, \( N = 909 \), sexual and/or physical abuse, \( N = 1,097 \), and the psychological variables depression, \( N = 1,254 \), and impulsivity, \( N = 1,122 \)), results supported moderation by 5-HTTLPR in the relationship between sexual and physical abuse and bulimic spectrum pathology, with some support for moderation by 5-HTTLPR in the relationship between traumatic life events and overall EDs. No interactions between 5-HTTLPR and psychological factors were identified. Study 2 aimed to conceptually replicate these results in a large, independent sample drawn from the Australian Temperament Project \((N = 650)\). Results showed no interaction between 5-HTTLPR and psychological factors (depressed mood or emotional control), but revealed a severity-dependent effect whereby there was some evidence of moderation by 5-HTTLPR in the relationship between severe, but not moderate-to-mild, parental physical punishment and adolescent EDI-2 Bulimia scores. The final investigation, Study 3, examined potential direct effects and GxE interactions between parenting and 5-HTTLPR to predict disordered eating from a ‘plasticity’ perspective, across two samples. Study 3a found an association between self-reported parental warmth and lowered EDI-2 Bulimia scores, and between self-reported parental use of physical punishment and greater EDI-2 Drive for Thinness scores \((N = 650)\), however no moderation by 5-HTTLPR was identified. Study 3b aimed to replicate these findings using an observational measure of parenting behaviours, and constituted the largest
study of observed parenting behaviours in the ED field \((N = 304)\), as well as the first GxE interaction study in the field to include observational measurement of parenting behaviours. Results showed an association between parental warmth and lower EDI-2 Drive for Thinness scores, but no association between parental hostility and disordered eating, or any GxE interactions. Overall, results across the three studies of the present thesis supported moderation by 5-\textit{HTTLPR} in the relationship between severe environmental stressors and eating pathology, but not between less severe environmental factors or psychological factors and ED outcomes. These results illustrate the important role of genetic factors in ED aetiology, carry implications for understanding the individual differences in response to potential environmental risk factors for EDs, and can be used to help inform ED prevention initiatives. Outstanding issues in investigation of candidate gene research in the ED field include sample size, publication bias, and multiple testing. Continued collaboration to increase sample sizes and careful selection of environmental and psychological measures is recommend for future GxE investigations, along with a move towards polygenic and genome wide investigative approaches.
ACKNOWLEDGEMENTS

This thesis would not have been possible without the help and support of many people over the past 3.5 years. Firstly, a massive thanks to my amazing supervisor, Dr Isabel Krug, who without a doubt deserves Supervisor of the Year award. Isabel’s dedication to her students is unparalleled. She has provided me excellent guidance throughout the course of my candidature, has supported me every step of the way, and has provided me with the best possible introduction to the ‘research world’. Isabel has always made herself available to meet with me and enthusiastically read drafts of my work – one email that particularly stands out, from May this year, was when she advised me she was due to give birth the next day but would do her best to look through my discussion in the morning, before the baby arrived!

I would also like to thank Dr Matthew Fuller-Tyszkiwicz for his help with the meta-analysis, in particular for the novel idea of seeking raw data and bringing on contributors as co-authors (thus allowing for the largest-to-date collaborative gene x environment study in the field and the forging of new relationships), and for his assistance with some of the more complicated data analysis for this part of the project.

Thank you also to the Australian Temperament Project for allowing me to join your team, collect genetic samples, and access the wonderfully rich longitudinal data gathered over the past 33 years. Thank you to Dr Craig Olsson for the chats about the future of genetic research in complex diseases, and to project manager Anna Booth, for showing me the ropes and being an amazing support throughout the many hours spent collecting data at the Royal Children’s Hospital. I would also like to extend a very heartfelt thank you to all the participants who so kindly underwent the unglamorous process of providing saliva samples and put their trust in us, in order to help increase our understanding of eating pathology.
Finally, thank you to my wonderful family and friends. Thank you to my friends in our eating disorders group at Melbourne University, who I’m sure have learned more about genetics than they’ve ever wished to know, to my PhD buddies, sharing in the ups and downs of the whole experience, and to my non-PhD friends, for still ‘getting it’. Thank you especially to my parents, who have been supportive beyond words, and to my partner, Geoff, without whom I may have never believed in myself enough to go down this road.
# Table of Contents

PREFACE .............................................................................................................................................. 16

1. CHAPTER 1........................................................................................................................................ 26
   1.1. Introduction to Eating Pathology .......................................................................................................... 26
   1.2. Overview of DSM-5 eating disorders ...................................................................................................... 26
   1.3. Disordered eating definition .................................................................................................................. 32
   1.4. Psychological comorbidity for EDs ........................................................................................................ 33
       1.4.1. Depressed mood .............................................................................................................................. 35
       1.4.2. Impulsiveness ................................................................................................................................. 37
   1.5. Chapter summary ................................................................................................................................... 41

2. CHAPTER 2.............................................................................................................................................. 42
   Environmental Risk Factors and Correlates of Eating Pathology ................................................................. 42
   2.1. Introduction to risk factors in Eating Disorders ..................................................................................... 42
   2.2. Methodology for identifying risk factors and correlates in psychopathology ...................................... 43
       2.2.1. Prospective longitudinal design ........................................................................................................ 45
       2.2.2. Cross-sectional designs .................................................................................................................. 47
       2.2.3. Summary of research designs ......................................................................................................... 48
   2.3. Current evidence for correlates and risk factors in eating pathology .................................................... 49
       2.3.1. Overview of environmental risk factors for and correlates of Anorexia Nervosa ......................... 50
       2.3.2. Overview of environmental risk factors for and correlates of Bulimia Nervosa ............................. 52
       2.3.3. Overview of environmental risk factors for and correlates of Binge Eating Disorder .................. 53
       2.3.4. Overview of environmental risk factors for and correlates of disordered eating ........................ 54
   2.4. Factors under investigation in the present thesis ................................................................................... 55
       2.4.1. Environmental correlates for eating pathology: Traumatic life events ........................................ 56
           2.4.1.1. Traumatic life events: Anorexia nervosa .................................................................................. 57
           2.4.1.2. Traumatic life events: Bulimia nervosa .................................................................................. 58
           2.4.1.3. Traumatic life events: Binge eating disorder ......................................................................... 59
           2.4.1.4. Traumatic life events: Disordered eating ............................................................................. 60
       2.4.2. Environmental correlates for eating pathology: Sexual and physical abuse ............................... 62
           2.4.2.1. Meta-analyses examining role of childhood abuse in clinical ED samples ........................... 64
           2.4.2.2. Summary of findings regarding childhood abuse in clinical eating disorder samples ........................... 69
       2.4.3. Sexual and physical abuse in community disordered eating samples ........................................... 71
       2.4.4. Environmental correlates for eating pathology: Parenting behaviours ....................................... 72
CHAPTER 6

5.2.5. 5-HTTLPR x Impulsiveness ................................................................. 173
5.2.6. Studies identified in the review measuring other genes (non-5-HTTLPR) .... 174
5.2.6.1. Dopamine-system polymorphisms .................................................. 175
5.2.6.2. Other polymorphisms: Bcl1 ........................................................... 179
5.2.6.3. Other polymorphisms: Val66Met ..................................................... 181
5.3. Quality evaluation of studies identified in the systematic review .................. 183
5.3.1. Sample size in gene x environment interaction studies ............................ 186
5.3.2. Controlling for the effect of confounders ............................................. 187
5.3.3. Further statistical limitations ............................................................ 188
5.4. Discussion of publication bias ................................................................ 190
5.4.1. Publication bias in the present systematic review ................................. 193
5.4.2. Summary of publication bias ............................................................. 196
5.5. Chapter summary ................................................................................. 196
6. CHAPTER 6 ......................................................................................... 198

Extended Discussion of Meta-Analysis and Methodology from Study 1 .............. 198
6.1. Identifying studies suitable for meta-analysis ......................................... 198
6.2. Contacting authors .............................................................................. 199
6.3. Integrating data sets ............................................................................ 201
6.4. Re-coding of data included in meta-analysis ........................................... 201
6.4.1. Coding of genotype ......................................................................... 202
6.5. Coding of variables in Analysis 1: Traumatic life events x 5-HTTLPR to predict eating disorder status ................................................................. 202
6.5.1. Coding eating disorder status in Analysis 1 ........................................ 203
6.5.2. Measuring traumatic life events .......................................................... 204
6.6. Coding of variables in Analysis 2: Sexual and/or physical abuse x 5-HTTLPR to predict bulimic status ................................................................. 207
6.6.1. Coding bulimic status in Analysis 2 .................................................... 207
6.6.2. Coding sexual and physical abuse ....................................................... 208
6.7. Coding of variables in Analysis 3: Depression x 5-HTTLPR to predict bulimic status ................................................................. 210
6.7.1. Coding the depression variable ......................................................... 211
6.7.2. Coding bulimic status in Analysis 3 .................................................... 212
6.7.3. Limitations of the data integration process in Analysis 3 ...................... 213
6.8. Coding of variables in Analysis 4: Impulsivity x 5-HTTLPR to predict disordered eating 214
6.8.1. Coding the disordered eating variable in Analysis 4 ............................ 215
13.5. Future directions of genetic research in the eating disorder field .........................362
13.5.1. Suggestions for addressing sample size issues ...........................................362
13.5.2. Polygenetic effects, genome wide association studies, and epigenetics ..........364
13.5.3. Future directions for candidate gene studies .............................................368
13.6. Final summary ..............................................................................................369
14. References ....................................................................................................371
15. Appendices ..................................................................................................i
**LIST OF TABLES**

*Table 1* DSM-5 Diagnostic Criteria for Anorexia Nervosa  

*Table 2* DSM-5 Diagnostic Criteria for Bulimia Nervosa  

*Table 3* DSM-5 Diagnostic Criteria for Binge Eating Disorder  

*Table 4* Summary of Findings of Six Identified Meta-Analyses Examining the Association Between Sexual and Physical Abuse and Overall EDs, AN, and BN.  

*Table 5* Studies Using Observational Methods to Investigate Family Interaction and Parenting Behaviours in EDs or Disordered Eating Samples.  

*Table 6* Summary of Studies Included in Each Meta-Analysis Examining the Association Between 5-HTTLPR and EDs.  

*Table 7* Studies Examining Candidate GxE Interactions in Eating Pathology  

*Table 8* Downs and Black (1998) Checklist for Methodological Quality, Adapted to Evaluate Studies Identified in a Systematic Review of the Role of Gene x Environment Interactions in Risk for Eating Pathology  

*Table 9* Number of Gene x Environment Interactions Tested in Each Study Identified in the Review and the Number of Significant Interactions Identified.  

*Table 10* Final Seventeen Life Events Included in Analysis 1 from each of the Three Studies  

*Table 11* Criteria to Determine Presence of Lifetime Physical and/or Sexual Abuse in each of the Five Studies Included in Analysis 2.  

*Table 12* Criteria to Determine Presence or Absence of Depressed Mood  

*Table 13* Year of Data Collection, Sample Size, and Response Rate for Each Survey Wave in the Australian Temperament Project  

*Table 14* Participation Outcomes for ATP Participants Eligible to Provide Saliva Samples Based on Participation in the Home Visit Study.

27  
30  
31  
70  
86  
103  
161  
184  
195  
206  
210  
212  
275  
279
Table 15  Correlations Between Self-Reported Parenting Behaviours, Observed Parenting Behaviours, EDI-2 Drive for Thinness and Bulimia scales, Participant BMI, and Participant Gender.

Table 16  Main Effects of Self-Reported Parental Warmth and use of Harsh Punishment in Predicting EDI-2 Bulimia and Drive for Thinness Outcomes in a Combined Model, Controlling for Gender and BMI (N = 650).

Table 17  Main Effects of Observed Parental Warmth and Hostility in Predicting EDI-2 Bulimia and Drive for Thinness Outcomes in a Combined Model, Controlling for Gender and BMI (N = 304).
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN</td>
<td>Anorexia Nervosa</td>
</tr>
<tr>
<td>AN-BP</td>
<td>Anorexia Nervosa Binge-Purge Subtype</td>
</tr>
<tr>
<td>AN-R</td>
<td>Anorexia Nervosa Restrictive Subtype</td>
</tr>
<tr>
<td>BED</td>
<td>Binge Eating Disorder</td>
</tr>
<tr>
<td>BIS</td>
<td>Barratt Impulsiveness Scale</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BN</td>
<td>Bulimia Nervosa</td>
</tr>
<tr>
<td>CDI</td>
<td>Children’s Depression Inventory</td>
</tr>
<tr>
<td>CFI</td>
<td>Camberwell Family Interview</td>
</tr>
<tr>
<td>CTI</td>
<td>Childhood Trauma Inventory</td>
</tr>
<tr>
<td>DEBQ</td>
<td>Dutch Eating Behaviours Questionnaire</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>EAT-26</td>
<td>Eating Attitudes Test-26</td>
</tr>
<tr>
<td>EATE</td>
<td>EATEATE Lifetime Diagnostic Interview</td>
</tr>
<tr>
<td>EDE</td>
<td>Eating Disorders Inventory</td>
</tr>
<tr>
<td>EDI-2</td>
<td>Eating Disorders Inventory-2</td>
</tr>
<tr>
<td>EDNOS</td>
<td>Eating Disorder Not Otherwise Specified</td>
</tr>
<tr>
<td>FBT</td>
<td>Family Based Treatment</td>
</tr>
<tr>
<td>GxE</td>
<td>Gene x environment</td>
</tr>
<tr>
<td>MADRS</td>
<td>Montgomery-Asberg Depression Rating Scale</td>
</tr>
<tr>
<td>MEBS</td>
<td>Minnesota Eating Behaviour Survey</td>
</tr>
<tr>
<td>ORF1</td>
<td>Oxford Risk Factor Interview</td>
</tr>
<tr>
<td>OSFED</td>
<td>Other Specified Feeding or Eating Disorder</td>
</tr>
<tr>
<td>PBI</td>
<td>Parental Bonding Instrument</td>
</tr>
<tr>
<td>PGC-ED</td>
<td>Eating Disorders Working Group of the Psychiatric Genomics Consortium</td>
</tr>
<tr>
<td>SCID</td>
<td>Structured Clinical Interview for DSM Disorders</td>
</tr>
<tr>
<td>SES</td>
<td>Socioeconomic status</td>
</tr>
<tr>
<td>TAQ</td>
<td>Traumatic Antecedents Questionnaire</td>
</tr>
</tbody>
</table>
PREFACE

Overview of thesis

This thesis with publication expands upon current understanding of how genetic factors play a role in eating disorders (EDs), by examining the role of candidate gene x environment (GxE) interactions in eating pathology. There is increasingly established evidence of a large genetic component in risk of developing an ED (40%-60%; Yilmaz, Hardaway, & Bulik, 2015). However, to date there is limited understanding regarding the specific genetic mechanisms that may underlie this relationship. Studies of genetic association examining whether certain genetic polymorphisms (i.e., variants of a gene) may be present in greater frequency in ED cases compared to controls have not provided conclusive evidence of any direct genetic associations (Culbert, Racine, & Klump, 2015). One possibility is that consideration of environmental factors is necessary to understand the functioning of genetic risk for EDs, which may operate via GxE interactions. GxE interactions refer to the process whereby certain genetic factors may increase risk for a given outcome under adverse environmental conditions (e.g., the experience of traumatic life events) but not in the absence of such environments, compared to individuals without the genetic risk factor. There is also some suggestion that certain alleles (i.e. specific variant of the gene) may increase responsiveness to both negative and positive environments, such that individuals with a polymorphism typically considered ‘risky’ may in fact show better outcomes under positive environmental conditions, compared to those with the ‘non-risky’ variant.

Most GxE interaction research in psychiatry has examined a polymorphism in the transporter region of the serotonin transporter gene (5-HTTLPR), with the short (s) allele
believed to be associated with greater risk of poor outcomes under challenging environmental conditions (Caspi et al., 2003; Trace, Baker, Penas-Lledo, & Bulik, 2013). Most investigations into GxE interactions thus far have been undertaken in the depression field, where two meta-analyses have found an association between 5-HTTLPR and traumatic life events in increasing risk of depressed mood (Karg, Burmeister, Shedden, & Sen, 2011; Sharpley, Palanisamy, Glyde, Dillingham, & Agnew, 2014). However, more recently these findings have been contested (Culverhouse et al., 2017; Risch et al., 2009). Other researchers have proposed that rather than constituting a ‘risk’ factor, 5-HTTLPR may be better conceptualised as a ‘plasticity’ factor, with individuals with the s-allele showing better outcomes under adaptive environments compared to those homogeneous for the long (l) allele (Belsky et al., 2009). In the ED field, little research has directly examined the role of GxE interactions from a risk perspective, with about half of published studies (N = 7) examining 5-HTTLPR, and none adopting a plasticity perspective (see Chapter 5).

The present thesis consists of three published studies investigating GxE interactions in eating pathology, and a number of accompanying introductory, methodology, and discussion chapters. The following three published studies are included:

**Study 1** featured a systematic review and combined-samples meta-analysis of current GxE interaction findings in the ED field. This study involved the integration of data from seven studies (Akkermann et al., 2012; Karwautz et al., 2011; Mata & Gotlib, 2011; Racine, Culbert, Larson, & Klump, 2009; Steiger et al., 2007; Stoltenberg, Anderson, Nag, & Anagnostopulos, 2012; van Strien, van der Zwaluw, & Engels, 2010) to perform four secondary data meta-analyses examining the following GxE interactions: 1) Whether 5-HTTLPR moderated the relationship between traumatic life events and ED status or
equivalent \((N = 909)\); 2) whether \(5-HTTLPR\) moderated the relationship between sexual and/or physical abuse and BN status or equivalent \((N = 1097)\); 3) whether \(5-HTTLPR\) moderated the relationship between depressed mood and bulimia nervosa status or equivalent \((N = 1254)\), and; 4) whether \(5-HTTLPR\) moderated the relationship between impulsiveness and ED status or equivalent \((N = 1122)\).

**Study 2** involved an attempt to conceptually replicate the findings of the meta-analysis in Study 1 by investigating the same, or similar, GxE interactions using an independent sample, drawn from the Australian Temperament Project (ATP). The ATP is a 35 year longitudinal study based in Victoria, Australia, which has followed the social and emotional development of a large cohort of individuals \((N = 1000+)\) from infancy to adulthood. This study involved five separate analyses, with \(N = 650\) for the first two analyses: 1) Whether \(5-HTTLPR\) moderated the relationship between depression and disordered eating; 2) whether \(5-HTTLPR\) moderated the relationship between emotional control and disordered eating, and \(N = 467\) for the remaining analyses; 3) whether \(5-HTTLPR\) moderated the relationship between sexual abuse and disordered eating; 4) whether \(5-HTTLPR\) moderated the relationship between mild-to-moderate parental physical punishment and disordered eating, and; 5) whether \(5-HTTLPR\) moderated the relationship between severe parental physical punishment and disordered eating.

Finally, **Study 3** aimed to examine GxE interactions from a plasticity perspective, to explore whether \(5-HTTLPR\) may be better conceptualised as conferring differential susceptibility as opposed to ‘risk’, per se. This was achieved by testing whether the relationship between observationally measured and self-reported parenting practices (specifically, parental warmth, hostility, and use of harsh punishment) and disordered
eating was moderated by 5-HTTLPR genotype across two studies (outlined below). This paper adopted a multi-source multi-method approach, by including both observationally measured and self-reported parenting behaviours. Study 3a examined the relationship between self-reported parental warmth and use of punishment and adolescent-reported disordered eating, including whether any relationships were moderated by 5-HTTLPR (N = 650). Study 3b aimed to replicate any relationships identified in Study 3a in a sub-set of participants (N = 304) who participated in a family interaction task that allowed for coding of observed parental warmth and hostility, measured via the Iowa Family Interaction Rating Scale (IFIRS; Melby & Conger, 2001).

This thesis provides an important overview of the current state of knowledge regarding candidate GxE interaction research in the ED field and builds upon this using a large sample from the ATP. Throughout the thesis, existing candidate GxE interaction research is synthesised and summarised to establish a clear picture of the most up-to-date findings. The thesis also provides an opportunity to critically evaluate and reflect upon candidate GxE research, allowing for careful discussion of the limitations and future directions of genetic research in the ED field. In addition to genetic findings, this thesis provides further insight into the important role of psychological and environmental factors in EDs. Importantly, sub-threshold ED samples are investigated throughout Studies 2 and 3, with findings relevant for initiatives aimed at preventing the development of full-blown EDs. Overall, this thesis constitutes the largest study of GxE interactions in the ED field. It investigates novel GxE interactions and tests the replicability of those previously published, attempting to shed light onto the mechanisms involved in the heritability of EDs, a question that remains unanswered in the current literature.
Research output

Based on the empirical work undertaken in the present thesis, the following publications and conference presentations have been produced:

Peer reviewed journal articles


1. This first publication constitutes Study 1 of the present thesis. VR was responsible for conducting all analyses and preparing all sections of the manuscript. DO contributed to the systematic review section, including searching, recording results evaluating the studies, and contributing to that section of the manuscript. IK and MFT contributed to study design and editing drafts of the manuscript, and MFT also contributed to the analyses section. Remaining authors were involved in the collection of data. All authors contributed to and approved the final manuscript.

Vanja Rozenblat, Joanne Ryan, Eleanor Wertheim, Ross King, & Isabel Krug. (2017). Investigating a role for the serotonin transporter 5-HTTLPR polymorphism in

2. The next publication forms Study 2 of the present thesis. VR was responsible for conducting all analyses and preparing all sections of the manuscript. IK, JR, EW, RK, and CO were involved in collecting data and revising the manuscript for important intellectual content. All authors contributed to and approved the final manuscript.


3. The final publication comprises Studies 3a & 3b of the present thesis. As in Study 2, VR was responsible for conducting all analyses and preparing all sections of the manuscript. IK, JR, EW, RK, PL, and CO were involved in collecting data and revising the manuscript for important intellectual content. All authors contributed to and approved the final manuscript.

**Conference presentations**

Vanja Rozenblat, Deborah Ng, Matthew Fuller-Tyszkiewicz, …, & Isabel Krug. (2016). A meta-analysis of gene (5-HTT) x environment interactions in eating pathology using
secondary data analyses. *ANZEAD’s 14th Annual Conference, Christchurch, 26-27th August.*


**Other publications undertaken during candidature**

**A) Journal Articles**

B) Book Chapters


C) Published Abstracts


Other conference presentations undertaken during candidature

A) Invited talks

Co-presented ‘Pub Discussion’ session on Eating Disorders for the Australian Association for Cognitive and Behavioural Therapy (AACBT), February 22nd, 2017

B) Oral presentations

Vanja Rozenblat, Laura Gorrie, Isabel Krug, & The ATP Consortium. (2017). The role of DAT1 and COMT in moderating risks for disordered eating in adolescence
associated with peer relationships. *Australia & New Zealand Academy for Eating Disorders Conference, Sydney, Australia, 1-3 of September. (Oral presentation).*


**C) Poster presentations**


Grants and awards received during candidature

2017    AED Student/Early Career Researcher Fellowship

2017    Graduate Higher Degree Travel Fellowship, The University of Melbourne

2013    Early Career Researcher Grant awarded to Dr Isabel Krug for project titled “Genetic sensitivity to the caregiving context in adolescents with disordered eating” (the present research).

2013    Research Training Program Scholarship (formerly Australian Postgraduate Award)
1. CHAPTER 1

1.1. Introduction to Eating Pathology

The first chapter of this thesis presents a broad background to EDs and disordered eating, followed by discussion of psychological factors identified as commonly comorbid with EDs. Particular focus is given to discussion of depression and impulsivity, two factors explored in some depth throughout the studies of the present thesis. Risk factors for EDs and the role of genes in ED aetiology are elaborated in subsequent Chapters (2 and 3).

1.2. Overview of DSM-5 eating disorders

EDs involve severe and harmful disturbances in eating behaviour that significantly impair physical health or psychosocial functioning. This may include a refusal to maintain a minimum healthy body weight, binge eating followed by inappropriate compensatory behaviour, binge eating without compensatory behaviour, or other distorted eating patterns (American Psychological Association [APA], 2013). According to the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5; APA, 2013), EDs are classified into a number of diagnostic categories, including: Anorexia Nervosa (AN), Bulimia Nervosa (BN), Binge Eating Disorder (BED), and Other Specified and Unspecified Feeding and Eating Disorders (OSFED & UFED). Other disorders, including as Pica, Rumination Disorder, and Avoidant/Restrictive Food Intake Disorder, are also classified as Feeding and Eating Disorders in the DSM-5 but are believed to have distinct underlying pathophysiology and patterns of psychopathology (e.g., no role of body weight or shape; Association, 2013) and are not the focus of the present thesis.
The most established and heavily studied EDs are AN and BN, with investigations of BED also on the rise following its inclusion as a separate diagnostic category in the DSM-5 (APA, 2013). AN involves a refusal to maintain at least a minimal body weight, intense fear of gaining weight, and disturbances in the perception of one’s body weight or shape (APA, 2013; see Table 1). It is typically divided into two main sub-types, a restrictive type (AN-R), which involves weight-control behaviours focussed on low calorie intake and exercise, and a binge-eating/purging type (AN-BP), which involves binge-eating followed by purging behaviours to maintain low body weight.

Table 1

*DSM-5 Diagnostic Criteria for Anorexia Nervosa*

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Restriction of body weight leading to significantly low body weight,</td>
</tr>
<tr>
<td>B</td>
<td>Intense fear of gaining weight or persistent behaviour that interferes with body weight,</td>
</tr>
<tr>
<td>C</td>
<td>Disturbance in way ones shape or weight is experienced, or, undue influence of shape or weight on self-evaluation, or, lack of recognition of seriousness of current weight</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restricting</td>
<td>Individual has not engaged in recurrent binge-purge behaviours during the last three months</td>
</tr>
<tr>
<td>Binge eating/purging</td>
<td>Individual has engaged in recurrent binge-purge behaviours during the last three months</td>
</tr>
</tbody>
</table>

*Note.* Table adapted from the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (APA, 2013).
Onset of AN is common during adolescence, with 12 month prevalence estimated at around 0.4% - 0.8% (Hoek, 2006; Stice, Marti, & Rohde, 2013) and one study estimating 3 month prevalence at 0.4% in an Australian sample (Hay, Girosi, & Mond, 2015). Lifetime prevalence is estimated at around 1.7% (Smink, Hoeken, Oldehinkel, & Hoek, 2014), although the number of affected individuals who do not seek healthcare is argued to be far larger (Hoek & Van Hoeken, 2003; Keski-Rahkonen et al., 2007). AN is also far more common in females than males, with many investigations into life-time prevalence citing risk ratio estimates of 20 and more (Lindberg & Hjern, 2003; Nicholls & Viner, 2009), although this may be in part due to lower recognition of the disorder in males (Griffiths et al., 2015).

AN may lead to a wide range of physical and social consequences. Physically, the extreme weight loss of AN is associated with cardiac abnormalities, osteoporosis, amenorrhea, constipation, and a range of other serious health issues resulting in frequent hospitalisation and a lower quality of life (Agh et al., 2016; APA, 2013; de Simone et al., 1994; Rigotti, Nussbaum, Herzog, & Neer, 1984). AN during adolescence is particularly injurious, with patients’ bodies still in the development and growth stage (van Elburg, 2015). Death is also a possible consequence, with AN associated with the highest mortality rate of any mental health disorder (Arcelus, Mitchell, Wales, & Nielsen, 2011; Chesney, Goodwin, & Fazel, 2014). This includes death due to both physical consequences of low weight and associated health problems (Millar et al., 2005), as well as death by suicide (Preti, Rocchi, Sisti, Camboni, & Mitto, 2011). The psychosocial consequences of AN are also vast, particularly as disorder onset is typically during a developmentally sensitive period. For example, AN is associated with greater absence from work and education,
social problems and withdrawal, and difficulties in social cognition (Arkell & Robinson, 2008; Caglar-Nazali et al., 2014; Treasure & Schmidt, 2013; Zipfel, Löwe, Reas, Deter, & Herzog, 2000). It is clear that AN is a damaging and serious mental health issue.

BN is another ED associated with significant impairment and psychological distress (Agh et al., 2016; APA, 2013). BN is characterised by recurrent episodes of binge eating (at least once per week, for a minimum of three months) followed by inappropriate compensatory behaviour (e.g., vomiting, laxative use, extreme exercise; APA, 2013; see Table 2). As in AN, the self-esteem of individuals with BN is unduly influenced by their weight or shape, however unlike AN, weight tends to be in the normal to above average range, with severity of BN determined by the frequency of compensatory behaviours (APA, 2013). Prevalence of DSM-5 BN is higher than AN, with 3 month prevalence in an Australian sample estimated at .66% (Hay et al., 2015), and lifetime prevalence by early adulthood estimated at 1.6% to 2.6% (Allen, Byrne, Oddy, & Crosby, 2013; Stice et al., 2013b). Like AN, BN commonly emerges in adolescence or young adulthood (Hudson, Hiripi, Pope, & Kessler, 2007), and is associated with increased risk of mortality as well as a range of social role impairments (e.g., education, relationships, employment; Keel & Brown, 2010; Kessler et al., 2014). It is also far more prevalent in females than males (Allen, Byrne, Crosby, & Stice, 2016).
Table 2

DSM-5 Diagnostic Criteria for Bulimia Nervosa

<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>E</td>
</tr>
</tbody>
</table>

Note. Table adapted from the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (APA, 2013).

BED is a recent addition to the DSM-5 as a disorder in its own right, and has the highest prevalence rate of the aforementioned disorders, at around 2.0 - 3.0 % (Cossrow et al., 2016; Stice et al., 2013b), although one study using an Australian sample reported a 3 month prevalence of 5.6% (Hay et al., 2015). BED involves recurrent episodes of binge eating (at least once per week, for a minimum of 3 months) without engaging in subsequent compensatory behaviour and is separate to but often associated with obesity (APA, 2013; see Table 3). Unlike AN and BN, the prevalence of BED is only slightly higher in females compared to males (Hay et al., 2015).
Table 3

*DSM-5 Diagnostic Criteria for Binge Eating Disorder*

<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>E</td>
</tr>
</tbody>
</table>

*Note.* Table adapted from the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (APA, 2013).

Finally, a diagnosis of ‘Other Specified Feeding or Eating Disorder’ (OSFED) may be applied when individuals experience clinically significant distress or impairment associated with feeding and eating issues, however symptoms do not meet full criteria for any disorder (APA, 2013). Examples include atypical AN (e.g., all criteria for AN are met besides underweight status) and purging disorder (engaging in purging behaviours in the absence of binge eating).

While AN, BN, and BED are defined as distinct categories in the DSM-5 (APA, 2013), there exist common underlying patterns of symptomatology linking the disorders (e.g., dietary restraint, purging, concern about weight and shape, etc.; Fairburn, Cooper, Doll,
Indeed, there is noted diagnostic fluidity between the disorders, with individuals initially diagnosed with AN likely to later meet criteria for BN (although crossover from BN to later AN is less common; Eddy et al., 2008), those with BED may go on to develop BN, or individuals who no longer meet strict criteria for AN or BN may become classified under OSFED (Castellini et al., 2011; Fairburn et al., 2000; Welch et al., 2016). Finally, some sufferers may not meet clinical criteria for any disorder, but still experience substantial eating disturbances that impact negatively on physical and psychosocial health and daily functioning (Stice, Marti, Shaw, & Jaconis, 2009).

1.3. Disordered eating definition

Disordered eating refers to unhealthy and/or restrictive eating patterns that do not meet the criteria for an ED, and includes behaviours such as extreme dieting, binge eating, and purging (Croll, Neumark-Sztainer, Story, & Ireland, 2002). Disordered eating is relatively common in adolescence, with around half of young females engaging in unhealthy eating behaviours, and roughly 20% of females engaging in extreme weight-control behaviours (Neumark-Sztainer, Wall, Eisenberg, Story, & Hannan, 2006; Neumark-Sztainer, Wall, Larson, Eisenberg, & Loth, 2011), with the figure estimated at 16.3% in an Australian sample (Hay et al., 2015). Despite not being considered a ‘clinical-level’ disorder, disordered eating is also associated with significant psychosocial impairment and comorbid psychopathology (Aspen et al., 2014; Zullig, Matthews-Ewald, & Valois, 2016). For example, disordered eating has been related to greater depressive symptoms, greater anxiety symptoms such as worry and stress, poorer physical health, reduced social
functioning, and lower self-reported quality of life (Becker et al., 2010; Doyle, Grange, Goldschmidt, & Wilfley, 2007; Rø, Bang, Reas, & Rosenvinge, 2012; Solmi, Hatch, Hotopf, Treasure, & Micali, 2014; Wade, Wilksch, & Lee, 2012).

Furthermore, patterns of disordered eating during childhood or early adolescence generally precede the emergence of later clinical-level eating pathology (Kotler, Cohen, Davies, Pine, & Walsh, 2001; Stice, Ng, & Shaw, 2010). A number of longitudinal studies have reported that, compared to controls, adolescents who engage in extreme dieting and other unhealthy weight control behaviours are more likely to report continued disordered eating or increased binge eating and use of compensatory behaviours such as self-induced vomiting and use of diet/laxative pills later in adolescence and young adulthood (Loth, MacLehose, Bucchianeri, Crow, & Neumark-Sztainer, 2014; Neumark-Sztainer et al., 2006b; Neumark-Sztainer, Wall, Story, & Standish, 2012). Extreme dieting behaviour has also been found to predict the later onset of EDs, with one prospective study finding that severe dieting in females was related to an 18-fold increase in risk for developing an ED, while moderate dieting was related to a five-fold increase in risk, compared to non-dieters (Patton, Selzer, Coffey, Carlin, & Wolfe, 1999). It is therefore evident that disordered eating is an important prodromal factor that may lead to the imminent onset of a clinical ED.

1.4. Psychological comorbidity for EDs

As with many other psychiatric disorders, EDs tend to be associated with various co-morbid psychopathologies, such as depression (Puccio, Fuller-Tyszkiewicz, Ong, & Krug, 2016), anxiety (Keski-Rahkonen & Mustelin, 2016), personality disorders (Martinussen et
al., 2016), and substance use disorders (Kanbur & Harrison, 2016). For example, a recent meta-analysis of 87 studies by Martinussen et al. (2016) reported a .52 prevalence of personality disorders for individuals with an ED compared to a prevalence of .09 in healthy controls. The most common disorders in the ED samples were Borderline Personality Disorder and Avoidance Personality Disorder, with a prevalence of .22 and .20, respectively. Likewise, substance use disorders occur at a greater frequency in those with clinical ED pathology compared to controls (Fouladi et al., 2015; Kessler et al., 2013), with an estimated comorbidity around 20-30% (Hudson et al., 2007; Mann et al., 2014) that is believed to be attributed to a range of shared aetiological factors, including personality factors such as impulsivity, the experience of trauma and adversity, and a shared genetic aetiology (Baker & Munn-Chernoff, 2014; Brewerton & Brady, 2014; Mann et al., 2014; Munn-Chernoff & Baker, 2016).

Indeed, a number of personality traits have been associated with EDs (e.g., impulsivity, perfectionism, neuroticism). Perfectionism is a personality trait that has some of the strongest support for a role in EDs, with the associated high standards, feelings of ineffectiveness, and rule-adherence believed to increase an individual’s propensity to aspire to unrealistic weight goals and persist with disordered eating behaviours to achieve these aims (Farstad, McGeown, & von Ranson, 2016; Forbush, Heatherton, & Keel, 2007; Machado, Gonçalves, Martins, Hoek, & Machado, 2014; Wade et al., 2008a; Wade, Wilksch, Paxton, Byrne, & Austin, 2015). Likewise, there is strong evidence for a link between neuroticism, the tendency experience negative emotions, and EDs and disordered eating (Atiye, Miettunen, & Raevuori-Helkamaa, 2015; Bulik et al., 2006; Luo, Forbush, Williamson, Markon, & Pollack, 2013; Miller, Schmidt, Vaillancourt, McDougall, &
Laliberte, 2006; Wade, Bulik, Prescott, & Kendler, 2004), although neuroticism is a common risk factor for a variety of mental health issues (e.g., depression, anxiety, non-specific mental distress; Jeronimus, Kotov, Riese, & Ormel, 2016). While there are a range of psychological factors that tend to be comorbid with eating pathology, this chapter will review evidence relating to depressed mood and impulsivity, with these variables forming a particular focus of the present thesis.

1.4.1. Depressed mood

Depression constitutes one of the strongest psychological comorbidities for EDs, with estimates ranging between 30% and 50% (Hudson et al., 2007; Swanson, Crow, Le Grange, Swendsen, & Merikangas, 2011). From a theoretical perspective, the relationship between these two constructs may exist for a variety of reasons (Puccio et al., 2016). It is possible that low mood during a depressive episode triggers the use of maladaptive coping styles, such as binge eating and purging (Goldschmidt, Wall, Loth, Le Grange, & Neumark-Sztainer, 2012; Heatherton & Baumeister, 1991). Indeed, one meta-analysis (Cardi, Leppanen, & Treasure, 2015) investigating the impact of experimentally induced-mood on food intake reported that induction of a negative mood was associated with increase food consumption by participants, and that this effect was particularly pronounced for participants with BED or who were restrictive eaters, compared to healthy controls. However, food consumption was also increased in the positive mood induction condition compared to the neutral mood, suggesting that food intake may, to an extent, be related to mood regardless of valence.
Another possibility is that depression may result as a consequence of an ED. This may occur due to a number of factors associated with EDs, including increased psychological distress, low self-esteem, guilt associated with binging, purging, or failure to adhere to restrictive dieting goals, or reduced social contact resulting in loss of positive environmental reinforcers (Levine, 2012; Martyn-Nemeth, Penckofer, Gulanick, Velsor-Friedrich, & Bryant, 2009; Tanofsky-Kraff et al., 2011). Other theorised links are more biological in nature, such as food deprivation associated with restrictive eating leading to mood dysregulation (Lieberman et al., 2017).

One meta-analysis explored the direction of the relationship between EDs and depression, by collating longitudinal investigations that examined the association between these two mental health disorders (Puccio et al., 2016). The inclusion of exclusively longitudinal studies allows for inferences regarding risk, unlike in most investigations of ED risk factors (e.g., sexual abuse, as described in the next chapter). Authors found that across the 30 studies identified, there was a significant effect of depression on subsequent ED diagnosis or disordered eating ($r = .16$, 95% CI: .10, .22). However, they also found a significant relationship between eating pathology and future depression ($r = .13$, 95% CI: .09, .17), suggesting a reciprocal relationship between the two constructs.

Meanwhile, in a meta-analysis by Luppino et al. (2010) investigating the longitudinal relationship between obesity or overweight status and depression, it was noted that obesity (OR: 1.55, 95% CI: 1.22, 1.98) and overweight status (OR: 1.27, 95% CI: 1.07, 1.51) were largely predictive of later depressive symptoms and depressive disorder, while the inverse relationship was less clear. Based on the differences between EDs and obesity, with the former more likely to be preceded by depression than the latter, it is possible that the
mechanisms through which depression may act as a risk on EDs may not be due to increases in food consumption evoking dysregulated attempts at weight control (as might be expected if depression was identified as a risk factor for obesity), but perhaps more likely due to changes in perceptions of oneself and one’s body that may arise as a consequence of distorted cognitions in depression.

In sum, based on current meta-analyses of longitudinal studies (Puccio et al., 2016; Luppino et al., 2010), there is emerging robust evidence that depression constitutes a risk factor for EDs and disordered eating, although it remains unclear how this varies according to ED diagnosis or type of eating pathology.

1.4.2. Impulsiveness

Another psychological factor that has been hypothesised to relate to EDs and onset of ED symptoms is impulsivity. Impulsivity is considered a stable personality trait and is incorporated in numerous models of personality (Cloninger, Svrakic, & Przybeck, 1993; Costa & McCrae, 1990; Eysenck & Eysenck, 1985). According to leading theoretical models (Whiteside & Lynam, 2001), impulsivity consists of four domains: negative urgency, premeditation, perseverance, and sensation seeking. Negative urgency refers to the tendency to engage in rash actions in response to strong negative emotions (e.g., binging on food when upset). Lack of premeditation refers to engaging in behaviours without careful thinking and planning, while lack of perseverance describes difficulty persisting with tasks until completion and avoiding boredom. Finally, the sensation seeking scale reflects an individual’s drive to seek excitement, adventure, and novel stimuli. However, somewhat different conceptualisations of impulsivity are also used, such as the Barratt Impulsiveness
Scale (BIS; Patton, Stanford, & Barratt, 1995), which includes attentional impulsiveness, motor impulsiveness, and non-planning impulsiveness.

Theoretically, higher impulsivity may constitute a risk for EDs, in particular BN, due to deficits in impulse control increasing engagement in binge eating and purging behaviours (Cassin & von Ranson, 2005; Robinson, Pearce, Engel, & Wonderlich, 2009; Rosval et al., 2006). A number of reviews and meta-analyses have investigated the empirical relationship between impulsivity and EDs (e.g., Stice, 2002; Waxman, 2009). Impulsivity, considered as a global variable, was significantly (but weakly) related to EDs in a meta-analysis by Stice (2002), which collated results from four longitudinal studies that did not themselves report significant findings. Similar results were reported in a subsequent systematic review of twelve mostly cross-sectional studies by Waxman (2009). Not all findings support this relationship, with three prospective longitudinal studies in Wonderlich, Connolly, and Stice (2004) analysing the relationship between impulsivity and later ED behaviour suggesting that impulsivity is not a risk factor for ED onset. However, these studies used author-composed scales rather than a well-established tool such as the BIS to measure impulsivity, with Cronbach’s alpha below .70 in two of the three studies. In light of mixed findings, it may be of more relevance to investigate the relationship between EDs and the unique facets of impulsivity, which may elucidate precisely which elements of impulsivity play a role in EDs.

Studies inspecting the facets of impulsivity individually lead to a somewhat clearer picture of the link between this personality trait and eating pathology. In particular, the negative urgency facet is most strongly linked to EDs, with higher negative urgency associated with increased binging, purging, and loss of control behaviours in both
individuals with AN and BN (Fischer, Smith, & Anderson, 2003; Hoffman et al., 2012). Possible explanations include that individuals high on negative urgency are possibly more likely to engage in binge eating in order to alleviate negative mood states (Pearson, Wonderlich, & Smith, 2015), with food consumption perceived by some as providing temporary positive experience (Fischer, Anderson, & Smith, 2004). Indeed, the expectancy that food consumption lowers negative affect mediates the relationship between negative urgency and binge eating (Racine & Martin, 2017), with this belief also prospectively associated with increased binge eating (Pearson, Zapolski, & Smith, 2015; Smith, Simmons, Flory, Annus, & Hill, 2007). Accordingly, increased state distress is associated with greater bulimic symptomatology (Smyth et al., 2007), as is trait distress (e.g., neuroticism; Peterson et al., 2010).

Overwhelmingly, the relationship between negative urgency and EDs has received strong empirical support. Farstad et al. (2016), who conducted a meta-analysis of 14 studies of clinical ED populations, identified negative urgency as elevated in all EDs (AN, BN, and BED). In a subsequent review by Culbert et al. (2015) examining community samples, negative urgency was prospectively related to increases in disordered eating, particularly binge eating and purging. In the most comprehensive meta-analysis on the topic, which included 50 studies (cross sectional and longitudinal) and focussed specifically on BN, Fischer, Smith, and Cyders (2008) found that negative urgency was by far the strongest predictor of BN ($R = .40$) compared to other impulsivity facets (sensation seeking [$R = .16$], lack of premeditation [$R = .20$], lack of perseverance [$R = .08$]). Furthermore, one twin study reported genetic associations between negative urgency and dysregulated eating
(binge eating and emotional eating; Racine et al., 2013), suggesting a possible biological link. Only weak genetic associations were noted for the other impulsivity facets.

There is also some evidence for a role of sensation seeking in EDs. The meta-analysis by Fischer et al. (2008) identified a small but significant relationship between sensation seeking and BN ($R = .16$, 95% CI: .13, .19), while Farstad et al. (2016) found that sensation seeking was lower in individuals with AN than controls, but higher in those with BN. This difference in sensation seeking levels between ED diagnoses aligns with the type of eating pathology involved, with individuals with AN showing greater restraint to food, a reward-inducing stimuli, while binging behaviour in individuals in BN may be partly ascribed to their desire for new and exciting stimuli or engagement in thrilling or excessive behaviours (Rossier, Bolognini, Plancherel, & Halfon, 2000). Similarly, persistence has been found to be weakly but significantly related to BN (Fischer et al., 2008; $R = .08$, 95% CI: .04, .12), but higher in those with AN compared to controls and individuals with BN (Farstad et al., 2016). This may be reflected in the high levels of ongoing self-control required to sustain the restrictive eating patterns typical of AN. Finally, Fischer et al. (2008) found that lack of premeditation was also weakly related to BN ($R = .20$, 95% CI: .14, .18), while there was mixed evidence for the role of this facet in EDs in Farstad et al. (2016). Premeditation may act as a moderator between the urge to binge and the ability to engage in dietary restraint (Steiger, Lehoux, & Gauvin, 1999).

A current limitation to the present literature investigating impulsivity in EDs is a paucity of longitudinal studies. It is therefore, once again, not possible to conclude whether impulsivity constitutes a risk factor for EDs, or a correlate that emerges concurrently with or as a result of an ED. To an extent, given that impulsivity is both a personality trait a form
of temperament, both which by definition are formed early in life and remain relatively stable (Blatný, Millová, Jelínek, & Osecká, 2015; Buss & Plomin, 2014), it is more likely that cross-sectional associations do represent risk-relationships as opposed to solely correlates. However, further longitudinal studies are necessary to support these, now well-established, cross-sectional findings.

1.5. Chapter summary

The present chapter provided an introduction to ED diagnostic criteria and epidemiology, touching upon some of the serious consequences of an ED diagnosis. At a sub-clinical level, disordered eating was also highlighted as a common precursor to EDs, which in itself can have a severe impact on physical and emotional health and daily functioning. Amongst the variety of comorbid psychological factors associated with EDs, discussion focused upon depression and impulsivity, which have accrued amongst the highest levels of support for a role in EDs. Although they are primarily considered to be comorbid factors, theory and findings conceptualising these traits as possible risk factors for EDs was presented. The following chapter will expand on the concept of risk for EDs and discuss current findings pertaining to a range of environmental factors that may increase susceptibility to maladaptive eating behaviours.
2. CHAPTER 2

Environmental Risk Factors and Correlates of Eating Pathology

2.1. Introduction to risk factors in Eating Disorders

The emergence of EDs is preceded by exposure to various risk factors that increase susceptibility to eating pathology. Such variables are diverse in nature and include individual psychological characteristics (e.g., depressed mood; Puccio et al., 2016; as discussed in the previous chapter), individual genetic or biological differences (see Chapter 3), or exposure to certain challenging environmental conditions (e.g., abusive environments; Trottier & MacDonald, 2017). Identifying the key risk factors that underlie ED development can help provide insight into causal mechanisms and facilitate development of appropriate prevention and treatment interventions. The present chapter will focus on a review of the environmental risk factors and correlates of eating pathology, with individual consideration of both clinical EDs and disordered eating.

Disordered eating is considered a prodromal risk factor for the development of later clinical-level eating pathology (Stice, et al., 2010). It is prevalent in adolescence and is associated with lowered quality of life and significant impairment across numerous domains (Ackard, Richter, Egan, Engel, & Cronemeyer, 2014; Jenkins, Hoste, Meyer, & Blissett, 2011). Establishing a strong understanding of disordered eating, a factor that precedes the onset of an ED, is a further avenue through which to better understand why particular individuals are more likely to develop eating disturbances. Better understanding the likely causes of disordered eating can assist clinicians in applying targeted prevention strategies in at-risk populations and may curb the number of individuals who proceed to
develop a full-threshold ED (Pennesi & Wade, 2016; Stice, et al., 2010). Awareness of risk factors for disordered eating can also be promoted in the wider community through education initiatives and health-promotion campaigns. This may increase understanding of the potentially serious consequences of extreme dieting, equip individuals to more readily perceive warning signs or disordered eating symptoms in friends and relatives, and perhaps increase access to support services by those experiencing disordered eating before they progress to a ‘full blown’ ED.

2.2. Methodology for identifying risk factors and correlates in psychopathology

Investigation of risk factors and correlates is largely an empirical endeavour, which hinges upon a strong theoretical understanding of precisely what denotes a risk factor and a correlate. Therefore, the theoretical underpinnings of risk factors will first be delineated, followed by an in-depth discussion of the risk factors and correlates thus far identified for EDs and disordered eating.

Kraemer et al. (1997) defines a risk factor as a “measureable characterisation of each subject in a specified population that precedes the outcome of interest” (pg 338), with a risk factor that may change spontaneously (e.g., weight or age) classified as a ‘variable’ risk factor, while one that is stable (e.g., year of birth, genes) termed a ‘fixed marker’. Where a variable risk factor is manipulable, and its manipulation is shown to exert a change on the outcome variable, this is considered a ‘causal risk factor’ (Kraemer et al., 1997). On the other hand, a ‘variable marker’ is a variable risk factor whose manipulation does not result in a change in the outcome variable (see Figure 1). Importantly, the term ‘cause’ is restricted for a necessary and sufficient condition for the disorder.
At present most studies in the ED field, including those in the present thesis, examine the ‘risk factor’ concurrently with the outcome variable (e.g., in a cross-sectional design). As these factors were not demonstrated to precede the outcome they cannot be classified as ‘risk factors’ per se, however if they satisfy all other requirements of a risk factor they are regarded as ‘concomitants’ or ‘correlates’ of the disease (Kraemer et al., 1997).

Figure 1. Guide for identifying risk factor status (Kraemer et al., 1997)
Kraemer et al. (1997) further specifies that to accurately determine a risk factor, it is necessary to measure the particular risk factor (e.g., childhood abuse) in the sample prior to the onset of the outcome under investigation (e.g., ED diagnosis), and then to observe how many participants subsequently develop the outcome of interest. A minimal requirement is that a statistically significant association between the risk factor and outcome is established, but it is also necessary for a measure of risk potency to be provided (e.g., odds ratio with confidence intervals). A number of study designs, discussed below, can be utilised to investigate whether a given variable meets these conditions.

2.2.1. Prospective longitudinal design

Prospective longitudinal studies are an ideal study design under which to examine the effects of a range of risk factors on ED outcomes (Jacobi, Hayward, de Zwaan, Kraemer, & Agras, 2004). Under this design a group of individuals can be observed from an initial time point where there are no cases of the disorder. The theorised risk factors can subsequently be measured and researchers can observe whether these are related to later disorder onset (Kraemer et al., 1997). Such study designs are also able to control for a wide array of factors that may influence the relationship between the risk factor and outcome under investigation (e.g., age, gender, BMI, other psychopathology, socio-economic status [SES], parental education, and so on). Longitudinal prospective studies are still somewhat limited by their observational nature, however exploration of risk factors for psychopathology is difficult and often not ethically feasible using an experimental design.

One major difficulty of using prospective studies to explore ED-related risk factors is the relatively low prevalence of EDs in the population. For example, in one study of 1010
school girls (Patton, Johnson-Sabine, Wood, Mann, & Wakeling, 1990), only 4 new cases of BN were identified at a one year follow up period. This percentage was somewhat larger in the Western Australia Pregnancy Cohort, where from $N = 1,597$ collected at birth, 6% of the sample met full or partial criteria for an ED (Allen, Byrne, Forbes, & Oddy, 2009). In the Avon Longitudinal Study of Parents and Children, where only self-reported AN and BN were considered, 247 women self-reported AN while 194 women self-reported BN, from a total of 14,663 women recruited in 1992 (Micali, Simonoff, & Treasure, 2009). These low numbers are unsurprising given the low population prevalence of diagnosed EDs, however they have the effect of greatly limiting the power of a given sample size in a longitudinal prospective study, and reduce the study’s ability to provide robust insights regarding risk factors.

There are a number of alternative study designs that may be employed to overcome this limitation. Firstly, it is possible to conduct prospective longitudinal studies using a dimensional measure of the outcome at hand in lieu of a categorical diagnosis (e.g., level of disordered eating or body dissatisfaction). This allows for the outcome variable to be represented by a range of values spanning from low to high on the outcome, with the risk factor assessed based on whether it is significantly associated with higher levels of the outcome variable. Besides increasing sample size by eliminating the presence of a strict ‘clinical ED group’, this has the advantage of identifying attenuated or sub-clinical symptoms (i.e. disordered eating) that may later develop into a clinical ED. This method has been adopted in a range of prospective longitudinal studies examining risk factors for disordered eating (e.g., Goldschmidt, Wall, Loth, & Neumark-Sztainer, 2015; Gonsalves, Hawk, & Goodenow, 2014; Haynos, Watts, Loth, Pearson, & Neumark-Stzainer, 2016;
Leon, Fulkerson, Perry, Keel, & Klump, 1999; Stice & Agras, 1998). As previously discussed, understanding the risk factors of these symptoms can help early intervention and prevent the development of ‘full blown’ EDs.

### 2.2.2. Cross-sectional designs

Another option involves adopting a cross-sectional design in lieu of the longitudinal approach. This provides a number of direct methodological benefits, for example, the option to compare a clinical cohort where the outcome of interest is present (e.g., individuals with AN sampled from an inpatient ED unit) to matched healthy controls without the disease (Schlesselman, 1982). The two groups may be contrasted on the risk factor(s) of interest, which are measured via retrospective means. This approach allows for larger samples of clinical populations, is less resource intensive, and produces quicker results compared to a large longitudinal prospective study (Mann, 2003). Furthermore, comparing risk factors using a clinical vs control sample involves comparison between those who clearly do have the disorder in question and those who do not, with conclusions gleamed from these studies often more clear-cut and easier to apply (e.g., in informing policies or treatment), compared to studies with dimensional measures of psychopathology, although the cross-sectional research design precludes investigation of causality (Kraemer et al., 1997).

Adopting a cross-sectional design using a community, as opposed to clinical, sample retains some of the aforementioned benefits, such as the ability to relatively quickly obtain large samples for analysis using fewer resources. It also contributes a range of other benefits to ED research. For example, using a dimensional measure of disordered eating
(e.g., the Eating Disorder Inventory-2 [EDI-2, Garner, 1991] which investigates drive for thinness, bulimic tendencies, and body dissatisfaction) in a community sample allows researchers to identify prodromal or attenuated/sub-clinical symptoms, which may be valuable for informing prevention and early intervention treatments (Rohde, Stice, & Marti, 2015). It also provides a more sensitive measure of eating pathology that takes into consideration symptom severity and type. For example, it allows conclusions to be drawn regarding what particular symptom or groups of symptoms (e.g., dietary restraint) are related to the risk factor or correlate at hand, while in contrast, investigating individuals on the basis of diagnosis vs control group does not allow for this nuanced understanding of the relationship between risk and outcomes. Investigating a community sample also provides the opportunity to target individuals who may not present for treatment, whereas clinical samples are subject to referral bias (Fairburn, Welch, Norman, O'Connor, & Doll, 1996). The latter point is particularly relevant in the ED field, where many individuals with the disorder never seek treatment (Keski-Rahkonen et al., 2007), possibly due to the numerous perceived barriers to treatment identified (e.g., stigma and shame, failure to realise severity of the disorder; Ali et al., 2016).

2.2.3. Summary of research designs

To sum, there are a variety of study designs and methods available for examining risk factors and correlates in eating pathology, each with associated strengths and limitations. Prospective longitudinal designs are the gold standard for investigating risk factors, however are often impractical due to their resource-intensive nature and the low prevalence of EDs in the general population. Therefore, many studies adopt a cross-sectional design as
an alternative model, using either using a case-control or community sample, although these studies are suited to identify correlates of EDs rather than risk factors per se. Nonetheless, preliminary risk studies tend to be cross-sectional in nature and aim to establish correlates of given diseases or outcomes (Kazdin, Kraemer, Kessler, Kupfer, & Offord, 1997), and can constitute an important first step in identifying candidate variables that can be examined in future longitudinal studies to confirm their risk factor status.

2.3. Current evidence for correlates and risk factors in eating pathology

Following on from discussion of risk factor theory and study design, an overview of currently identified environmental risk factors and correlates of EDs will be provided. Evidence will first be presented relating to the identified risk factors and correlates with the most empirical support for EDs overall, followed by discussion of risk factors for individual ED diagnoses and for disordered eating. Discussion will then focus on current evidence for the role of traumatic life events, sexual abuse, physical abuse, depression, impulsivity, and parenting behaviours as risks and correlates for eating pathology, which will be a focus of the present thesis. Genetic risk factors and correlates are discussed in the subsequent chapter. Risk factors such as body dissatisfaction, dieting, importance of weight and shape, etc., are not purely environmental however will be included in the current discussion for sake of completeness. Likewise, although psychological factors, such as depression and impulsivity, were explored in the previous chapter, their roles will be briefly mentioned below, where relevant, in the overview of risk factors for each ED.

Only a small number of risk factors have been identified for EDs (considered overall) using prospective longitudinal designs (Stice, 2016). Those with the most support include
an individual’s levels of neuroticism (Bulik et al., 2006; Cervera et al., 2003; Ghaderi & Scott, 2000), negative affect (Beato-Fernández, Rodríguez-Cano, Belmonte-Llario, & Martínez-Delgado, 2004; Leon et al., 1999; Rohde et al., 2015), body dissatisfaction (Beato-Fernández et al., 2004; Jacobi et al., 2011; Rohde et al., 2015; Stice, Marti, & Durant, 2011), thin-ideal internalisation (Jacobi et al., 2011; Rohde et al., 2015), and social pressure to be thin (McKnight Investigators, 2003; Rohde et al., 2015).

When considering studies that assess correlates of eating pathology, which are largest in number due to the aforementioned resource-intensive nature of conducting prospective research, a broader picture is presented. Identified correlates of EDs range from factors specific to the individual (e.g., perfectionism, neuroticism, low self-esteem, overvaluation of shape and weight; Jacobi et al., 2004) to environmental influences (e.g., critical comments about weight, social ideation of thinness; Groesz, Levine, & Murnen, 2002; Pike et al., 2008), and genetic vulnerability (see Culbert et al., 2015, for an overview).

### 2.3.1. Overview of environmental risk factors for and correlates of Anorexia Nervosa

Individual studies have identified numerous risk factors for AN. Those with the most empirical support as risk factors from prospective longitudinal studies include various perinatal factors, such as premature birth and complications during delivery such as cephalhematoma (Cnattingius, Hultman, Dahl, & Sparén, 1999; Lindberg & Hjern, 2003), infant feeding problems and a history of undereating (Nicholls & Viner, 2009), personality factors such as perfectionism (Tyrka, Waldron, Graber, & Brooks-Gunn, 2002) and neuroticism (Bulik et al., 2006), as well as parental psychiatric problems (Lindberg &
Hjern, 2003; Nicholls & Viner, 2009). This list of variables remains quite scarce, and, due to various limitations in existing longitudinal research, the role of most of these factors as true ‘risks’ for AN remains contested (Stice, 2016).

Cross-sectional and retrospective analyses have also provided support for the aforementioned factors as correlates of AN: for example, perfectionism (Cassin & von Ranson, 2005; Farstad et al., 2016; Machado et al., 2014; Pike et al., 2008; Wade et al., 2008a), family history of ED or parental psychiatric illness (Machado et al., 2014; Pike et al., 2008), and neuroticism (Cassin & von Ranson, 2005). Meanwhile, various additional factors have also been identified as correlates of AN, including those directly related to weight, such as weight concern and exposure to critical comments about weight or shape (Machado et al., 2014; Pike et al., 2008), personality and temperament characteristics such as negative affectivity, need for organisation, reward dependence, and emotional regulation (Cassin & von Ranson, 2005; Oldershaw, Lavender, Sallis, Stahl, & Schmidt, 2015; Pike et al., 2008; Wade et al., 2008a), cognitive features such as difficulties in set-shifting (Westwood, Stahl, Mandy, & Tchanturia, 2016), as well as some suggestion of a role for antecedent life events and sexual and physical abuse (Caslini et al., 2016; Machado et al., 2014; Pike et al., 2008). Family factors such as unresolved family disagreements, problem parenting, parental demands, family discord, and foster care have also been identified as potential correlates of AN (Machado et al., 2014; Pike et al., 2008), although some studies have found no evidence of a role for factors such as foster care and authoritarian parenting (Nicholls & Viner, 2009). The potential role of parenting in AN and EDs is further explored later in the present chapter.
Fairburn, Cooper, Doll, and Welch (1999) argue that many identified risk factors for AN and other EDs may in fact represent risk for overall psychiatric illness, although studies that have compared AN groups to both psychiatric and healthy control groups have found a number of specific correlates for AN (Machado et al., 2014; Pike et al., 2008; e.g., perfectionism, family dynamic/discord, weight and eating concerns, and family EDs, with mixed evidence for parental demands, family psychiatric disorder, and family weight and eating concerns).

Overall, the most common profile of an individual at risk of AN typically includes elevated perfectionism and holding high personal standards and expectations, coupled with negative views of one’s own weight and shape, parental ED or weight and shape issues, genetic liability (see Chapter 3), as well as exposure to a number of general environmental and personal risk factors for psychiatric illnesses.

2.3.2. Overview of environmental risk factors for and correlates of Bulimia Nervosa

Research investigating risk factors in BN includes only a relatively small number of prospective longitudinal studies, with evidence for risk factors including dieting and fasting (Patton et al., 1999; Stice, Davis, Miller, & Marti, 2008), social pressure to be thin and body dissatisfaction (Stice et al., 2011), weight and eating concern (Allen et al., 2016), low food intake during childhood (Kotler et al., 2001), and negative affect (Tyrka et al., 2002), with one review arguing that the risk factor with the strongest prospective evidence for a role in BN is dieting (Stice, 2016). Some of the most commonly identified correlates of BN, based on studies using cross-sectional designs and retrospective report, include teasing
or critical comments regarding shape or weight (Fairburn, Welch, Doll, Davies, & O'Connor, 1997; Gonçalves, Machado, Martins, & Machado, 2014), parent psychiatric illness (Boumann & Yates, 1994), obstetric complications such as low birth weight (Raevuori, Linna, & Keski-Rahkonen, 2014), sexual abuse (Hilbert et al., 2014), and novelty seeking and neuroticism (Atiye et al., 2015; Cassin & von Ranson, 2005; Wade et al., 2004).

Many of these risk factors and correlates have also been identified in AN (e.g., weight and eating concern, dieting, exposure to critical comments regarding weight or shape, negative emotionality, parent psychiatric illness, etc.; Jacobi et al., 2004), suggesting a similar pattern of underlying pathology. One possible key differentiating factor between the two disorders is that BN appears more likely to be preceded by patterns of binge eating behaviour while AN is more likely to be preceded by dieting behaviour (Hilbert et al., 2014).

### 2.3.3. Overview of environmental risk factors for and correlates of Binge Eating Disorder

Fewer studies have investigated risk factors for BED, a new addition to EDs in the DSM-5 (APA, 2013). Given its recent inclusion in the DSM-5, there remains a lack of prospective longitudinal studies investigating possible risks for BED, with just one study showing some support for a role of social pressure for thinness (Stice et al., 2011), and another recent study finding support for a host of variables, including thin-ideal internalisation, body dissatisfaction, dieting, overeating, and negative affect (Stice, Gau, Rohde, & Shaw, 2017). These studies constitute a promising start in identifying risk factors
for BED, although require independent replication to provide further evidence for the initial findings. In terms of correlates of BED, one meta-analysis of 16 studies by Linardon (2017) found that dietary restraint, weight, shape and eating concerns may play a role in BED, while other studies have identified factors such as comorbid psychiatric disorders (depression and anxiety) and stressful life events (Degortes et al., 2014; Kessler et al., 2013).

The large overlap in aetiological factors between EDs, particularly between AN and BN, and between BN and BED (Hilbert et al., 2014), is not surprising given the closely linked nature of the disorders. AN and BN both involve a disruption of eating directly linked to body image concerns, while BN and BED both involve binge eating with loss of control. As such, many individuals with AN later proceed to develop BN (Eddy et al., 2008), or with BN go on to develop BED (Hilbert et al., 2014), indicating a fluidity between the disorders and also other forms of eating pathology. This further complicates identification of specific risk factors for the individual forms of ED identified in the DMS-5 (APA, 2013). Indeed, given the diagnostic movement and symptom cross-over between the ED diagnoses (Tozzi et al., 2005), it may be equally fruitful to analyse antecedents of underlying disordered eating pathology (such as drive for thinness or engaging in binge-purge behaviours).

2.3.4. Overview of environmental risk factors for and correlates of disordered eating

Risk factors for disordered eating have more empirical support from prospective longitudinal studies compared to risk factors for clinical EDs, largely due to the relatively
less intensive nature of assessing community samples in such designs (Lilenfeld, Wonderlich, Riso, Crosby, & Mitchell, 2006). Under these investigations, risk factors with the most longitudinal support for a role in disordered eating behaviours, such as binge eating and purging, restrictive eating, and also risk of developing an ED, include thin-ideal internalisation, greater social pressure to be thin, body dissatisfaction, dieting and weight concerns, low self-esteem, negative affect or emotionality, and neuroticism (Goldschmidt et al., 2015; Gonsalves et al., 2014; Haynos et al., 2016; Jacobi et al., 2004; Leon et al., 1999; Martin et al., 2000; Stice & Agras, 1998).

Amongst the numerous identified correlates of disordered eating, those with the most support include weight and appearance teasing, (Menzel, 2010), media exposure (Grabe, Ward, & Hyde, 2008; Levine & Murnen, 2009), other psychiatric disorders (e.g., anxiety and mood disorders; Solmi et al., 2014), personality and temperament traits including neuroticism (Eggert, Levendosky, & Klump, 2007; Luo et al., 2013; Miller et al., 2006; Wade et al., 2000a), perfectionism (Forbush et al., 2007; Petrie, Greenleaf, Reel, & Carter, 2009), impulsivity (Lee-Winn, Townsend, Reinblatt, & Mendelson, 2016), emotional regulation difficulties (Ambwani, Slane, Thomas, Hopwood, & Grilo, 2014; Cooper, O'Shea, Atkinson, & Wade, 2014), and, more recently identified, the occurrence of multiple victimisation experiences (Hasselle, Howell, Dormois, & Miller-Graff, 2017).

2.4. Factors under investigation in the present thesis

Following on from the overview of broad correlates and risk factors for AN, BN, BED, and disordered eating, subsequent discussion will explore in greater detail existing research relating to the specific environmental correlates and risk factors investigated in the present
thesis. The environmental variables under examination, traumatic life events, sexual and physical abuse, and parenting behaviours, have overwhelmingly been assessed using cross-sectional or retrospective design, and thus are discussed in terms of ‘correlates of’ EDs. In contrast, the psychological factors under present investigation, depression and to a lesser extent, impulsivity, have been examined in longitudinal studies and thus inferences may be tentatively made in terms of risk.

2.4.1. Environmental correlates for eating pathology: Traumatic life events

Amongst the many factors proposed to be involved in the development of an ED, the experience of traumatic life events (also referred to as ‘adverse events’ or ‘life stressors’), has been empirically investigated in numerous studies (e.g., Meiser & Esser, 2017; Speranza, et al., 2003; Fairburn et al., 1997). For the purposes of the present review, traumatic life events will be conceived as broad measure of childhood adversity that is typically determined holistically in terms of total number of events, or by assessing trauma across certain ‘domains’, such as difficulties at home, poor health, experience of abuse, etc. Traumatic life events may include, but are not limited to, childhood abuse.

One theory is that exposure to stress related to adverse life events may provoke the use of certain maladaptive coping strategies (Heatherton & Baumeister, 1991), such as engaging in binge eating as a distraction technique, or restrictive eating to exercise control in a domain not related to the adverse event. Indeed, links have been identified between adverse life events and changes in body weight and dietary restraint (Johnson, Cohen, Kasen, & Brook, 2002), and events such as childhood bullying and increased disordered eating behaviour (Wade, Gillespie, & Martin, 2007). Moreover, the experience of traumatic
life events is associated with a host of physiological changes that may increase individual susceptibility to a range of negative outcomes (Berens, Jensen, & Nelson, 2017).

A collection of studies investigating the role of traumatic life events in EDs and disordered eating are discussed below. To date, all but one identified study (Johnson et al., 2002) have used a cross-sectional design, with either a semi-structured interview or questionnaire employed. Results of these studies thus examine whether traumatic life events are a correlate of EDs, as prospective longitudinal studies are required to test whether this variable may constitute a risk factor (Kraemer et al., 1997).

2.4.1.1. Traumatic life events: Anorexia nervosa

From the studies identified in a non-systematic albeit thorough review of the literature, three studies examined whether AN patients differed from healthy controls in terms of number of traumatic life events experienced (Gowers, North, Byrum, & Weaver, 1996; Horesh et al., 1995; Speranza et al., 2003). Horesh et al. (1995) included both males and females in their study ($N = 140$) and queried events that had occurred more than one year prior to diagnosis in the following domains: Bad experiences with parents (including physical punishment), separations, deaths, and bad experiences with family. T-tests revealed that the overall total life events score, calculated by summing each event, and bad experiences with parents were significantly higher in the AN group compared to healthy controls. The AN group also scored significantly higher on this domain compared to a non-ED psychiatric inpatient group. AN patients scored higher on bad experiences with other family members than did the control group, although did not differ from the psychiatric controls on this domain.
Also testing both males and females (N = 35), Gowers et al. (1996) assessed events experienced by patients with AN in the year before disorder onset, compared to psychiatric and healthy controls. Events assessed included, but were not limited to, parental death, divorce/separation, ill health, burglary, peer ill health or death, loss of friendship, change of school, physical illness, assault, and other. No differences were found between groups, although there was a trend for AN patients to report a high number of ‘extremely negative’ life event compared to controls.

Finally, testing a female-only sample of patients with AN, BN, and healthy controls (N = 391), Speranza et al. (2003) found that traumatic life events, including parental divorce, separation from family, adoption, severe medical or psychiatric illness, death of close family member, and physical or sexual abuse, significantly predicted membership of the AN or BN groups compared to controls.

2.4.1.2. Traumatic life events: Bulimia nervosa

A few studies have examined the role of traumatic life events in patients with BN compared to controls (Day et al., 2011; Fairburn et al., 1997; Raffi, Rondini, Grandi, & Fava, 2000; Speranza et al., 2003; Welch, Doll, & Fairburn, 1997). Speranza et al. (2003), discussed above, found a significant difference between ED groups (AN & BN) and controls in number of traumatic life events experienced. Another study, by Welch et al. (1997) measured a broad array of disruptive life events in a female-only sample (N = 306), with a linear trend between number of life events and greater likelihood of BN. In particular, sexual abuse (OR: 6.20, 95% CI: 1.70, 22.72) and pregnancy (OR: 16.0, 95% CI: 2.00, 127.9) most strongly differentiated the BN group from controls, followed by
physical abuse, a major house move, change in family structure, and significant physical illness. Major illness of close relative, friend, or partner, bereavement, commencement or dissolution of a new significant relationship did not significantly differentiate the two groups. Raffi et al. (2000) also tested a female only sample (N = 60) on a list of 64 traumatic life events in the 6 months preceding disorder onset, and finding a significant difference between BN patients and controls.

However, not all studies have reported significant findings, with Fairburn et al. (1997) finding no difference between a BN group and a healthy control group or a psychiatric control group across five traumatic events, including parental death, change of parent figure, parental chronic illness, frequent house moves, and severe personal health problems. Similar results were also found in Day et al. (2011), who reported no difference between BN and Eating Disorder Not Otherwise Specified (EDNOS)-BN patients and controls in their experience of those same disruptive events tested by Fairburn et al. (1997). It is possible, however, that the lack of difference between the groups is due to the relatively limited subset of events measured compared to those included other studies.

2.4.1.3. Traumatic life events: Binge eating disorder

One study has examined the role of traumatic life events in BED. Pike et al. (2006) compared the number of traumatic life events in the year before disorder onset in a group of women with BED, a psychiatric control group, and a healthy control group. Life events were measured by the Oxford Risk Factor Inventory (ORFI; Fairburn et al., 1998) and were grouped into two composite variables, ‘major stress from school, work, or other source’ and ‘concerns about safety’. They found that women with BED reported a greater number of
adverse life events than did both healthy controls and psychiatric controls. Compared to the healthy controls, BED patients were more likely to have experienced major changes in circumstance and relationship (e.g. death of a close relative, house move) and physical abuse.

### 2.4.1.4. Traumatic life events: Disordered eating

Finally, a number of studies identified in the present review have examined the relationship between traumatic life events and disordered eating. Using a prospective longitudinal design, Johnson et al. (2002) examined the relationship between eating pathology and history of childhood maltreatment (sexual or physical abuse or neglect), maladaptive parental behaviour (e.g., punishment, possessiveness, loud arguments, difficulty controlling anger towards child), and other childhood adversities, including death of a parent, disabling parental accident or illness, living in an unsafe neighbourhood, low parental education, parental separation or divorce, peer aggression, low family income, school violence, family member victim of crime, and upbringing by a single parent. Physical neglect, sexual abuse, and childhood adversity were associated with greater likelihood of an ED, as well as numerous disordered eating problems, including obesity, fluctuations in weight, strict dieting, and self-induced vomiting. Meanwhile, low parental affection was associated with greater likelihood of extensive fasting and use of medication to lose weight, while experiencing three or more types of maladaptive parenting behaviour was associated with a three times greater likelihood of having an ED compared to those who did not experience any maladaptive parenting. Results of this study indicate that
physical neglect, sexual abuse, child adversity, and maladaptive parenting behaviour or low parental warmth may all be variable risk factors for disordered eating symptoms.

Other studies have investigated the relationship between disordered eating and traumatic life events using a cross-sectional design. Smyth, Heron, Wonderlich, Crosby, and Thompson (2008) examined whether levels of restrictive eating behaviours and binge-purging behaviours at two time points were predicted by traumatic life events, including the death of a loved one, parental divorce, a sexual event, a violent event, a non-personal event, or another traumatic event, as well as the severity of each event, to create a total severity index. At the first time-point, they found that restricted eating was predicted by more severe violent trauma but not by other events, while binge-purging was predicted by a number of events, including death of a loved one, divorce/separation of parents, and violent trauma. Similar results were obtained when analysing disordered eating symptoms 6 months later. Unlike in Johnson et al. (2002), sexual trauma was not related to either type of disordered eating behaviour. Notably, as traumatic life events were measured retrospectively during the first time point as opposed to the second time-point, results of this study suggest these events are a ‘correlate’ of disordered eating symptoms.

Loth, van den Berg, Eisenberg, and Neumark-Sztainer (2008) tested a sample of 1708 male and female adolescents and found that levels of binge eating and extreme weight control behaviours (e.g., vomiting, diet pills, laxatives) were associated with experiencing a greater number of stressful life events. Events measures included 12 stressful life events (e.g., motor-vehicle accident, parental divorce), coded on a 0, 1, 2, 3+ scale. Meanwhile, McEwen and Flouri (2009) tested adolescents (N = 203) from a socio-economically disadvantaged background in England and found that life events, as measured via a list of
25 possible stressors, were associated with greater disordered eating symptoms, including when controlling for gender, emotional regulation, and paternal warmth, control and overprotection. Also adopting a retrospective measure of traumatic life events, Meiser and Esser (2017) found that reporting traumatic events during a semi-structured interview (in the domains of parents/family, school/education, leisure/friends, romantic relationships, health, and others), was associated with greater disordered eating symptoms in a community sample of 922 adolescents in Germany.

Overall, there is a moderate amount of research supporting the role of traumatic life events in AN, BN, and disordered eating. A systematic review or meta-analyses of these studies would help increase clarity of findings. Importantly, given the predominant use of cross-sectional research in this literature, particularly when clinical samples are under investigation, further high-quality prospective studies are desirable to allow inferences regarding whether traumatic life events can truly be considered a ‘risk factor’ for EDs, or whether they are better considered as correlates or consequences of EDs.

2.4.2. Environmental correlates for eating pathology: Sexual and physical abuse

As a construct distinct from number of traumatic life events, the experience of sexual and physical abuse has received substantial attention as a potential risk factor for EDs (Caslini et al., 2016). Sexual abuse that involves physical contact during childhood is not uncommon, with prevalence estimates for females around 15% and somewhat lower estimates for males, based on a number of meta-analyses (Barth, Bermetz, Heim, Trelle, & Tonia, 2013; Ji, Finkelhor, & Dunne, 2013; Stoltenborgh, van IJzendoorn, Euser, & Bakermans-Kranenburg, 2011) and longitudinal studies (Finkelhor, Shattuck, Turner, &
Hamby, 2014). A variety of theoretical links between these events and EDs are argued to be relevant, including the role of reduced self-esteem following an abuse experience increasing risk for body dissatisfaction and ED behaviours (Cervera et al., 2003), dissociation following abuse related to increased propensity to engage in binge eating episodes (Engelberg, Steiger, Gauvin, & Wonderlich, 2007; Perry, Pollard, Blakley, Baker, & Vigilante, 1995), and a need to re-gain control in response to sexual abuse that may manifest in restrictive eating behaviours (Slade, 1982). These factors may all also be relevant to the experience of general traumatic life events, with increased stress leading to use of maladaptive food-related coping strategies. However, effects of sexual and physical abuse may be exacerbated compared to other events due to the interpersonal nature of the trauma. This may be due to a variety of mediating factors, such as increased rejection sensitivity (De Paoli, Fuller-Tyszkiewicz, & Krug, 2017), shame and social anxiety (Grabhorn, Stenner, Stangier, & Kaufhold, 2006), or emotional dysregulation (Burns, Fischer, Jackson, & Harding, 2012).

Amongst studies investigating the role of childhood and adolescent sexual abuse in EDs, a number of studies discuss whether or not sexual abuse can be considered a specific risk factor for EDs, or rather constitutes a risk for psychopathology more broadly (Fairburn et al., 1997). While there is certainly some value in discussing and clarifying this point, where possible, it is also worthwhile noting that many well-established risk factors for EDs, such as dieting and body-image issues, have also been identified as risks for other psychiatric disorders (e.g., for depression; Stice, Hayward, Cameron, Killen, & Taylor, 2000). Furthermore, like most risk factors for EDs, sexual abuse is not argued to be a sole risk, but rather to contribute to overall heightened vulnerability to EDs, along with a myriad
of other factors (Smolak & Murnen, 2002). The argument, therefore, that sexual abuse may not be a specific risk factor for EDs does not invalidate its potentially important role in the development of eating pathology.

2.4.2.1. Meta-analyses examining role of childhood abuse in clinical ED samples

To date, six meta-analyses have examined the relationship between sexual and/or physical abuse and EDs (Caslini et al., 2016; Chen et al., 2010; Molendijk, Hoek, Brewerton, & Elzinga, 2017; Norman et al., 2012; Rind, Tromovitch, & Bauserman, 1998; Smolak & Murnen, 2002). Three examined sexual abuse only (Chen et al., 2010; Rind et al., 1998; Smolak & Murnen, 2002), one examined physical and emotional abuse (Norman et al., 2012), and another two examined all three types of abuse (Caslini et al., 2016; Molendijk et al., 2017). The earliest meta-analysis, by Rind et al. (1998), included a range of studies pertaining to numerous psychiatric outcome variables (e.g., EDs, depression, anxiety). Ten of the studies related to EDs, with meta-analysis suggesting that child sexual abuse was related to EDs, \( r = .06, 95\% \text{ CI:} .02, .10 \). Studies included were restricted to college samples with the aim of developing an understanding of child sexual abuse in the general population rather than in clinical samples, however it was not clear which specific studies were included in the analysis.

A second, more comprehensive meta-analysis analysis by Smolak and Murnen (2002) included 53 studies using female samples. They found that there was a small but significant relationship between sexual abuse and ED symptoms as well as sexual abuse and BN across both clinical and non-clinical samples. Most studies included a measure of disordered eating (e.g., EDI-2 or the Eating Attitudes Test-26 [EAT-26]; Garner, 1991; Garner,
Olmsted, Bohr, & Garfinkel, 1982), or BN diagnosis. Effect sizes for those diagnosed with BN versus controls, and a ‘general’ ED group, varied substantially due to different methodology applied across the studies (from $r = -.27$ to $r = .43$).

These results were corroborated by a meta-analysis of ten ED studies by Chen et al. (2010), which found a significant association between sexual abuse and lifetime diagnosis of an ED in a mixed-gender sample, OR: 2.72, 95% CI: 2.04, 3.63. Authors also found an association between severity of sexual abuse and ED diagnosis likelihood. However, the meta-analysis did not differentiate between different types of ED diagnoses or eating pathology. To clarify findings for AN and BN specifically, it is necessary to inspect the individual ten studies included in the review (Aglan, Kerfoot, & Pickles, 2008; Brown, Russell, Thornton, & Dunn, 1997; Cachelin, Schug, Juarez, & Monreal, 2005; Deep, Lilienfeld, Plotnicov, Pollice, & Kaye, 1999; Dinwiddie et al., 2000; Pettigrew & Burcham, 1997; Steiger et al., 2000; Striegel-Moore, Dohm, Pike, Wilfley, & Fairburn, 2002; Stuart, Laraia, Ballenger, & Lydiard, 1990; Welch & Fairburn, 1996).

Of the ten studies included purportedly related to EDs in Chen et al. (2010), six investigated diagnosis of AN (Aglan et al., 2008; Brown et al., 1997; Cachelin et al., 2005; Dinwiddie et al., 2000; Pettigrew & Burcham, 1997). However, full text for three of these studies (Brown et al., 1997; Cachelin et al., 2005; Pettigrew & Burcham, 1997) was unavailable. Through reading the abstract of one of these studies (Brown et al., 1997), it appeared that there was an association between sexual abuse and EDs in an inpatient population. However, it was not possible to determine which ED diagnosis was related to sexual abuse or to thoroughly assess the methodology of this study. Another study included in Chen et al. (2010) did not appear to include any published mention of ED (Aglan et al.,
2008), while it was also not possible to make any inferences regarding EDs and sexual abuse from published data in the fifth study, by Dinwiddie et al. (2000). Only one study featured in the meta-analysis by Chen et al. (2010) included accessible full-text and clear, published results relating to AN (Deep et al., 1999). In this study, Deep et al. (1999) found that those with restrictive AN reported higher rates of sexual abuse compared to healthy controls (\(p < .03\); although BN patients with a history of substance disorder reported the greatest rates of sexual abuse). There was no difference between those with AN and those with BN without a substance use disorder history. In sum, the evidence from Chen et al. (2010) regarding the relationship between sexual abuse and AN is unclear.

Four of the studies in Chen et al. (2010) related to BN (Deep et al., 1999; Steiger et al., 2000; Stuart et al., 1990; Welch & Fairburn, 1996). As mentioned, Deep et al. (1999) found a relationship between sexual abuse and BN, with stronger effects for those participants with BN and comorbid substance use disorder. In another study, also investigating comorbidity, Steiger et al. (2000) found a greater prevalence of sexual abuse history in BN patients compared to controls, although only for BN patients with comorbid Borderline Personality Disorder (APA, 1994). Stuart et al. (1990) did not find a difference in rates of sexual abuse between women with BN compared to those with depression and healthy controls, although the sample sizes involved were small (\(n = 30\) with BN) and a trend was evident. Welch and Fairburn (1996) found that sexual abuse was more common in participants meeting BN criteria than healthy controls, but found no differences between BN patients and a group of psychiatric controls. The final study from Chen et al. (2010) investigated BED (Striegel-Moore et al., 2002), and reported links between sexual abuse and BED diagnoses, with sexual abuse differentiating black women from both healthy and
psychiatric controls, but only from the former in white women. Overall, there is some evidence from Chen et al. (2010) of a relationship between sexual abuse and BN and BED, however results across the studies included suggest that this relationship may be contingent upon other psychiatric comorbidities or due to general psychiatric issues, rather than related to BN specifically.

A subsequent meta-analysis examining a range of psychological outcome variables, by Norman et al. (2012), included five studies testing the relationship between childhood physical and emotional abuse and EDs. Results showed a significant relationship between EDs and physical (OR: 2.58, 95% CI: 1.17, 5.70) and emotional (OR: 2.56, 95% CI: 1.41, 4.65) abuse, and authors claimed a five-fold increase in BN for those who had experienced physical abuse. Authors noted that BN risk was greater as physical abuse increased in severity. No studies in the meta-analysis tested specifically for AN. Authors concluded there was ‘robust’ evidence for a link between physical abuse and EDs, but only ‘weak/inconsistent’ evidence for the relationship with emotional abuse.

A recent meta-analysis, by Caslini et al. (2016), examined the relationships between childhood sexual abuse, physical abuse, emotional abuse, and ED diagnosis. Thirty-two published studies featuring case-control designs with mostly female participants were included in the review. They reported a significant relationship between any child abuse and any ED. When examining sexual abuse specifically, there was a significant relationship with AN (13 studies; OR: 1.92, 95% CI: 1.13, 3.28), BN (26 studies; OR: 2.73, 95% CI: 1.96, 3.79), and BED (6 studies; OR: 2.31, 95% CI: 1.66, 3.20), although the relationship with AN did not withstand correction for publication bias. Childhood physical abuse was also found to be associated with AN (4 studies; OR: 3.35, 95% CI: 1.43, 7.85), BN (9
studies; OR: 3.44, 95% CI: 2.56, 4.61), and BED (4 studies; OR: 3.10, 95% CI: 2.49, 3.88). Childhood emotional abuse was assessed based on two studies for each diagnosis, with significant relationships identified for BN (OR: 4.15, 95% CI: 2.71, 6.37), and BED diagnoses (OR: 3.70, 95% CI: 2.07, 6.60), but not for AN (OR: 2.13, 95% CI: .37, 12.31).

Finally, the most recently published meta-analysis, by Molendijk et al. (2017), also examined the relationship between sexual abuse, physical abuse, and emotional abuse and EDs. Results largely corroborated those of Caslini et al. (2016), although also included comparison with psychiatric controls. Molendijk et al. (2017) included a total of 82 studies, finding that compared to healthy controls, there was a significant relationship between sexual abuse and any ED (OR: 2.23, 95% CI: 1.79, 2.78), BN (OR: 2.57 , 95% CI: 1.62, 4.01), AN-BP subtype (OR: 3.09, 95% CI: 1.57, 6.08), BED (OR: 1.88 , 95% CI: 1.38, 2.55), and EDNOS (OR: 5.11, 95% CI: 1.94, 13.64) but not AN -R subtype (OR: 1.50, 95% CI: .89, 2.54). There was also a significant relationship between physical abuse and any ED (OR: 2.66, 95% CI: 2.18, 3.25), BN (OR: 3.78, 95% CI: 2.25, 6.32), AN-BP (OR: 3.06, 95% CI: 1.22, 7.67), AN-R (OR: 2.78, 95% CI: 1.13, 6.78) and BED (OR: 2.57, 95% CI: 1.99, 3.31), but not EDNOS (OR: 1.38, 95% CI: .74, 2.54), as well as emotional abuse and any ED (OR: 2.98, 95% CI: 2.30, 3.87), BN (OR: 5.13 , 95% CI: 2.80, 9.40), AN (OR: 3.81, 95% CI: 2.05, 7.08), and BED (OR: 2.44, 95% CI: 1.73, 3.43), with EDNOS and AN subtypes not assessed in the last analysis. Childhood maltreatment was more commonly reported by ED patients compared to psychiatric controls (with depressed mood or substance abuse; OR: 1.31, 95% CI: 1.08, 1.58), with no differences according to type of abuse or ED diagnosis, which runs contrary to some of the earlier meta-analyses that
reported no difference in rates of abuse between ED patients and psychiatric controls (Fairburn et al., 1997).

Overall, results from the two most recent meta-analyses, by Caslini et al. (2016) and Molendijk et al. (2017) include the most up-to-date summary of studies, are coherently and transparently presented, and should therefore be viewed as the authority on the current state of knowledge regarding childhood abuse in EDs.

### 2.4.2.2. Summary of findings regarding childhood abuse in clinical eating disorder samples

In sum, a total of five meta-analytic reviews (Caslini et al., 2016; Chen et al., 2010; Molendijk et al., 2017; Rind et al., 1998; Smolak & Murnen, 2002) have examined the relationship between EDs and sexual abuse, while three reviews have examined EDs and physical abuse (Caslini et al., 2016; Molendijk et al., 2017; Norman et al., 2012). Four meta-analyses found support for a relationship between overall EDs or ED symptoms and sexual abuse (Chen et al., 2010; Molendijk et al., 2017; Rind et al., 1998; Smolak & Murnen, 2002). When inspecting results according to ED diagnoses, evidence for a relationship between BN and sexual abuse is moderate, with all meta-analyses that specifically investigated this relationship reporting significant findings (Caslini et al., 2016; Molendijk et al., 2017; Smolak & Murnen, 2002), although there is some suggestion this may be associated with general psychiatric vulnerability or comorbidity (Fairburn et al., 1997). In contrast, published support for a relationship between AN and sexual abuse is weak, with positive findings only apparent in two meta-analyses, although results in Caslini et al. (2016) did not withstand adjustment for publication bias while Molendijk et al. (2017) reported significant findings only for the AN-BP but not AN-R sub type.
The potential role of physical abuse has been less commonly explored in relation to eating pathology. Across the three meta-analyses that examined this relationship (Caslini et al., 2016; Molendijk et al., 2017; Norman et al., 2012), the latter two found robust evidence for a relationship between physical abuse and overall EDs (not analysed in Caslini et al., 2016), while all studies found a strong relationship between physical abuse and BN. The relationship between physical abuse and AN was only reviewed by Molendijk et al. (2017) and Caslini et al. (2016), with significant findings in both studies. These somewhat preliminary findings therefore suggest a relationship between physical abuse and EDs, however more studies and continued meta-analyses are required to provide robust evidence (see Table 4 for summary).

Table 4

Summary of Findings of Six Identified Meta-Analyses Examining the Association Between Sexual and Physical Abuse and Overall EDs, AN, and BN.

<table>
<thead>
<tr>
<th>Meta-analysis</th>
<th>N</th>
<th>ED overall</th>
<th>AN</th>
<th>BN</th>
<th>ED overall</th>
<th>AN</th>
<th>BN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rind et al., 1998</td>
<td>10</td>
<td>Y</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Smolak &amp; Murnen, 2002</td>
<td>53</td>
<td>Y</td>
<td>-</td>
<td>Y</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chen et al., 2010</td>
<td>10</td>
<td>Y</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Norman et al., 2012</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Y</td>
<td>-</td>
<td>Y</td>
</tr>
<tr>
<td>Caslini et al., 2016</td>
<td>32</td>
<td>-</td>
<td>M</td>
<td>Y</td>
<td>-</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Molendijk et al., 2017</td>
<td>82</td>
<td>Y</td>
<td>M</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

Notes. N = Number of studies included in meta-analysis. Y = yes, evidence of an association. M = mixed evidence of association, see written text for further information. N = no evidence of association. - = Association not tested.
2.4.2.3. Sexual and physical abuse in community disordered eating samples

Evidence of a role for sexual and physical abuse has also been found in sub-clinical populations. To the author's knowledge, no systematic reviews or meta-analyses to date have examined the precise relationship between abuse and disordered eating, however a brief inspection of the literature suggests that sexual and physical abuse appear to be correlates of disordered eating pathology. For example, in a community sample of 9,943 adolescents, Neumark-Sztainer, Story, Hannan, Beuhring, and Resnick (2000) found that both physical abuse (boys, OR: 2.74, 95% CI: 1.82, 4.13; girls, OR: 2.27, 95% CI: 1.75, 2.96) and sexual abuse (boys, OR: 6.17, 95% CI: 3.80, 10.02; girls, OR: 2.29, 95% CI: 1.75, 3.00) were associated with disordered eating, even after adjusting for familial and psychological factors, demographics, and the experience of other forms of abuse. Similarly, the experience of sexual abuse or any abuse was associated with a greater level of binge eating behaviours in a population representative sample of $N = 2,960$ (Ericsson et al., 2012), while in another community sample of 477 women, where approximately half had experienced childhood sexual abuse, Romans, Gendall, Martin, and Mullen (2001) reported a relative risk [RR] for women with AN having reported sexual abuse of $RR = 5.00, 95\% CI: 1.44, 17.37$, and $RR = 2.55, 95\% CI: 1.05, 6.18$ for women with BN reporting sexual abuse. In regards to severity of abuse, Messman (2007) found that childhood sexual and physical abuse were related to increased fear of weight gain and bulimic behaviours, with greater severity of abuse associated with a greater level of disordered eating symptomatology. There is also some evidence for a link between childhood emotional
abuse and disordered eating, as reflected by greater scores on the EDI-2 scale (Kennedy, Ip, Samra, & Gorzalka, 2007).

However, not all findings are in concordance with these results. For example, a (non-systematic) review by Gardner (2011) found only mixed evidence for the role of sexual abuse in body size estimation, while results from Mazzeo and Espelage (2002) suggested that childhood physical and emotional abuse were only indirectly related to disordered eating, with some studies reporting no links at all between sexual abuse and disordered eating symptoms (Hodson, Newcomb, Locke, & Goodyear, 2006; Hund & Espelage, 2005).

Nonetheless, most of the aforementioned studies with larger sample sizes do tend to support an association between sexual and physical abuse and disordered eating attitudes and behaviours, reflecting the patterns of association present for clinical-level EDs (particularly, BN). However, at present evidence for both sexual and physical abuse in EDs and disordered eating can only be discussed in terms of ‘correlates’ rather than ‘risk factors’, given the lack of prospective studies in this area. Future studies planning to extend knowledge regarding the role of abuse in the development of EDs should adopt longitudinal designs.

2.4.3. Environmental correlates for eating pathology: Parenting behaviours

Research has also highlighted the important role of family in the development of eating pathology. Direct food-related family factors, such as parental ED status and dieting (Haynos et al., 2016; Machado et al., 2014), the child’s perception of parental concern about their body size (Agras, Bryson, Hammer, & Kraemer, 2007), parental encouragement of weight-control behaviours (Helfert & Warschburger, 2011; Rodgers & Chabrol, 2009),
and family comments or teasing about shape or weight (Machado et al., 2014; Rodgers, Paxton, & Chabrol, 2009; Wade et al., 2007) have all been related to disordered eating or an ED, although much of this research remains cross-sectional (Ventura & Birch, 2008) and is not unequivocally supported by longitudinal research (e.g. Loth et al., 2014). Family and parenting behaviours that are outwardly unrelated to food or body image have also been linked to eating pathology. For example, EDs and disordered eating symptoms have been related to exposure to high parental expectations and parental control (Wade et al., 2007), family dysfunction and negative caregiving qualities (Anastasiadou, Medina-Pradas, Sepulveda, & Treasure, 2014; Holtom-Viesel & Allan, 2014), poor parent-child communication (Haynos et al., 2016; Kichler & Crowther, 2008), and low parental care (Tetley, Moghaddam, Dawson, and Rennoldson, 2014).

Non-food related parenting behaviours, such as parental care, may be related to eating pathology via associations with factors such as increased positive adolescent self-esteem (Orth, 2017) or healthy eating attitudes (Swarr & Richards, 1996), with supportive and warm parenting likely to help children better cope with challenging life events or exposure to risks for eating pathology (McVey, Pepler, Davis, Flett, & Abdolell, 2002). Conversely, low care and greater parental restriction are inversely related to these protective factors (Zhu, Luo, Cai, Li, & Liu, 2014). A substantial body of research examining the role of parenting behaviours in EDs has examined parenting variables using the Parental Bonding Instrument (PBI; Parker, Tupling, & Brown, 1979). The PBI measures parenting behaviours according to two dimensions, care and control, and involves retrospective reporting by the ‘child’ regarding their experiences up to the age of 16 years. Each parent is classified either as ‘high’ or ‘low’ on care and overprotectiveness/control. The PBI has
been reported to have good reliability and validity (Parker et al., 1979; Xu, Morin, Marsh, Richards, & Jones, 2016) and findings of studies assessing the use of this tool in clinical and community samples will be discussed below. The following segments will also discuss research findings relating to parental warmth, and parental use of harsh punishment and hostility, three of the parenting behaviour variables under investigation in the present thesis.

2.4.3.1. Parental warmth and control in clinical eating disorder samples

Tetley et al. (2014) reported a systematic review of all studies investigating parenting behaviours via the PBI in a clinical ED sample. Inclusion criteria included use of the PBI in a clinical ED group with a non-clinical comparison group in studies published from 1999 until 2012. This time period was selected to follow on from an earlier review by Ward, Ramsay, and Treasure (2000), who reported preliminary findings that parents of AN and BN patients were lower on care than controls, but results regarding parental control were mixed. Inclusion criteria in Tetley et al. (2014) also allowed for studies using measurement tools that closely approximated the PBI and also measured parental care and overprotection. Twenty four studies were identified, all using a cross-sectional design. Tetley et al. (2014) reported that across studies reviewed, both maternal and paternal care tended to be lower in those with AN, BN, BED, and EDs overall, compared to non-clinical controls. Similar results were obtained for parental control, with women with AN, BN, and in a ‘general’ ED category reporting greater parental control compared to non-clinical controls. When analyses were restricted to only the studies with the best methodological practices, differences between the clinical and control groups on parental care and control were even more pronounced. When AN and BN patients were compared, no differences in
parental warmth or care were evident. Tetley et al. (2014) further compared ED patients to psychiatric controls, and also found largely no differences in levels of parental care and control. Results suggest that these parental behaviours may be related to both ED pathology and psychopathology more broadly, a conclusion that is supported by existing research (Yap & Jorm, 2015).

A systematic review by Caglar-Nazali et al. (2014) published in the same year also investigated findings from the PBI care (25 studies) and overprotection (26 studies) scales, but additionally included a meta-analysis of studies identified in the review. The authors reported that individuals with an ED experienced lower parental care ($d = .53, 95\% CI: .41, .65$) and greater parental over-protection ($d = .33, 95\% CI: .21, .45$) relative to healthy controls. Although findings were not reported separately for ED diagnoses and maternal and paternal behaviours, the meta-analysis supported the overall pattern of results reported in the systematic review by Tetley et al. (2014).

Since Tetley et al. (2014) and Caglar-Nazali et al. (2014), a number of additional studies investigating the PBI in clinical ED samples have been published, all using a cross-sectional design (e.g., Grenon et al., 2016; Halvorsen, Rø, & Heyerdahl, 2013; Horesh, Sommerfeld, Wolf, Zubery, & Zalsman, 2015). Horesh et al. (2015) found evidence of lower parental care and higher parental control/protection amongst those with an ED compared to healthy controls, but that paternal care and overprotection did not differ between ED patients and psychiatric controls, supporting findings from the systematic review by Tetley et al. (2014). Another study reported that body dissatisfaction amongst those with an ED was related to lower maternal care (Grenon et al., 2016), while Halvorsen et al. (2013) found that AN patients and their siblings did not differ in their retrospective
assessments of parental care/warmth and control, suggesting that although the PBI measures perceived parenting, it provides a measure of parental behaviours that is stable within-families with clinical ED patients.

Findings from the PBI are largely reflected in other research investigating a variety of family interaction styles (e.g., ED families are characterised by greater conflict and enmeshment, and lower cohesion, adaptability, and expressiveness; Cunha, Relvas, & Soares, 2009; Laghi et al., 2015; Laliberté, Boland, & Leichner, 1999; Vidović, Jureša, Begovac, Mahnik, & Tocilj, 2005), although these patterns of findings are not uncontested (e.g., Fisher & Bushlow, 2015; Holtom-Viesel & Allan, 2014). Nonetheless, the overall picture suggests that ED families tend to be characterised by greater parental control and restriction of the child coupled with reduced levels of positive family bonds.

2.4.3.2. Parental warmth and control in community disordered eating samples

The PBI has also been used to investigate disordered eating symptoms in community samples. As in studies of clinical ED populations, no prospective studies have investigated the role of parental care/warmth and control/overprotection in disordered eating. A number of cross-sectional studies have assessed the relationship between parental care, parental control, and disordered eating in large community samples. Lampis, Agus, and Cacciarru (2014) found that greater EDI-2 (Garner, 1991) Drive for Thinness, Body Dissatisfaction, and Bulimia were all related to parenting that was low on care and high on control. In partial support of this finding, Cella, Iannaccone, and Cotrufo (2014) found that Drive for Thinness was related to lower paternal care and higher maternal control, while Abebe, Lien,
Torgersen, and von Soest (2012) reported no differences in parental care or control between individuals reporting binge or purging behaviour and those who did not.

In a very large university sample \(N = 5,000\), Tseng, Gau, Tseng, Hwu, and Lee (2014) found lower parental care and greater parental overprotection amongst those with ED symptoms compared to controls. Amongst studies investigating medium-sized university-based samples, greater disordered eating was related to lower parental care and greater parental control (Hossein zad, Jabbarov, Mosazade, Azizi, & Mostafayev, 2013), while Patton, Beaujean, and Benedict (2014) found a relationship between maternal care and body dissatisfaction. Pace, Cacioppo, and Schimmenti (2012) found that binge eating symptoms were related to lower paternal care, but unrelated to paternal control. They did not assess maternal parenting factors. In a smaller community-based study, Young and Cooper (2013) corroborated these results by finding that binge eating symptoms were associated with lower parental care and high parental overprotection. Overall, clear links have been identified between reduced parental care, greater parental protection, and a range of disordered eating symptoms using the PBI. However, there is far less research examining other potentially important parenting behaviours in disordered eating samples, such as the mechanisms via which parents exert control (e.g., harsh punishment, parental hostility).

2.4.3.3. Parental hostility and use of harsh punishment in eating disorders

Parental hostility and use of harsh punishment may plausibly also play a role in adolescent EDs or disordered eating. Parental hostility, which includes behaviours such as parental verbal criticism and use of punitive punishment (e.g., threatening, yelling) to
control the child, is considered dimensionally opposite to parental warmth (Schaefer, 1959). Parental use of harsh punishment is a closely related construct that consists of parental practices that lie on a continuum, from hostility and harsh verbal punishment, to physical punishment and relatedly, physical abuse. The two parental behaviours, hostility and harsh punishment, are associated with a myriad of negative outcomes. Indeed, meta-analytic findings suggest that hostile parenting is related to outcomes such as increased child delinquency (Hoeve et al., 2009), depression (McLeod, Weisz, & Wood, 2007; Yap, Pilkington, Ryan, & Jorm, 2014), increased relational aggression (Kawabata, Alink, Tseng, Van Ijzendoorn, & Crick, 2011), and anxiety (Yap et al., 2014). Meanwhile, parental use of harsh punishment, including physical punishment, has been associated with depression (Wang & Kenny, 2014), conduct issues and other externalising behaviour (Bender et al., 2007; Hoskins, 2014), and personality disorders (Hallquist, Hipwell, & Stepp, 2015). Both parental hostility and use of harsh punishment are under investigation in Study 3 of the present thesis.

There are a number of theories suggesting why hostile parenting and parental use of harsh punishment may be associated with an increased risk for developing an ED. Firstly, this may function in a similar manner to the experience of traumatic or stressful life events. According to the Transactional Model of Coping and Stress (Folkman, 1984), avoidance is a common response to stress. Disordered eating behaviours, such as binge eating or restriction of caloric intake, may be conceptualised as avoidance strategies for dealing with stress associated with the home environment. They may also be aimed at increasing positive affect in response to stress, either through the direct psychological and physiological ‘reward’ of food intake, or through less direct routes, such as the anticipated
feeling of satisfaction following weight loss. According to another model, the Interpersonal Theory of Adolescent Development (Muuss, 1996; Sullivan, 1953), bulimic behaviours may arise as a consequence of negative-self-image and self-directed hostility that occurs in response to a dysfunctional early family environment (Friedman, Wilfley, Welch, & Kunce, 1997; Striegel-Moore, 1993). Following this theory, a hostile or conflict-filled upbringing may result in adolescents internalising this hostility, which manifests in binge or purging behaviours.

Empirically, a small number of studies have examined parental use of physical punishment and other punitive discipline techniques in EDs. Findings from these studies suggest that parents of those with BN have been found to use more physical punishment and punishment perceived as harsh compared to families of controls (Rorty, Yager, & Rossotto, 1995), and use more threats and coercion compared to families of healthy and psychiatric controls (Stuart et al., 1990). However, one systematic review of three studies (Czaja, Hartmann, Rief, & Hilbert, 2011; Decaluwé, Braet, Moens, & Van Vlierberghe, 2006; Hartmann, Czaja, Rief, & Hilbert, 2012) investigating the role of harsh punishment in binge eating reported inconclusive findings, suggesting further investigation is necessary (Saltzman, 2016). More extreme forms of parental punishment, which include physical abuse, have received somewhat more attention in the ED field and a more detailed discussion of these studies is featured earlier in the present chapter.

Meanwhile, investigation into the role of hostile parenting in the ED field is even more limited. While ‘adverse parenting’ overall has been related to ED aetiology (Fairburn & Harrison, 2003), the specific role of parental hostility in the ED field or in relation to disordered eating outcomes has only been touched upon in studies examining ‘expressed
emotion’ (Vaughn & Leff, 1976), and to an extent, parenting styles (Baumrind, 1967, 1971). Studies of expressed emotion measure five domains of parental behaviour (critical comments, hostility, emotional over-involvement, positive remarks, and warmth; Brown, Birley, & Wing, 1972), and have largely found that families of patients with an ED tend to have higher levels of expressed emotion than control families (Anastasiadou et al., 2014; Schmidt, Tetzlaff, & Hilbert, 2015; Zabala, Macdonald, & Treasure, 2009), with high expressed emotion in families associated with poorer treatment outcomes in ED patients (Hooley, 2007; Wearden, Tarrier, Barrowclough, Zastowny, & Rahill, 2000). No research relating to parental expressed emotion has been conducted with non-clinical disordered eating samples.

When inspecting the elements of expressed emotion individually, parental criticism has been associated with greater ED symptoms and treatment drop out (Eisler, Simic, Russell, & Dare, 2007; Rienecke, Accurso, Lock, & Le Grange, 2016; Schmidt et al., 2015), with a number of studies finding that maternal criticism and hostility were related to poorer treatment outcomes following therapies involving a prominent role for parents (e.g., Family Based Therapy; FBT), but not in treatments that primarily focus on the adolescent (Eisler et al., 2007; Rienecke et al., 2016). Given that FBT is a widely-used therapy with empirical support for treating EDs (Couturier, Kimber, & Szatmari, 2013; Lock et al., 2010), family hostility appears to play an important role in ED maintenance and recovery. However, little is known regarding the role of parental hostility in ED aetiology or the development of disordered eating symptomatology.

The role of parental hostility in EDs and disordered eating is also partly reflected by research investigating parenting styles (Baumrind, 1967, 1971). This theory categories
parents into one of four behaviour groups that reflect the means by which parents address their child’s need for both nurturance and limit setting. Authoritative parenting is considered to be high on both care and monitoring. Meanwhile, authoritarian parenting is conceptualised as low on warmth and care but high on monitoring, control, and hostile behaviour. Permissive parenting tends to be high on care and warmth but low on monitoring, while the neglecting style encompasses parenting that is both low on care and monitoring.

Studies have largely found that authoritarian parenting has been associated with greater eating pathology (Jáuregui Lobera, Bolaños Ríos, & Garrido Casals, 2011; Zubatsky, Berge, & Neumark-Sztainer, 2015). Meanwhile, authoritative parenting, which while being high on control and demands is also high on warmth, has not shown such an association with EDs or disordered eating (Enten & Golan, 2008, 2009; Haycraft & Blissett, 2010). These findings suggest that it is reasonable to expect that warmth acts as a potential protective factor (although further prospective studies are required to confirm risk factor status). Conversely, as authoritarian parenting often involves punitive punishments and hostile behaviour (Buri, 1991; Moilanen, Rasmussen, & Padilla-Walker, 2015), it may be that along with increased control, the hostile element of this parenting style contributes to the relationship between eating pathology and authoritarian parenting. Indeed, the relationship between hostile parenting and disordered eating in one study was shown to be greater than the association between adolescent autonomy and disordered eating (Pruitt, 2015), suggesting that the effects of parental hostility may be similar, if not greater, than that of parental control. While this result awaits replication, it provides early suggestion that the effects of parental hostility may play a notable role in adolescent disordered eating.
2.4.3.4. Limitations to self-report questionnaires measuring parent behaviours

One caveat of research investigating parental warmth and hostility is that it has largely utilised questionnaire-based measurement of parenting behaviour or family interactions. In addition, questionnaires have generally been completed retrospectively (e.g., by university students or clinical ED patients recalling their childhood family environment). For example, all studies in the systematic review of papers investigating parenting via the PBI by Tetley et al. (2014) used retrospective reporting of parenting behaviours following ED diagnosis. Such research is limited in its ability to provide an accurate picture of family interactions before or during the period in which adolescents are most prone to developing eating pathology. This is because retrospective responses to questionnaires may be influenced by factors such as general forgetting or biased recall following ED diagnosis (e.g., reading significance into certain aspects ‘after the event’, or current relationship with parents influencing perception of past relationship; Gardner, 2000). Responses to questionnaires are also inherently subjective, as they are reported in an idiosyncratic manner (e.g., participants may have different perceptions of ‘high’ warmth or hostile behaviour). Patient-report parenting questionnaires therefore measure perception of parenting behaviours. Conversely, parent-reported parenting behaviours may be influenced by factors such as limits in parental self-awareness and socially-desirable responding. For example, in non-ED literature, Browne (2000) measured parenting behaviour using both self-report questionnaires and observational methodology. They found limited correspondence between parent responses on the questionnaire and their actual observed behaviours. Comparable results were reported by Kallstrom-Fuqua (2004), who found
substantial differences between mothers’ self-reported parenting attitudes and their parenting behaviours as observed in a parent-child interaction task (e.g., mothers exhibited significantly poorer limit-setting skills than they self-reported). There are no studies in the ED literature that have quantified differences in self-reported and observationally measured family interaction styles, however it is reasonable to assume that a similar pattern of results would exist.

2.4.3.5. Observational measurement of parenting behaviours

A different method of measuring parenting behaviours includes using observational techniques. This form of measurement typically involves measuring observable parent behaviours during child-parent interaction tasks and coding these behaviours according to systematic, well-established scoring systems. Observation of parents and adolescents is typically made during a problem-solving task, or a task designed to elicit some level of conflict. Observation can be recorded on a number of levels, such as recording content and themes of verbalisations, frequency of events, or qualities of the interaction (e.g. warmth, hostility, reciprocation; Aspland & Gardner, 2003). Types of measurement are typically classified as either macro-level analysis, which includes focus on frequency, intensity, and quality of parent behaviours overall (e.g., warmth, intrusiveness), or micro-level analysis, which focusses on the content of specific utterances (Murray et al., 2015). Most studies have used macro-analytic techniques, which are simpler and considered to have greater clinical utility (Mash & Foster, 2001).

Observational coding of parent and child behaviours has many advantages over questionnaire-based research. Firstly, as behaviours are coded according to established
guidelines by a trained observer, they are defined in a consistent and reliable manner (Aspland & Gardner, 2003). Through this process, all parental behaviour is measured in the same unit, as opposed to self or child-report measurements, which can vary substantially between individuals depending on their individual perception of the given behaviours (Prescott et al., 2000). Furthermore, they overcome issues related to limited self-awareness, selective responding, or other individual-specific biases, particularly when measurement occurs at a time when the family is experiencing discord. Finally, it has been suggested that observed behaviours are stronger predictors of future outcomes compared to self or parent-reported behaviours (Patterson & Forgatch, 1995).

Observational assessment has faced some criticism relating to ecological validity (Bronfenbrenner, 1977), as parent-child interaction is observed in a ‘laboratory setting’, away from the usual context of the family’s interaction. Furthermore, adults and children are conscious that they are under observation and this knowledge may prompt them to alter their behaviours (‘observer reactivity’). However, despite this, the behaviours of interest are often displayed even in these ‘contrived’ laboratory settings (Shriver, Frerichs, Williams, & Lancaster, 2013), and under many conditions parents are limited in their ability to ‘control’ the behaviour of their child or modify their own behaviours (Aspland & Gardner, 2003; Gardner, 2000).

In the ED field, a very limited number of studies have used observational techniques to study (non-mealtime) parenting behaviours. All studies to the author’s knowledge that have used this design thus far in the ED field are featured in Table 5. Table 5 excludes studies of expressed emotion, with findings of studies examining expressed emotion discussed above. Studies of expressed emotion tend to assess this constructs either via questionnaires, or the
Camberwell Family Interview (CFI; Vaughn & Leff, 1976), with coding based on content of the interview and tone of voice, generally using an audio-tape (e.g., Dare, Grange, Eisler, & Rutherford, 1994). Rather, present discussion centres on studies that have used family-interaction tasks (e.g. family problem solving) to elicit examples of parental behaviour and other family interaction variables.

Observational studies of family interaction and parenting behaviour in the ED field thus far have tended to use tasks designed to provoke some level of family conflict or disharmony, allowing for analysis of how families interact in emotionally or interpersonally challenging situations (e.g., conflict resolution tasks or family role-play of a contentious issue; Gardner, 2000). However, there are a multitude of limitations to the existing studies. Of the seven studies outlined in Table 5, Thomas, Hoste, and Le Grange (2012) did not use a conflict task, but rather conducted a structured interview and only coded family members’ observed levels of individuation and connection, while Stasch and Reich (2000) did not administer an experimental task per se, but instead observed a series of family therapy sessions and measured the conflict inherent in this setting. Therefore, only five (Blair, Freeman, & Cull, 1995; Humphrey, 1989; Kog & Vandereycken, 1989; Lattimore, Wagner, & Gowers, 2000; Ratti, Humphrey, & Lyons, 1996) of the seven studies utilised a purpose-specific interaction task from which to derive their observational data.
Table 5

*Studies Using Observational Methods to Investigate Family Interaction and Parenting Behaviours in EDs or Disordered Eating Samples.*

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample N, diagnosis, age in years (SD)</th>
<th>Task</th>
<th>Coding/Variables</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humphrey (1989)</td>
<td>$N = 74$ (50 AN/BN, 24 controls) $M = 18.0$ years (2.2)</td>
<td>Problem solving role play*</td>
<td>SASB observational system</td>
<td>AN more restrictive, BN more hostility and enmeshment</td>
</tr>
<tr>
<td>Blair et al. (1995)</td>
<td>$N = 87$ (27 AN, 20 CF, 31 controls). $M = 18.7$ years (2.8)</td>
<td>Problem solving task*</td>
<td>Enmeshment* /overprotection* &amp; weak boundaries*</td>
<td>AN families more enmeshed, overprotective, poor problem solving</td>
</tr>
<tr>
<td>Ratti et al. (1996)</td>
<td>$N = 44$ (13 substance use, 14 BN, 17 controls) $M = 17.3$ years (1.9)</td>
<td>10-minute role play*</td>
<td>SASB observational system</td>
<td>BN patients less attached and autonomous than controls</td>
</tr>
<tr>
<td>Lattimore, Wagner, &amp; Gowers (2000)</td>
<td>$N = 34$ (20 AN, 14 psychiatric controls)</td>
<td>Family discussion task*</td>
<td>PACES* (conflict management)</td>
<td>More ‘destructive’ communication in AN dyads</td>
</tr>
<tr>
<td>Study</td>
<td>Sample Size</td>
<td>Research Design</td>
<td>Measurement Tools</td>
<td>Findings</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
<td>----------------</td>
<td>-------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Stasch &amp; Reich (2000)</td>
<td>N = 20 (BN, with both parents, and one BN sibling)</td>
<td>Goettinger Familieninteraktion task</td>
<td>Conflict, Cohesion, Expression, Interpersonal boundaries</td>
<td>Greater conflict amongst parents and mother-BN daughter dyads</td>
</tr>
<tr>
<td>Thomas, Rienecke Hoste, &amp; Le Grange (2012)</td>
<td>N = 54 (1 male)</td>
<td>Semi-structured interview (SCFI)</td>
<td>Individuation scale of the SIRQ, and measure of Connection*</td>
<td>Several sig. negative correlations between BN symptoms and indviduation and connection</td>
</tr>
</tbody>
</table>

*Indicates scale devised by authors. PACES = Parent Adolescent Conflict Evaluation Scheme (devised by authors); SASB = Structural Analysis of Social Behaviour (Benjamin, Foster, Roberto, & Estroff, 1986); SCFI = Standardised Clinical Family Interview (devised by authors); SIRQ = Scale of Intergenerational Relationship Quality (L. Wakschlag, Chase-Lansdale, & Brooks-Gunn, 1991). Participants were all female unless indicated otherwise. Table excludes Humphrey, Apple, and Kirschenbaum (1986), whose primary focus was comparing the psychometric properties of two scales, and full text was unavailable for Humphrey (1987), however methodology closely reflected Humphrey (1989).

There was also a high level of variation in the measurement tools used to code the interactions and derive the variables of interest. The majority of the tools used had poor or untested psychometric properties. For example, Kog and Vandereycken (1989) used an ad-hoc measure, which although designed for the purpose of their study and tested in a pilot study (Kog, Vandereycken, & Vertommen, 1985), was self-described as only ‘acceptable’ and had no established psychometric properties. Furthermore, their outcome measure was based on written material produced during the observation, as opposed to parental behaviour during the interaction. Blair et al. (1995) also utilised a self-devised measure to examine behaviour during the family interaction tasks, which again had sub-optimal inter-
rater reliability. Lattimore et al. (2000) coded interactions using the Parent-Adolescent Conflict Evaluation Scheme (PACES), which only had ‘fair’ inter-rater reliability and was developed for the purposes of the study, but has not since been used. Thomas et al. (2012) used two author-created measures of connectivity and the Scale of Intergenerational Relationship Quality to assess interaction data, however the latter was primarily developed for use with mothers and grandmothers, and normed in an African American minority population (Wakschlag, Chase-Lansdale, & Brooks-Gunn, 1996), which was not the population used in their study. Stasch and Reich (2000) microanalysed therapy sessions using the Gottinger Familieninteraktions-Skalen, for which there is little available information regarding reliability and validity. Only Humphrey (1989) and Ratti et al. (1996) used a well-established measurement technique, the Structural Analysis of Social Behaviour (SABS) model, which has relatively good psychometric properties (Benjamin, 1996). To the writer’s knowledge, these are the only observational studies conducted to date that attempt to assess family interactions in eating pathology.

As a consequence of the inconsistent method of analysing the observational data, there has only been moderate agreement regarding which interaction variables are relevant, and in all cases these variables (e.g., warmth, connection, conflict avoidance) have been constructed in a different manner. There was some consistency in results, for example, Humphrey (1989) found that fathers in ED families were more watching and managing, and Thomas et al. (2012) reported less respect of interpersonal boundaries between ED adolescents and their parents, both which correspond to similar findings from questionnaire studies (e.g., Calam, Waller, Slade, & Newton, 1990; Gutzwiller-Jurman, 1999). However, overall findings from these studies were largely inconsistent or conflicting. For example,
Kog and Vandereycken (1989) found that ED families displayed less conflict and greater cohesion than controls, while Lattimore et al. (2000) reported that ED families displayed more conflict than controls. The studies also reported conflicting findings regarding differences in interpersonal boundaries between AN and BN families.

Further limitations of these previous observational studies include their use of small samples sizes, with the largest study featuring $N = 87$ (Blair et al., 1995). Regarding gender differences, the majority of the five studies examined female participants only (excluding Kog and Vandereycken, 1989, Blair et al., 1995, and Thomas et al., 2012, although the latter two studies included only one and two male participants, respectively), and those who did include males in their sample did not compare gender differences in the relationship between family interactions and eating pathology. Given the existing aetiological differences of eating pathology between males and females (e.g., males are more likely to develop EDs earlier than females, who show a substantial increase in ED symptoms at puberty; Cotrufo, Cella, Cremato, & Labella, 2007), it is also likely that the relationship between family interaction patterns and disordered eating will vary between males and females. Finally, none of the studies investigated disordered eating in a community sample. Studies of parenting behaviours in clinical samples are limited by the fact that it is not possible to conclude whether certain parental behaviours preceded, and were risk factors for the ED diagnosis, or whether they arose in response to child ED pathology. In contrast, examining parenting interactions in a community sample consisting of individuals with a range of disordered eating symptoms can provide a more nuanced understanding of the relationship between the two constructs, with findings potentially relevant for prevention and early intervention of clinical-level EDs.
In sum, previous observational studies of family interactions in eating pathology populations are limited. Only three studies (Blair et al., 1995; Humphrey, 1989; Kog & Vandereycken, 1989) administered a problem-solving family interaction task. All but two studies (Humphrey, 1989; Ratti et al., 1996) used poor, unestablished, or inappropriate instruments to code the observational data. There was no agreement on relevant variables or how these variables were to be constructed, gender differences were not examined, and results from the studies were largely conflicting and do not shed light on families with sub-threshold eating pathology. There is therefore a need for a large-scale, community-based observational study of both female and male participants to answer outstanding questions regarding the role of parental behaviour in disordered eating.

2.5. Chapter summary

This chapter introduced risk factor theory and presented evidence regarding the current risk factors and correlates identified for EDs and disordered eating pathology. In particular, focus was on traumatic life events, sexual and physical abuse, and parental behaviours, with cross-sectional research largely supporting the role of these variables as correlates for eating pathology. However, several gaps in the literature were identified, particular regarding the role of parental hostility and use of harsh punishment.

While consideration of environmental and psychological correlates of EDs is an important step, it does not offer a complete picture in ED aetiology. Namely, it remains unclear why certain factors may pose a risk for particular individuals but not for others. These differences are likely due to a range of individual factors that may increase or decrease susceptibility to environmental or psychological risks (or correlates) for EDs.
Investigation of biological risk factors, in particular, the role genetics, may offer insight into some of the current gaps in our understanding of why, amongst individuals exposed to the aforementioned risk factors, only a select few proceed to develop pathological eating behaviours. Evidence regarding the genetic underpinnings of eating pathology, and how genes many contribute to the overall picture of ED risk, will be considered in the subsequent chapter.
3. CHAPTER 3

The Role of Genes in Eating Pathology

Amongst the numerous risk factors and correlates identified for EDs, the influence of genetic factors has garnered a large amount of empirical support. Numerous twin studies have revealed a sizable genetic contribution to EDs, with heritability estimates around 40 – 60% (Yilmaz et al., 2015), highlighting the important role of genes in this particular type of pathology. However, little is known regarding which genetic factors are of most relevance to ED development. The following chapter will focus on exploring current findings relating to heritability in the ED field. It will begin by exploring the results of twin studies that provide broad heritability estimates. It will then focus on exploring the evidence thus far accumulated regarding the specific genetic mechanisms proposed to be involved in EDs. This will include a brief discussion of the biology of EDs, following by exploration of research that has assessed both the role of direct genetic association and GxE interactions in EDs, with a focus on the serotonin transporter 5-HTTLPR polymorphism. The chapter will conclude by introducing the first study of the present thesis, a systematic review and secondary data meta-analysis of studies investigating GxE interactions in the ED field.

3.1. Evidence for the heritability of eating disorders

Strong evidence for the heritability of EDs has been accumulated thus far via family and twin studies. In family studies, heritability estimates are typically calculated by comparing the risk of a relative of someone with an ED developing that particular ED compared to an individual in the general population (Thornton, Mazzeo, & Bulik, 2011). If
a relative displays elevated risk, this is taken to indicate a level of heritability present in the disorder aetiology, although does not preclude a role for shared family environments. In twin studies, risk of an ED amongst monozygotic twins is compared to risk amongst dizygotic twins, under the assumption that both twin pairs experience roughly the same level of shared environmental exposure, but monozygotic twins theoretically have (almost) identical DNA while dizygotic twins share, on average, only 50% of their genes. Estimates based on twin methodology are divided into three components: risk due to additive genetic effects, shared environmental effects, and unique environmental effects (Thornton et al., 2011). Using these approaches, studies thus far estimate a heritable component of roughly 28 – 74% for AN (Dellava, Thornton, Lichtenstein, Pedersen, & Bulik, 2011; Klump, Miller, Keel, McGue, & Iacono, 2001; Kortegaard, Hoerder, Joergensen, Gillberg, & Kyvik, 2001; Wade, Bulik, Neale, & Kendler, 2000), around 60% for BN (Bulik et al., 2010; Kortegaard et al., 2001), and 39 - 45% for BED (Javaras et al., 2008; Mitchell et al., 2010). Meanwhile, heritability estimates for specific disordered eating behaviours have been estimated only by a small number of studies. Mixed results have been found for restrained eating (Trace et al., 2013), and estimates for binge eating and vomiting (17% - 70% and 8% - 72%, respectively; Root et al., 2010; Sullivan, Bulik, & Kendler, 1998; Wade, Treloar, & Martin, 2008), with more consistency in investigations of drive for thinness in females (44% - 50%; Keski-Rahkonen et al., 2005; Rutherford, McGuffin, Katz, & Murray, 1993). Self-induced vomiting was also revealed to have a large heritable component in a recent study by Peterson et al. (2016). Overall, twin studies indicate a substantive genetic component to a range of ED symptoms and diagnoses.
While the results of these twin studies suggest a notable heritable component to EDs, they also contain wide variations in their estimations of the precise genetic contribution. This identified between-study variation in estimates of the same phenotype may be due to a number of factors, including variations in measurement error or differences in assessment of EDs/precise disordered eating behaviours and environmental factors, which together increase heterogeneity amongst the studies. Other issues that twin studies may encounter include the fact that measurement error can often inflate the estimation for non-shared environmental factors, while estimations of heritability may inadvertently contain variance due to the effects of gene-environment interactions (Capusan et al., 2017). Furthermore, such studies depend on the assumption of random mating, which has not always proven to be the case in some psychiatric conditions (e.g., Nordsletten et al., 2016). Lastly, the aforementioned studies in the ED field have almost exclusively assessed individuals of European ancestry and thus may not be generalizable to all populations. Nonetheless, despite the issues faced and variability of estimates between studies, current results provide strong evidence for at least a moderate role of genes in EDs.

While the estimates provided by twin studies indicate a moderate to high level of risk due to additive genetic effects, they have also consistently highlighted the important role of non-shared environments in ED risk. For example, non-shared environmental factors accounted for 44% (AN; Dellava et al., 2011), 61% (BED; Javaras et al., 2008), and 56% (purging disorder; Munn-Chernoff et al., 2015) in studies investigating heritability across a range of EDs. These findings suggest, therefore, that genetic factors do not present a full picture of ED aetiology and that investigation into individual environmental factors is essential in order to fully understand ED onset. Indeed, results from some twin studies
suggest that the effects of certain environmental stressors may strengthen estimates of additive genetic effects in eating pathology (e.g., weight-related peer teasing; Fairweather-Schmidt & Wade, 2017). However, classical twin studies tend to focus on comparing ‘shared’ and ‘non-shared’ environmental factors rather than investigating which particular environmental factors appear to be involved in EDs (Røysamb & Tambs, 2016).

Furthermore, twin studies do not offer insight into which specific genetic factors may underlie these heritability estimates, and thus, despite growing evidence supporting the role of genetics in EDs, the mechanisms involved in ED heritability remain unknown. Investigation of molecular genetics can provide a further avenue from which to uncover the precise genetic factors that play a role in EDs, and present an opportunity to investigate the role of interactions between specific genetic and environmental factors in ED risk.

3.2. Biological and molecular basis of eating disorders and disordered eating

Developing an understanding of EDs at a molecular level can provide guidance regarding which genes may be most heavily implicated in eating pathology (Scherag, Hebebrand, & Hinney, 2010). Investigation of specific genetic factors should be guided by a strong theoretical basis and existing research findings, in order to best identify appropriate candidate genes and minimize chances of spurious findings (Duncan & Keller, 2011). As such, it is important to consider the biological basis of eating behaviours and other psychological factors believed to be involved in eating pathology.

Although primarily viewed as a psychological disorder, EDs have a substantial biological component. At a basic level, biological processes in the brain are involved in the desire to eat and in the perception of food as a pleasurable activity. For example, exposure
to pleasant food activates regions linked with emotional processing, such as the orbitofrontal cortex, striatum, and amygdala (Berridge, 2009). Moreover, activation of these centres is directly associated with the caloric density of food, highlighting the naturally biologically rewarding nature of eating (Val-Lailet et al., 2015). In contrast, aversion to or fear of food, as is seen in AN, has been related to hyper-arousal of emotional and fear systems in response to food-related stimuli (Friederich, Wu, Simon, & Herzog, 2013). There are also individual biological differences in the manner in which individuals respond to food. For example, differences have been identified in the level of activation of reward centres in response to external food stimulants, such as palatable food commercials or food smells, and these individual differences are predictive of subsequent weight gain (Sun, Veldhuizen, Wray, De Araujo, & Small, 2013; Yokum, Gearhardt, Harris, Brownell, & Stice, 2014). There is also some suggestion that this relationship is genetically moderated (Stice, Yokum, Bohon, Marti, & Smolen, 2010). More recently, there are emerging findings that disorders such as AN may have a metabolic basis (Duncan et al., 2017) or be related to individual differences in gut microbes (Glenny, Bulik-Sullivan, Tang, Bulik, & Carroll, 2017). These various biological processes suggested to play a role in eating pathology likely implicate individual differences at the genetic level, with growing evidence that specific brain neurotransmitters may be involved.

Indeed, the strongest evidence for specific biological factors involved in eating and ED pathology is represented by studies examining neurotransmitters. Differences in the functioning of brain neurotransmitters are evident in those with an ED compared to unaffected controls (Wang et al., 2011). One major neurotransmitter that exemplifies this is dopamine, which is involved in feeding and reward-sensitivity. For example, in one study
by Wang et al. (2011), following administration of a mild stimulant, individuals with BED showed greater dopamine release in the striatum compared to healthy controls. This effect was independent of participant BMI and suggests a specific role for dopamine in the compulsive behaviour of binge eating. Studies of dopamine levels in individuals with AN report greater dopamine release following restrictive eating, which may act as a reinforcer for dieting and facilitate this behaviour to become habitual (Södersten, Bergh, Leon, & Zandian, 2016). However, it is also hypothesised that brain-structure abnormalities relating to dopamine regulation may be present in individuals prior to AN onset and thus constitute a risk factor (O’Hara, Campbell, & Schmidt, 2015). In animal models of EDs, food restriction has been associated with greater release of dopamine upon eventual food consumption, while purging behaviours have been related to reduced release of a neurotransmitter (acetylcholine) associated with satiety (Avena & Bocarsly, 2012). Meanwhile, in experimental research, ingestion of dopamine-releasing substances (e.g., some medications) has been associated with compulsive eating (Nirenberg & Waters, 2006).

Serotonin is another brain neurotransmitter that has been strongly implicated in EDs and disordered eating behaviours. Serotonin plays a role in mood and regulating energy balance, with increases in serotonin levels in the brain associated with reduced food intake (Leibowitz & Alexander, 1998; Spiegelman & Flier, 2001). Serotonin activity is altered in patients with AN and BN compared to healthy controls, and remains so following recovery (Trace et al., 2013). For example, individuals with AN have been found to have reduced levels of a precursor molecule for serotonin in their cerebrospinal fluid, while increased levels are found in individuals who have recovered from AN (Kaye, 2008; Kaye,
Gwirtsman, George, & Ebert, 1991). These serotonergic differences are hypothesised to be related to differences in emotional regulation, impulse control, anxiety, and body image distortions amongst individuals with AN and BN (Bailer et al., 2007; Kaye et al., 2005). They have also been argued to reflect hereditary effects or the influences of developmental stressors (e.g., childhood abuse; Steiger, 2004). Finally, the key role of serotonin in EDs is reflected in the fact that selective serotonin reuptake inhibitors (SSRI) have support in treatment of BN (Tortorella, Fabrazzo, Monteleone, Steardo, & Monteleone, 2014).

Given the strong theoretical and empirical findings supporting the role of the dopamine and serotonin neurotransmitters in ED behaviours, genes related to the functioning of these neurotransmitters and related systems have been most commonly assessed in investigations of specific candidate genes in the ED field. Research examining candidate genes in EDs is introduced below.

3.3. Candidate genes in eating disorders and disordered eating

Investigations into the relationship between candidate genes and EDs have aimed to narrow down the precise genetic mechanisms involved in eating pathology. Unlike simple traits, inheritance of complex psychiatric disorders is believed to be polygenic, that is, expressed via a combined expression of a large number of genes (Civelek & Lusis, 2014). The genes involved are also likely to be expressed via interaction with other genes (GxG interactions) or with environmental factors (GxE interactions). As such, although there is not believed to be a causal ‘ED gene’, some genetic polymorphisms (i.e. variants of a gene) may be associated with a somewhat higher risk of developing an ED (Hinney et al., 2017),
and identifying these variants may provide greater insight into the genetic and biological basis of EDs.

Early candidate gene studies focussed on uncovering direct genetic association, by comparing the frequency of particular candidate genes in affected versus non-affected samples (Hinney, Remschmidt, & Hebebrand, 2000). Under this approach, between-group differences in allele frequency suggest that the candidate gene in question may be related to ED risk. Candidate genes investigated using this methodology generally have been selected due to accumulating evidence regarding their role in general psychopathology, or due to their role in broader eating behaviours, weight regulation, mood, or stress reactions (Rask-Andersen, Olszewski, Levine, & Schiöth, 2010). A large number of different polymorphisms have been investigated so far as potential risk factors for EDs (Scherag et al., 2010). In a review by Trace et al. (2013), authors reported mixed evidence for a number of genes in the serotonergic (e.g., 5-HTTLPR, 5-HT2A), dopaminergic (DRD2, DRD4, catechol-O-methyltransferase gene [COMT]) and opioidergic (OPRD1) systems, as well as genes involved in appetite regulation, weight regulation, and food intake. For example, while early studies found an association between COMT and AN (Frisch et al., 2001; Mikołajczyk, Śmiarowska, Grzywacz, & Samochowiec, 2006), these results did not withhold subsequent meta-analytic examination (Brandys et al., 2012). However, most polymorphisms have so far only been investigated in a handful of studies, with most investigations limited by small sample sizes and failures to replicate (Trace et al., 2013). These studies require consistent and independent replication before any specific polymorphisms can be considered an established risk factor for EDs.
3.3.1. Introduction to 5-HTTLPR in eating disorders and disordered eating

The polymorphism that has received the most attention to date in candidate gene studies is 5-HTTLPR. This is a degenerate repeat polymorphism in the promoter region of the 5-HTT gene, which is responsible for transporting serotonin from synaptic spaces into presynaptic neurons, in the serotonergic neurons of the central nervous system. The actions of 5-HTTLPR facilitate neuronal communication. The promoter region initiates the transcription (activity) of the gene, and is most commonly conceptualised as comprising two alleles, one short (s), with fourteen repeated elements, and the other long (l), with sixteen repeated elements. Together they give rise to three genotypes, s/s, s/l, and l/l. Presence of one or two copies of the s-allele has been associated with reduced serotonin transcription (Lesch et al., 1996). While some studies have also argued that the l-allele can be further divided into a high function and low function variant, results investigating 5-HTTLPR using a triallelic definition have largely been unconvincing (e.g., Clarke, Flint, Attwood, & Munafo, 2010; Parsey et al., 2006; Solmi et al., 2016) and thus this approach will not be a focus of the present thesis.

Investigation of 5-HTTLPR in candidate gene research is a logical first point of focus in the ED field for numerous reasons. Firstly, it is theoretically linked to EDs at a biological level and has some early (albeit mixed) empirical evidence for a role in eating pathology. Selecting candidate genes on the basis of theoretical and past empirical evidence is important to minimize the risk of false positives that commonly plague studies of genetic association (Duncan & Keller, 2011).
As a genetic factor that influences serotonin reuptake efficiency, the 5-HTTLPR s-allele has been associated with numerous eating-related outcomes, including mood regulation, appetite, and weight regulation (Culbert et al., 2015). Previous studies have identified relationships between 5-HTTLPR and greater BMI (Sookoian, Gianotti, Gemma, Burgueño, & Pirola, 2008) and restrained eating (Sanhueza, Herrera, Salazar, & Silva, 2011) in community samples, and with higher EDI-2 Drive for Thinness scores in both clinical (Frieling et al., 2006) and community (Akkermann, Paaver, Nordquist, Oreland, & Harro, 2008) samples. One limitation to two of these studies is that they were assessed in very small samples (N = 132 in Sanhueza et al., 2011; N = 45 in Frieling et al., 2006). 5-HTTLPR has also been related to ED comorbidity and other individual differences amongst those with an ED. For example, 5-HTTLPR has been associated with increased risk of diagnostic crossover amongst patients with EDs (Castellini et al., 2012) and with increased affective instability amongst individuals with BN (Steiger et al., 2005). Amongst those with binge-eating tendencies in a community sample, 5-HTTLPR has also been associated greater impulsivity, anxiety, and higher levels of binge eating (Akkermann, Nordquist, Oreland, & Harro, 2010). These findings exemplify the potential links between 5-HTTLPR and EDs, and suggest that 5-HTTLPR may be an important aetiological factor for eating pathology.

### 3.3.2. Meta-analyses of the direct association between 5-HTTLPR and eating disorders in clinical samples

So far, five meta-analyses have investigated the relationship between 5-HTTLPR and EDs in clinical populations (Calati, De Ronchi, Bellini, & Serretti, 2011; Chen, Qian, Pu,
Ge, & Wu, 2015; Lee & Lin, 2010; Polsinelli, Levitan, & De Luca, 2012; Solmi et al., 2016). The first meta-analytic review (including 8 studies) was conducted by Lee and Lin (2010), who found a significant association between AN and 5-HTTLPR (OR: 1.41, 95% CI: 1.20, 1.66), but not between BN and 5-HTTLPR. Two subsequent meta-analyses (including an additional 6 and 9 studies; see Table 6) found similar results (Calati et al., 2011; Chen et al., 2015). Calati et al. (2011) reported a significant interaction between 5-HTTLPR and EDs considered overall (OR: 1.35, 95% CI: 1.00, 1.81; although this was no longer significant when studies deemed to introduce bias were removed). They also reported a significant interaction between 5-HTTLPR and AN (OR: 1.35, 95% CI: 1.07, 1.71), but not BN. However, a few notes of caution must be observed for this meta-analysis. Firstly, authors included a number of studies that tested clinical samples without a comparison group, and used ‘virtual’ control groups when including these studies in their meta-analysis. Secondly, throughout the results section authors describe comparing ‘genotypes’ (i.e. s-allele versus l/l genotype) rather than comparing genotypes across clinical ED and control groups. It is unclear whether results were indeed analysed in this manner only, or whether this is a consequence of unclear written communication. Findings in Chen et al. (2015) also indicated an association between 5-HTTLPR and AN (OR: 1.35, 95% CI: 1.09, 1.67) but not BN, which is unsurprising given this meta-analysis consisted of almost entirely the same group of studies as Calati et al. (2011; see Table 6). The only additions were two studies, one which was an un-published doctoral dissertation by the first author, and the second study published in Mandarin with no full text available in English, hence unable to be assessed for the present purposes. Overall, it is unclear what the review by Chen et al. (2015) adds to the literature. The fourth meta-analysis, by Polsinelli et al.
(2012), focussed specifically on the relationship between 5-HTTLPR and BN. Across the five studies identified by the review (see Table 6), a meta-analysis revealed no significant associations, both when testing the dominant model (s/s & s/l versus l/l genotypes) or when investigating an additive model (comparing s/s to s/l to l/l genotypes).

The largest and most recent meta-analysis on this topic is by Solmi et al. (2016; 20 studies). Authors tested both biallelic and triallelic models of 5-HTTLPR, both by comparing allele frequencies between clinical ED and control groups (when possible), and by examining s-allele versus l-allele frequency amongst clinical participants. They reported no association between 5-HTTLPR and ED overall, BN, or AN, contradicting three of the past meta-analyses that reported associations between 5-HTTLPR and AN (Calati et al., 2011; Chen et al., 2015; Lee & Lin, 2010). Given that this meta-analysis included the largest number of studies, and is the most up-to-date investigation published, these results cast serious doubt over the significant associations between 5-HTTLPR and AN reported in previous meta-analyses.

Table 6

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hinney et al., 1997</td>
<td>208</td>
<td>*</td>
<td>*</td>
<td></td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Di Bella et al., 2000</td>
<td>226</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Sundaramurthy et al., 2000</td>
<td>228</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>AN</td>
<td>BN</td>
<td>X</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>Fumeron et al., 2001</td>
<td>215</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>De Luca et al., 2003#</td>
<td>302</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lauzurica et al., 2003</td>
<td>245</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matsushita et al., 2004</td>
<td>195</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urwin &amp; Nunn, 2005^</td>
<td>106</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wonderlich et al., 2005 ^</td>
<td>178</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chen, 2006</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frieling et al., 2006^</td>
<td>45</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monteleone et al., 2006a</td>
<td>219</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monteleone et al., 2006b</td>
<td>138</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rybakowski et al., 2006</td>
<td>225</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribasés et al., 2008^</td>
<td>82</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steiger et al., 2008a^</td>
<td>111</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steiger et al., 2008b</td>
<td>98</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steiger et al., 2009</td>
<td>278</td>
<td>*</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Martásková et al., 2009</td>
<td>137</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ehrlich et al., 2010</td>
<td>152</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karwautz et al., 2011</td>
<td>256</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Castellini et al., 2012</td>
<td>351</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yue et al. 2012</td>
<td>?</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Original data, Solmi et al. (2016)</td>
<td>976</td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Total N studies**
|       | 8   | 15  | 5   | 17  | 20  |

**Evidence for 5-HTTLPR**

<table>
<thead>
<tr>
<th>Estimates overall</th>
<th>N/A</th>
<th>✓</th>
<th>N/A</th>
<th>N/A</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN</td>
<td>✓</td>
<td>✓</td>
<td>N/A</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>BN</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**Notes.** * indicates study included in meta-analysis. # indicates unpublished abstract. X indicates no association between 5-HTTLPR and the outcome. ✓ indicates an association between 5-HTTLPR and the outcome. N/A indicates the outcome was not assessed in the meta-analysis.
In addition, a number of limitations across all the meta-analyses must be noted. Firstly, the sample sizes across almost all studies included in these reviews were very low for studies of genetic association. A substantial number of studies in each meta-analysis included sample sizes less than \( N = 100 \), with most other samples in the low hundreds. These samples, in most part, are clearly too small for assessment of genetic association (Dick et al., 2015; Duncan & Keller, 2011). Inclusion of numerous small studies in meta-analytic reviews also exacerbates issues related to heterogeneity (Thompson & Higgins, 2002). Furthermore, neither Lee and Lin (2010) nor Chen et al. (2015) included an assessment of the quality of studies identified by their reviews. Finally, the issue of publication bias was inadequately addressed across all studies other than Polsinelli et al. (2012). Lee and Lin (2010) reported testing for publication bias, although used the regression model, which has low power to detect bias (Duval & Tweedie, 2000), and reported no publication bias was present but did not display any data to support this claim. Calati et al. (2011) and Solmi et al. (2016) stated that publication bias was measured used the funnel plot method, however results of this test were never reported in either study, so it is unclear whether publication bias affected findings or not. Finally, Chen et al. (2015) did not report any assessment of publication bias. This is a major issue affecting almost all the meta-analyses, as publication bias is a noted limitation affecting studies of genetic association (de Vries, Roest, Franzen, Munafò, & Bastiaansen, 2016; Duncan & Keller, 2011), with such issues argued to most strongly affect studies with small sample sizes (Ioannidis, 2005). These issues will be discussed in depth in Chapter 5.
3.3.3. Direct associations between 5-HTTLPR and disordered eating in community samples

While there are some conflicting results in studies testing clinical ED samples, research investigating the association between 5-HTTLPR and disordered eating has produced few direct links at all (e.g., Akkermann et al., 2008; Munn-Chernoff et al., 2012). Akkermann et al. (2008) found that 5-HTTLPR genotype was not associated with drive for thinness or binge eating in a sample of boys and girls (N = 772), while in a subsequent study Akkermann et al. (2010) did not find an association between 5-HTTLPR and binge eating in a sample of 484 women. Similarly, Munn-Chernoff et al. (2012) found no association between disordered eating and seven polymorphisms related to the serotonin system, including 5-HTTLPR, in a sample of 465 individuals (twins or family members). However, some studies have found contrary results. In a sample of 132 females, Sanhueza et al. (2011) found an association between the s/s genotype and restrictive eating, while Matsushita, Nakamura, Nishiguchi, and Higuchi (2002) reported an association between the l-allele and disordered eating in a sample of 179 Japanese women, a result that runs contrary to existing trends. It is noteworthy that the studies reporting associations between 5-HTTLPR and disordered eating contained substantially smaller sample sizes than those reporting no association. As there is limited research examining the direct effects of 5-HTTLPR in non-clinical samples, no systematic reviews or meta-analyses have summarised findings to date.
3.3.4. Introduction to gene x environment interactions

Thus far, results of direct genetic association studies have been inconsistent, and plagued with failures to replicate. Equally, while some environmental factors have garnered strong support for a role in risk of EDs (e.g., critical comments about weight or shape; Machado et al., 2014), there remains substantial individual variation in susceptibility to these environmental factors. As such, an emerging area of research concerns the interaction between genes and environmental factors in the development of eating pathology. It is possible that such interactions may explain some of the inconsistencies in studies of direct genetic association, as well as differences in outcome amongst individuals exposed to certain environmental risk factors. The GxE interaction approach proposes that individuals with particular genetic vulnerability may be at greater risk of developing disordered eating in adverse environments compared to individuals with low-risk gene variants. For example, amongst two individuals who have experienced similar levels of adverse life events, only the individual with the ‘risky’ genotype may subsequently experience an increased risk of developing an ED (Stoltenberg et al., 2012).

As with studies of direct association, 5-HTTLPR has been the polymorphism most commonly studied within a GxE framework, with presence of one or two copies of the low-function s-allele believed to constitute a ‘risky’ genotype. 5-HTTLPR is an appropriate candidate for GxE research for a number of reasons, particularly in light of findings suggesting it is implicated in the functioning of numerous areas associated with the human stress response (Moffitt, Caspi, & Rutter, 2005). For example, it is related to reduced grey matter volume in limbic regions (Pezawas et al., 2005), which are associated with emotional processing and behavioural motivation. It has also been implicated in regulating
hypothalamic-pituitary-adrenocortical (HPA) axis activity (Gotlib, Joormann, Minor, & Hallmayer, 2008). The HPA axis is involved in reactions to stress and a number of other processes, including those relating to mood and emotions (Pariante & Lightman, 2008). 5-HTTLPR has also been associated with trait anxiety (Schinka, Busch, & Robichaux-Keene, 2004; Sen, Burmeister, & Ghosh, 2004), and individuals with lower serotonin activity are believed to be hypersensitive to threatening stimuli (Heinz et al., 2005). It should be noted that 5-HTTLPR operates within a system of genes that have also been identified as part of the stress-response system that may alter individual susceptibility to environmental stressors (e.g., genes coding for the neurotransmitters dopamine, norepinephrine, epinephrine, and other genes coding for serotonin; van Eekelen et al., 2012). Therefore, 5-HTTLPR should not be misconstrued as the only gene contributing to altered environmental susceptibility. However, in light of existing theoretical support and early empirical evidence of a role for 5-HTTLPR in individual response to varied environmental stressors (Caspi et al., 2003), it is an important starting point in the investigation of how genes and the environment interact to influence eating pathology.

A compromised ability to cope with environmental stressors may increase risk of an ED or disordered eating through a number of mechanisms. It may increase the propensity of individuals to engage in coping behaviours, such as disordered eating. As discussed earlier, binge eating, purging, and food restriction have been conceptualised as mechanisms for coping with stress under a number of theoretical models (Corstorphine, Mountford, Tomlinson, Waller, & Meyer, 2007; Heatherton & Baumeister, 1991). For example, binge eating has been described as an ‘avoidance’ strategy that temporarily distracts the individual from negative affect, while caloric restriction is believed to reduce stress by
increasing perceived control in a different domain (Heatherton & Baumeister, 1991). Other effects may be more tentatively linked to EDs. For example, difficulty coping with environmental stressors may result in greater likelihood of depressed mood or anxiety (Slavich & Irwin, 2014), which may inadvertently increase risk for eating pathology. Indeed, the role of 5-HTTLPR in increasing susceptibility to environmental stressors has been investigated in depth across numerous other mental health areas, in particular, depression (Culverhouse et al., 2017).

GxE interaction research exploded in popularity in the psychiatric field following a seminal study by Caspi et al. (2003), who reported a significant interaction between 5-HTTLPR and the experience of traumatic life events in predicting depressed mood, and in a closely related study where Caspi et al. (2002) found an interaction between MAOA gene (related to aggression and mood symptoms; Fan et al., 2010; Shih & Thompson, 1999) and childhood maltreatment in predicting the development of antisocial problems. These studies, particularly Caspi et al. (2003), prompted a vast number of replication attempts, with five meta-analyses now reporting on whether there is an interaction between 5-HTTLPR and adverse life events in predicting depression (Culverhouse et al., 2017; Karg et al., 2011; Munafò, Durrant, Lewis, & Flint, 2009; Risch et al., 2009; Sharpley et al., 2014). Three of the meta-analyses reported no evidence of an interaction (Culverhouse et al., 2017; Munafò et al., 2009; Risch et al., 2009), while two report a significant 5-HTTLPR x life events interaction in predicting depression (Karg et al., 2011; Sharpley et al., 2014). Authors of the latter two analyses have argued that the earlier meta-analyses by Risch et al. (2009) and Munafò et al. (2009) excluded relevant studies by making their inclusion criteria too restrictive, thus accounting for the discrepant findings, although others have contested
the results of Karg et al. (2011) due to issues such as publication bias and inclusion of studies that introduce bias to the meta-analysis (Duncan, Pollastri, & Smoller, 2014). As such, conclusions regarding the traumatic life events x 5-HTTLPR interaction in the depression field remain contested, with arguments relating to publication bias and insufficient sample sizes on one hand (Duncan & Keller, 2011) meeting criticisms regarding quality of measuring outcome and environmental variables on the other (Moffitt & Caspi, 2014).

3.3.5. Gene x environment interactions in eating disorders and disordered eating

A number of studies have applied the GxE interaction framework to exploring risk relating to EDs. Studies have largely focussed on 5-HTTLPR (e.g., Karwautz et al., 2011; van Strien et al., 2010b), although many have also explored other genes, such as BDNF, DRD2, and NR3C1 (Akkermann, Hiio, Villa, & Harro, 2011; Groleau et al., 2012; Steiger et al., 2011). As in the depression field, published studies have started to accumulate evidence of an interaction between 5-HTTLPR and poor environments in relation to eating pathology. For example, in a clinical ED sample, Karwautz et al. (2011) reported that amongst sister-pairs discordant for AN, problematic parenting styles increased AN risk only for those with one or two copies of the 5-HTTLPR s-allele, but not those homogenous for the l-allele. Investigations of disordered eating in community samples have also found evidence of GxE interactions, with reports of 5-HTTLPR moderating the relationship between environmental stressors such as traumatic life events and adolescent disordered eating (Akkermann et al., 2012; Stoltenberg et al., 2012). For example, Akkermann et al.
(2012) found that adverse life events, particularly sexual abuse, were related to an increase in binge eating and drive for thinness in adolescent girls who carried the s-allele of 5-HTTLPR.

Studies of other genetic polymorphisms (e.g. the DRD2 Taq1A polymorphism, the NR3C1 Bc11 polymorphism) have also reported evidence of GxE interactions (Akkermann et al., 2011; Steiger et al., 2011). Steiger et al. (2011) found that individuals with the c-allele of the NR3C1 Bc11 polymorphism who had experienced childhood abuse were more likely to have a BN diagnosis compared to those with the g-allele. However, numerous studies have also returned null findings, such as (Groleau et al., 2012), who did not find any interactions between a number of environmental risk factors (childhood sexual, physical, or emotional abuse) and a number of dopamine-system genes (DRD2, DAT1, COMT).

Relatedly, a number of studies in the ED field have examined psychological characteristics in lieu of environmental stressors within a GxE interaction framework. For example, van Strien et al. (2010b) found that individuals with the s-allele of 5-HTTLPR reporting greater depressive symptoms also scored higher on a measure of emotional eating, while individuals with equivalent levels of depressed mood but who did not carry the s-allele did not show elevated levels of emotional eating. Meanwhile, Racine et al. (2009) examined interactions between 5-HTTLPR and impulsivity and drive for thinness, although found no interaction effects. Such investigations are prompted by findings suggesting that depressed mood or some personality factors may be related to genetic factors that also predict ED-related pathology. For example, investigating a sample of 59 women with a BN-spectrum disorder, Steiger et al. (2005) found that the s-allele of 5-HTTLPR was associated with poorer inhibition on a go/no go discrimination test, and
greater likelihood of comorbid borderline personality disorder (of which a key symptom is impulsivity). Given the heritability of personality factors such as impulsivity (around 50%; Bezdjian, Baker, & Tuvblad, 2011), these findings may reflect both GxE (or gene x personality) and GxG interactions. Despite some early null findings in GxE studies (Racine et al., 2009), the aforementioned differences in psychological traits amongst ED patients indicate there may be a role for GxE interactions and that these should undergo further investigations with increased methodological vigour.

3.4. Plasticity in gene x environment interactions

Whereas research into GxE interactions initially focussed on investigating the interaction between ‘risky’ polymorphisms and environmental factors and their association with negative outcomes, the GxE interaction framework has also been conceptualised under a ‘plasticity’ model (Belsky et al., 2009). Under this differential susceptibility model, a particular allele that may interact with negative environments to increase risk of poor outcomes may lead to better outcomes in the presence of positive or neutral environments, compared to those with the non-plasticity allele. For example, individuals with the s-allele who under environmental conditions considered ‘risky’ for a disorder (e.g., parental use of harsh discipline; Wang & Kenny, 2014) may show a greater propensity to develop negative outcomes, but may show better outcomes than their l/l counterparts under adaptive environmental conditions (e.g., high parental warmth; Tetley et al., 2014).

Belsky et al. (2009) argue that prior research guided by the diathesis-stress model has focussed too heavily on adverse outcomes, thereby potentially overplaying the role of certain poor environmental factors while under-recognising the benefits of advantageous
environments. Indeed, as will be seen from the subsequent chapters of the present thesis, little to no candidate gene research has involved exploration of potentially beneficial environments in reducing ED risk. Aside from the fact that such findings in genetic studies would offer a more ‘hopeful’ approach to understanding ED susceptibility and prevention efforts, the plasticity theory is also logical from an evolutionary perspective. From this standpoint, the fact that certain ‘risky’ s-alleles have not been eliminated through evolutionary processes supports the notion that they may confer a selective advantage under particular environmental conditions, rather than merely increasing risk for pathology (Belsky & Pluess, 2009).

3.4.1. Plasticity findings in non-eating disorder fields

The plasticity theory has been supported by findings from a number of studies. Pluess, Belsky, Way, and Taylor (2010) examined the relationship between negative life events, 5-\textit{HTTLPR}, and levels of neuroticism in $N=118$ individuals. Amongst those who had experienced a greater number of negative life events, they found that individuals with an s/s genotype showed higher levels of neuroticism than individuals with the l/l genotype, with participants with the s/l genotype displaying intermediate levels of neuroticism. However, individuals with the s/s genotype showed lower levels of neuroticism in the presence of positive life events, a relationship that was not found for those homogenous for the long allele. A similar pattern was observed in Kuepper et al. (2012), who found that an increase in positive life events was associated with lower levels of neuroticism and increased life satisfaction for individuals with the s/s genotype. This pattern was observed to a lesser extent in those with the s/l phenotype, but there were no associations between positive life
events and lower neuroticism or greater life satisfaction for individuals with the s/l structure. Taken together, these findings illustrate the idea that the s-allele, rather than being a risk factor per se, may be better conceptualized as increasing an individual’s reactivity to both positive and negative environments.

A variable relevant to ED aetiology that allows for examination of the GxE plasticity hypothesis in relation to mental health outcomes is parenting. Differences in parenting behaviours have been implicated in numerous child behavioural and development outcomes (Sanders, Kirby, Tellegen, & Day, 2014; although this must be understood as distinct from parents causing mental health problems or being blamed for conditions such as EDs; see previous chapter for discussion relating to EDs), and parenting can be assessed on a scale ranging from more to less positive, thus providing a good candidate environmental factor with which to examine possible genetic plasticity.

Parenting has been examined under the plasticity model in a handful of studies assessing non-ED outcomes. For example, a meta-analysis by Bakermans-Kranenburg and van Ijzendoorn (2011) found that dopamine-system genes DRD2 (A1 allele) and DRD4 (7-R allele) were associated with increased risk for a range of negative outcomes in children exposed to adverse rearing conditions, but better outcomes in more positive family environments. In a study of N = 1,586 adolescents, Belsky and Beaver (2011) found that self-regulation was predicted by an interaction between supportive or unsupportive parenting and a cumulative plasticity score involving a number of genes previously identified as conferring plasticity (DAT1, DRD2, DRD4, 5-HTTLPR, and MAO-A; Belsky et al., 2009). Hankin et al. (2011) reported similar findings in a series of three studies (total N = 1874) examining whether 5-HTTLPR moderated the relationship between supportive
parenting and levels of positive affect, with participants with the s/s genotype showing lower positive affect in unsupportive conditions, but higher positive affect in an environment with high levels of parental support.

However, some more recent findings have reported contrary results (Van Assche et al., 2016; van Roekel, Engels, Verhagen, Goossens, & Scholte, 2011). For example, Van Assche et al. (2016) examined a range of parenting behaviours, including control, support, and physical and non-physical punishment, and found no interaction with 5-HTTLPR (under either the plasticity or diathesis stress models) in predicting childhood depressive symptoms ($N = 797$). Similarly, van Roekel et al. (2011) did not identify any GxE interaction between 5-HTTLPR and parental support in predicting future depressive symptoms in a sample of $N = 306$ adolescents. Notably, both these studies failed to find a GxE interaction when assessing a positive environmental factor (supportive parenting). In depth discussion regarding the possible explanations for discrepancies between these studies and those identifying significant GxE interactions involving plasticity are discussed in further depth in Chapter 12. Thus far, GxE interactions have not been investigated from a plasticity perspective by any study in the ED field.

### 3.5. Introduction to Study 1

The first study of the present thesis (Study 1) will constitute a systematic review of all studies examining GxE interactions in the ED field, in order to present a clear picture of current findings. This will help summarise existing knowledge and elucidate areas in candidate GxE research that require further investigation or that have not adequately been examined within the ED field, as well as provide insight regarding limitations to existing
studies and future directions. This will be followed, in the same study, by a secondary data meta-analysis of eligible studies, by combining entire data sets from studies that have examined the same polymorphism (5-HTTLPR) and environmental variable (traumatic life events, sexual and physical abuse, depression, and impulsivity). Study 1 is presented in Chapter 4, while the subsequent Chapters (5 – 6) expand on Study 1 by featuring a systematic review of all polymorphisms investigated within a GxE framework in the ED field (e.g, DRD2, DAT1) and include further discussion of methodological issues and the meta-analytic findings.
4. CHAPTER 4

Study 1

This chapter features the original manuscript for the following published study, which constitutes Study 1 of the present thesis:


The only changes to the manuscript presented in the current chapter include formatting for consistency with the present thesis. To view the published version of Study 1, please see Appendix A.

**Author contributions**

VR was responsible for conducting all analyses and preparing all sections of the manuscript (80%). DO contributed to the systematic review section, including searching, recording results evaluating the studies, and contributing to that section of the manuscript. IK and MFT contributed to study design and editing drafts of the manuscript, and MFT also contributed to the analyses section. Remaining authors were involved in the collection of data. All authors contributed to and approved the final manuscript.
A systematic review and secondary data analysis of the interactions between the serotonin transporter 5-HTTLPR polymorphism and environmental and psychological factors in eating disorders


aDepartment of Psychological Sciences, The University of Melbourne, Parkville 3010, Australia
bSchool of Psychology, Deakin University, Geelong, 3220, Australia
cDepartment of Psychology, Estonian Centre of Behavioural and Health Sciences, University of Tartu, Tartu 50410, Estonia
dSocial, Genetic, and Developmental Psychiatry Research Centre, Institute of Psychiatry, King’s College, London SE5 8AF, United Kingdom
eBehavioural Science Institute, Radboud University Nijmegen, 6500 HE Nijmegen, The Netherlands
fDepartment of Psychiatry, University Hospital of Bellvitge – IDIBELL,08907, Spain; CIBER Fisiopatologia Obesidad y Nutricion (CIBERobn), Instituto Salud Carlos III, Madrid, Spain
gDonders Institute for Brain, Cognition, and Behaviour, Centre for Neuroscience, Radboud University Medical Centre, Department of Cognitive Neuroscience, 6525 EZ Nijmegen, The Netherlands
hDepartment of Child and Adolescent Psychiatry, Medical University of Vienna, 1090 Vienna, Austria
iDepartment of Psychology, Michigan State University, East Lansing, Michigan 48824-1116, United States
jDepartment of Psychology, University of Wisconsin-Milwaukee, Wisconsin, United States
kEating Disorders Continuum, Douglas Institute, Montreal; Psychiatry Department, McGill University, Montreal, Canada
lDepartment of Psychology, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, United States
mThe Institute of Psychiatry, Psychology, and Neuroscience, King’s College, London SE5 8AF, United Kingdom

Key words: Eating disorders, gene-environment interaction, 5-HTTLPR, meta-analysis, systematic review, bulimia nervosa.
ABSTRACT

Objectives: To summarize and synthesize the growing gene x environment (GxE) research investigating the promoter region of the serotonin transporter gene (5-HTTLPR) in the eating disorders (ED) field, and overcome the common limitation of low sample size, by undertaking a systematic review followed by a secondary data meta-analysis of studies identified by the review. Method: A systematic review of articles using PsycINFO, PubMed, and EMBASE was undertaken to identify studies investigating the interaction between 5-HTTLPR and an environmental or psychological factor, with an ED-related outcome variable. Seven studies were identified by the systematic review, with complete data sets of five community (n=1750, 64.5% female) and two clinical (n=426, 100% female) samples combined to perform four secondary-data analyses: 5-HTTLPR x Traumatic Life Events to predict ED status (n=909), 5-HTTLPR x Sexual and Physical Abuse to predict bulimic symptoms (n=1097), 5-HTTLPR x Depression to predict bulimic symptoms (n=1256), and 5-HTTLPR x Impulsiveness to predict disordered eating (n=1149). Results: Under a multiplicative model, the low function (s) allele of 5-HTTLPR interacted with traumatic life events and experiencing both sexual and physical abuse (but not only one) to predict increased likelihood of an ED and bulimic symptoms, respectively. However, under an additive model there was also an interaction between sexual and physical abuse considered independently and 5-HTTLPR, and no interaction with traumatic life events. No other GxE interactions were significant. Conclusion: Early promising results should be followed-up with continued cross-institutional collaboration in order to achieve the large sample sizes necessary for genetic research.
INTRODUCTION

Over the past decade, etiological models of eating disorders (EDs) have increasingly acknowledged the role of genetics, with twin studies estimating a notable heritable component (approximately 40-60%; Bulik, 2006, 2010; Fairweather-Schmidt & Wade, 2015; Trace et al., 2013). Investigations so far have not consistently identified specific candidate genes associated with increased ED risk, suggesting that hereditary factors in EDs may not operate via simple genetic association (Trace et al., 2013). Hence, studies are now increasingly examining whether environmental factors moderate the influence of candidate genes on risk for pathological eating behaviour. Gene x environment (GxE) interaction research in the ED field is still relatively novel, with early studies identifying potential candidate genes associated with ED risk under specific environmental conditions (e.g., history of abuse; Steiger et al., 2012). In anticipation of the increased popularity of this research focus, it is timely to evaluate the current state of evidence and to highlight existing limitations, in order to guide the direction and methods of future GxE studies in eating pathology.

Previous research examining genetic influences on eating pathology has primarily focused on genes in the serotonin and dopamine systems linked to functions relevant to EDs, including appetite, mood, and reward sensitivity (e.g., SLC6A4, HTR2A, DRD2, DRD4, DAT1, and COMT; see Culbert et al., 2015, and Trace et al., 2013, for a review). Direct genetic association studies have not provided a clear picture of the links between specific genes and EDs or disordered eating symptoms, with many initial significant findings failing to achieve consistent replication (see Calati et al., 2011; Culbert et al., 2015; Lee & Lin, 2010; Scherag et al., 2010; Trace et al., 2013).
One reason for a lack of direct association between allele frequency and ED risk may be that this relationship is moderated by environmental factors. Under the diathesis-stress model of GxE interactions, individuals carrying a ‘risk’ allele may be more susceptible to EDs when exposed to environmental stressors, but show no differences in outcome in the absence of challenging environmental circumstances, compared to those without the risky genotype (Caspi et al., 2003; Monroe & Simons, 1991). The role of GxE interactions in psychology has gained increasing attention since Caspi and colleagues (2003) found that stressful life events increased susceptibility to depression for those with one or two copies of the short (s) allele of the serotonin transporter gene (5-HTTLPR polymorphism).

Studies have since largely focussed on 5-HTTLPR due to its biological relevance to psychiatric disorders (with the s-allele reducing serotonin transporter transcription efficiency; Heils et al., 1996), and early significant findings in the depression literature (Karg et al., 2011). Despite substantial research investigating GxE interactions with 5-HTTLPR and other polymorphisms, many studies are limited by small sample size, and replicability remains a major issue (see Duncan et al., 2014 for a review; Risch et al., 2009). Furthermore, most studies to date failed to control for confounding influences on the GxE interaction by not including all required covariate x gene and covariate x environment contrast terms in the regression model (Keller, 2014). Studies examining case-control samples have also tended to evaluate the GxE effect using logistic regression and have thus tested departures from a multiplicative model of interaction, which is believed to be less biologically plausible than an additive model (Rothman, 1976; Rothman & Greenland, 1998).
GxE studies of candidate genes in eating pathology have been scarce. A recent review by Culbert et al. (2015) highlighted the heterogeneity of candidate GxE research in eating pathology. Their investigation identified five studies examining candidate GxE interactions with eating pathology outcome variables. Two studies reported a significant GxE interaction for 5-HTTLPR (Karwautz et al., 2011, parenting styles; Akkermann et al., 2012, traumatic life events), while one study investigating a psychological factor did not (Racine et al., 2009, impulsivity). The two remaining studies examined other genes (NR3CI x childhood abuse, Steiger et al., 2011; BDNF x restricted food intake; Akkermann et al., 2011), finding significant interactions to predict bulimia nervosa (BN) spectrum pathology. This paper presents a good start in summarising candidate GxE literature in eating disorders (although it was not a systematic review and thus omitted several studies, e.g., Stoltenberg et al., 2012; van Strien et al., 2010), and reflects the growing focus on gene x environment interactions in the eating disorders field.

While candidate GxE research in eating pathology is still in its infancy, it is not premature to consider how to best utilise academic resources to avoid the pitfalls GxE research has faced in other fields, such as lack of consistent replication and small sample sizes (Dick et al., 2015). This will aid greater accuracy in GxE findings, which is a vital step in increasing understanding of how individual differences at the genetic level can influence susceptibility to eating pathology. In the depression field, a protocol for a collaborative meta-analysis to achieve these aims has been published (N = 33,761), with authors aiming to re-analyse their data using a standardised analysis script to increase consistency of analytic methods and phenotypic definitions (Culverhouse et al., 2013).
Future collaborations could integrate complete datasets for combined re-analysis rather than relying on summary statistics. No such study has been undertaken in the ED field so far.

Thus, the present study aims to provide a systematic, detailed overview and re-analysis of current GxE studies investigating 5-HTTLPR in eating pathology, to clarify the current state of knowledge and to encourage future research to build upon this via continued focus on replication of published findings and multi-institute collaborations to achieve larger sample sizes. Specifically, it will examine, via a systematic review, existing studies that have analysed how the interaction between 5-HTTLPR and an environmental or psychological factor influences ED status or sub-threshold ED symptomatology. Secondary data meta-analyses to re-analyse GxE interactions using larger sample sizes with appropriate control of confounding variables as per Keller (2014) will then be performed by aggregating the results of three or more existing studies in a series of smaller analyses. Each analysis will be tested according to the multiplicative model of interaction, for consistency with prior research, and also according to the additive model of interaction, because of the possibility that this better represents and may be more sensitive to identifying gene x environment interactions. This study will be reported according to PRISMA guidelines where applicable (Moher et al., 2010).

**Systematic review**

**Inclusion criteria and search strategy**

The databases PsycINFO, PubMed, and EMBASE were searched through to January 2016 by two authors (V.R. and D.O) using the search terms ("eating disorder*" or “disordered eating” or “anorexi*” or “bulimi*” or “binge eating” or "emotional eating" or
“dietary restraint”) + (“gene environment interaction” or “gene” or “allele”), limited to "human only" and English language. Inclusion criteria included testing an interaction between 5-HTTLPR and an environmental or psychological factor, with eating pathology as the outcome variable. Eating pathology included a clinical-level diagnosis or a measure of disordered eating (e.g., dieting, body dissatisfaction). Studies examining body mass index (BMI) or weight gain as the outcome variable, or examining twin samples rather than candidate genes, were excluded to maintain a focussed investigation of 5-HTTLPR. While not technically an ‘environmental’ factor, psychological factors were included in the search as in many cases such variables are implicated in the aetiology of EDs and may influence how a genetic variant modifies risk for EDs (e.g., impulsivity in BN, Steiger et al., 2005). Indeed, many studies have investigated psychological factors within a GxE framework both in the ED literature (Akkermann et al., 2011; Racine et al., 2009; Mata & Gotlib, 2011; van Strien et al., 2010) and in other psychopathologies (Lu et al., 2011; Mandelli et al., 2009; Wang et al., 2013). Results were limited to published studies. A total of 1353 papers were initially identified (701 duplicates), with 35 selected for closer reading. Of these, 7 papers met criteria for the systematic review, with a summary provided in Figure 1.
Figure 1. Flow chart depicting selection of papers for systematic review and secondary data meta-analyses based on analysis of 5-HTTLPR x environment interaction with an ED-related outcome variable.
Quality appraisal

Quality of each study in the systematic review was evaluated using Downs and Black’s (1998) framework. As this tool was created to assess clinical trials, criteria were adapted to evaluate GxE research in eating disorders, with 14 non-applicable criteria excluded. A brief description of the items is presented, with notes in parentheses detailing changes in their current application:

1) Clear description of the hypothesis/aim/objectives; 2) Clear description of main outcomes in introduction/method; 3) Participant characteristics clearly described (as appropriate for GxE and ED research); 4) Clear description of main findings; 5) Characteristics of participants lost to follow-up described; 6) Exact probability values reported (or confidence intervals included); 7) Participants representative of population (including clinical, but not convenience samples); 8) Any “data-dredging” explicitly noted; 9) Appropriate statistical tests used; 10) Main outcome measures valid and reliable; 11) Participants in different groups (if case-control study) recruited contemporaneously; 12) Adequate adjustment for (potential) confounding variables (e.g., BMI; according to Keller (2014), this requires inclusion of all covariate x gene and covariate x environment interaction terms in the model); and 13) Sufficient power (to detect a GxE interaction, as guided by Duncan & Keller, 2011).

Studies were evaluated independently by two coders, V.R. and D.O., and cross-checked for consistency. Discrepancies were discussed amongst the raters with a third author (I.K.) consulted where necessary. Another author with particular expertise in statistical methods in psychology (M.F.T.) additionally evaluated criterion 9. To avoid biases or conflicts of interest, no other co-authors provided input to the evaluation.
Table 1 presents results of the quality evaluation. Discrepancy between coders was lower than 5%. The evaluation found that studies largely adopted valid and reliable methods with good reporting of results. The main issues pertained to insufficient power to detect the small-to-medium effect sizes likely involved in GxE interactions (Duncan & Keller, 2011), and that no study properly controlled for potential confounds on the interaction effect by including covariate x gene and covariate x environment interaction terms (Keller, 2014). Some studies tested three-category polymorphic groupings using cross-product terms in regression models, which was recently suggested to be statistically flawed due to the possibility of both false positive and negative results (Aliev et al., 2014). Nonetheless, the studies present promising initial findings and constitute good building blocks for continued GxE analyses in the field.

Table 1
Downs and Black (1998) Checklist for Methodological Quality, Adapted to Evaluate Studies Identified in a Systematic Review of the Role of 5-HTTLPR x Environment Interactions in Risk for Eating Pathology

<table>
<thead>
<tr>
<th>Study</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steiger et al.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Racine et al.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>X</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mata &amp; Gotlib</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>van Strien et al.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Karwautz et al.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Akkermann et al.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stoltenberg et al.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1 = Criteria met  0 = Criteria not met  X = Unable to determine  N/A = Criteria not applicable. A description of each item is provided under the heading Quality Appraisal in the Method section.
Summary of findings

The systematic review identified 7 studies (see Table 2). Samples were from North American or European countries and $n$ varied from 50 to 584. Participants were mainly adolescents and young adults, with mean age spanning from 13.4 years to 25.6 years. Five studies investigated community samples (total $N = 2017$, 78.0% female), with two of these studies investigating mixed gender samples. Two studies examined clinical ED patients ($N = 348$, 100% female), with one of these a discordant sister-pair sample ($N = 128$ controls, 100% female).

Three studies found a significant 5-HTTLPR x Traumatic Life Events interaction, although each predicted a different ED pathology; two disordered eating (Akkermann et al., 2012 – EDI-2 Bulimia subscale only; Stoltenberg et al., 2012 – EAT-26 total score) and one Anorexia Nervosa (AN) diagnosis (Karwautz et al., 2011). Notably, unlike in Akkermann et al. (2012) and Stoltenberg et al. (2012), Karwautz et al. (2011) found an interaction only when analysing risky parenting styles and not for broader traumatic life events. One study found a significant sexual abuse x 5-HTTLPR interaction (Akkermann et al., 2012, predicting EDI-2 Bulimia and Drive for Thinness scales), while the other did not (Steiger et al., 2007, predicting BN-spectrum clinical diagnosis). Neither study reported a significant physical abuse x 5-HTTLPR interaction. Mata and Gotlib (2011) and van Strien et al. (2010) both reported a significant depression x 5-HTTLPR interaction in predicting overeating and emotional eating, respectively, although this effect was only for the s/s genotype in the former study. Racine et al. (2009) found no interaction between 5-HTTLPR or $HTR2A$ (T102C polymorphism) and impulsivity or dietary restraint in predicting binge eating or emotional eating symptoms.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Total No. of Participants (No. Women)</th>
<th>Mean age, yrs (SD)</th>
<th>Clinical sample</th>
<th>5-HTTLPR Genotype %</th>
<th>Outcome (measures)</th>
<th>Environmental factor</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steiger et al., (2007)</td>
<td>92 (92)</td>
<td>25.4 (6.4)</td>
<td>BN</td>
<td>LL LS SS</td>
<td>34 47 20 BN (EDE, EAT-26, DSM-IV diagnosis)</td>
<td>Childhood sexual/physical abuse, (CTI)</td>
<td>No significant interactions</td>
</tr>
<tr>
<td>Racine et al., (2009)</td>
<td>344 (344)</td>
<td>19 (1.4)</td>
<td>No</td>
<td>27 56 17</td>
<td>Binge eating (MEBS), Emotional eating (DEBQ)</td>
<td>Impulsivity (Barratt Impulsiveness Scale) Dietary restraint (EDE-Q/DEBQ composite)</td>
<td>No significant interactions</td>
</tr>
<tr>
<td>Mata &amp; Gotlib (2011)</td>
<td>50 (50)</td>
<td>13.9 (1.9)</td>
<td>No</td>
<td>28 44 28</td>
<td>Overeating (EDI-C)</td>
<td>Depression (CDI)</td>
<td>Interaction between s/s (but not s/l) genotype and depression</td>
</tr>
<tr>
<td>van Strien et al., (2010)</td>
<td>584 (295)</td>
<td>13.4 (0.6)</td>
<td>No</td>
<td>32 50 18</td>
<td>Emotional eating (DEBQ)</td>
<td>Depression (Depressive Mood List)</td>
<td>Interaction b/w s-allele and depression on DEBQ scores</td>
</tr>
<tr>
<td>Karwautz et al., (2011)</td>
<td>256 (128 discordant sister-pairs)</td>
<td>25.6 (8.4)</td>
<td>Half AN</td>
<td>38 43 18</td>
<td>AN (EATATE-I interview, DSM-IV diagnosis)</td>
<td>Life events (Oxford Risk Factor Inventory)</td>
<td>Interaction between s-allele and life events, specifically problematic parenting styles</td>
</tr>
<tr>
<td>Akkermann et al., (2012)</td>
<td>252(252)</td>
<td>17.8 (0.5)</td>
<td>No</td>
<td>103 (l/l) 136 (s/-)</td>
<td>Drive for thinness, Bulimia (EDI-2)</td>
<td>Life events (self-devised scale), including sexual, physical, and emotional abuse</td>
<td>Interaction between s-allele and life events on bulimia only (interaction with sexual abuse for both outcomes, none for physical abuse)</td>
</tr>
<tr>
<td>Stoltenberg et al., (2012)</td>
<td>439 (284)</td>
<td>22.5 (6.2)</td>
<td>No</td>
<td>33 46 21</td>
<td>Disordered eating (EAT-26)</td>
<td>Life events (Traumatic Antecedent Questionnaire)</td>
<td>Interaction between s-allele and traumatic events for females only</td>
</tr>
</tbody>
</table>
Notes. GxE = gene x environment interaction; AN = anorexia nervosa; BN = bulimia nervosa; CDI = Children’s Depression Inventory (Kovacs, 1985); CTI = Childhood Trauma Interview (Fink et al., 1995); DEBQ = Dutch Eating Behavior Questionnaire (van Strien, 2002); EAT-26 = Eating Attitudes Test (Garner et al., 1982); EATATE-I = EATATE Lifetime Diagnostic Interview (Anderluh et al., 2009); EDE = Eating Disorders Examination (Fairburn & Cooper, 1993); EDI-2 = Eating Disorders Inventory-2 (Garner, 1991a); EDI-C = Eating Disorders Inventory for Children and Adolescents (Garner, 1991b); MEBS = Minnesota Eating Behavior Survey (von Ranson et al., 2005). Results are significant at \( p < .05 \) unless otherwise specified.
Secondary Data Meta-Analyses

Method

Inclusion criteria

From the final 7 studies identified through systematic review, those that tested equivalent environmental or psychological variables were considered for a secondary data meta-analysis. Six suitable studies were identified (see Figure 1). Data from one additional study (Richardson et al., 2008) were included in the secondary data analysis but not the systematic review, as it contained relevant variables (drawing from the same larger sample as Steiger et al., 2007), but did not explicitly analyse the GxE interaction with an ED-specific outcome in their publication. Authors were contacted with a request to provide data for re-analysis and an invitation to join the present study. All authors contributed their data via email attachment as an SPSS or Microsoft Excel file. Variables sought included participant ID, age, gender, BMI (if assessed), 5-HTTLPR genotype, and item-level data for the environmental and ED variables.

Design

Data from six studies (Akkermann et al., 2012; Karwautz et al., 2011; Racine et al., 2009; Richardson et al., 2008; Stoltenberg et al., 2012; Steiger et al., 2007; and van Strien et al., 2010) were combined to test four separate secondary data analyses: 5-HTTLPR x Traumatic Life Events to predict ED diagnosis or equivalent, 5-HTTLPR x Sexual and/or Physical Abuse to predict a BN-spectrum ED or equivalent, 5-HTTLPR x Depression to predict BN-spectrum ED or equivalent, and 5-HTTLPR x Impulsiveness to predict ED diagnosis or equivalent.
Data synthesis

Full data sets from each study were provided. Overlapping participant data in Steiger et al. (2007) and Richardson et al. (2008) were removed by contributing authors prior to sending their data. Karwautz et al. (2011) was part of a European multi-centre collaboration (The European Project) and data for the present study were drawn from the larger unpublished sample, including additional data from clinical BN patients. Data from The European Project were only included if they contained item-level responses to the Oxford Risk Factor Inventory (ORFI; Fairburn et al., 1998) to ensure consistent measurement of the environmental factor ‘traumatic life events’ across studies. Item-level data were unavailable from some participating research centres and therefore the present sample size does not match Karwautz et al.’s (2011) publication, but includes additional participants with a BN diagnosis.

Prior to combining datasets according to the below-mentioned procedures, missing data were imputed at the item-level where necessary using the median value (Tabachnick & Fidell, 2013), with missingness lower than 5%. Participants with missing genetic data or summary scales (where item-level data were unavailable), were excluded from the analyses.

Measures of environmental and psychological factors used across studies were heterogeneous to varying extents. Most studies utilised different scales to assess ED status or examined different elements of disordered eating. Therefore, a complex process was necessary to integrate the variables to achieve compatibility for combined analysis, which is summarised below. Participant 5-HTTLPR genotypes across each study were coded as s-allele present (s/s and s/l genotypes) or s-allele absent (l/l genotype), as the s-allele is argued to function in a genetically dominant manner (Lesch et al., 1996).
**Analysis 1 - Traumatic life events:** Traumatic life events were determined according to 17 events (e.g., traumas/accidents, abuse, major health problems) that overlapped between scales used in Akkermann et al. (2012; self-devised scale), and Stoltenberg et al. (2012; Traumatic Antecedent Questionnaire, Herman & van der Kolk, 1987). Fourteen of these events overlapped with The European Project (ORFI; Fairburn et al., 1998) data, which was scaled to match the 18-item (0-17 events) solution.

ED status or equivalent was determined by a total score above 5 and 3 on the Drive For Thinness and Bulimia Scales of the Eating Disorder Inventory-2 (EDI-2; Garner, 1991a), respectively, which are the recommended scale-level cut-offs for clinical-level disordered eating (Nevonen & Broberg, 2001; Norring & Sohlberg, 1988). In Stoltenberg et al. (2012), ED status or equivalent was determined by a total score of 20 or greater on the Eating Attitudes Test-26 (EAT-26; Garner et al., 1982), the established cut-off for likely clinical-level eating pathology. A semi-structured clinical interview, the EATATE (Anderluh et al., 2009), was used to identify ED diagnosis based on DSM-IV criteria (American Psychiatric Association [APA], 2000) in the European Project.

**Analysis 2 - Sexual and physical abuse:** Sexual abuse and physical abuse were coded dichotomously in the European Project and Akkermann et al. (2012), and re-coded into yes/no format in Richardson et al. (2008) and Steiger et al. (2007) if participants endorsed anything above ‘low’ sexual or physical abuse, and in Stoltenberg et al. (2012) if abuse was ‘occasional’ or greater. BN status or equivalent was established based on whether participant responses to items on the EDI-2 (Garner, 1991a) in Akkermann et al. (2012) and on the EAT-26 (Garner et al., 1982) in Stoltenberg et al. (2012) endorsed DSM-IV (APA, 2000) BN-criteria, namely, engaging in regular binge eating, with loss of control, and engagement in compensatory behaviour. In addition, participants whose scores on the EDI-2 Bulimia scale and EAT-26 Bulimia and Food Preoccupation scale were substantially elevated, suggesting
likely clinical-range BN, were classified in the BN group. BN was determined according to the EATATE (Anderluh et al., 2009) and DSM-IV criteria (APA, 2000) in the European Project, and by the Eating Disorders Examination (EDE; Fairburn & Cooper, 1993) in Steiger et al. (2007) and Richardson et al. (2008).

**Analysis 3 - Depression:** Depression was coded dichotomously in the European Project using the ORFI (Fairburn et al., 1998) and in Richardson et al. (2008) as determined by the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-I, First et al., 1996). Dimensional measurements were obtained in Akkermann et al. (2012) via the self-report version of the Montgomery-Åsberg Depression Rating Scale (MADRS-S; Montgomery & Åsberg, 1979), and van Strien et al. (2010) via the Depressive Mood List (Kandel & Davies, 1982). For compatibility with the European Project and Richardson et al. (2008), these were dichotomised. A cut-off score of 15 was selected for the MADRS-S according to research examining criterion validity (Svanborg & Åsberg, 2001; Svanborg & Ekselius, 2003). No cut-off score has been established for the Depressive Mood List. However, as there was complete overlap between these measures, participant scores on the Depressive Mood List were scaled to match MADRS-S responses and the same cut-off value was applied. BN status or equivalent was determined as in Analysis 2 for the European Project, Richardson et al. (2008), and Akkermann et al. (2012). BN status was based on participant responses to categorical questions investigating binge frequency, loss of control, and engagement in compensatory behaviours in van Strien et al. (2010).

**Analysis 4 - Impulsiveness:** All studies assessed impulsiveness using the Barratt Impulsiveness Scale (BIS-11; Patton et al., 1995). ED status or equivalent was determined as in Analysis 1 for Akkermann et al. (2012) and Stoltenberg et al. (2012), while for Racine et al. (2009) this was determined by a mean score of 2.3 or greater on a self-report version of
the EDE (EDE-Q; adopted from Fairburn & Cooper, 1993), as suggested by Mond and colleagues (2004).

**Statistical analyses**

Analyses were conducted using binary logistic regression to test main and interaction effects of 5-HTTLPR and the environmental or psychological factor in predicting ED/BN status. 5-HTTLPR was coded according to presence or absence of the low-function s-allele. In light of past findings suggesting the s-allele operates in a genetically dominant manner (e.g., Lesch et al., 1996), and in order to avoid issues relating to multiple testing, genotype grouping (s/s, s/l, l/l) was not investigated. All analyses controlled for age by including the age, age x environment, and age x 5-HTTLPR terms to the overall model. BMI was also controlled for where data were available. These interaction terms are necessary to adequately control for potential confounders, although have been omitted from most GxE investigations in psychiatry to date (Keller, 2014). It was not possible to control for sex due to frequency distribution issues in the logistic regression. When examining sex differences by comparing a female only sub-sample to the overall sample in each analysis (a male-only sub-sample was not possible to due to frequency distribution), results were similar across all analyses, therefore only results for the larger, complete sample are displayed.

Finally, whereas the interaction term between gene and environmental or psychological factor is sufficient for testing a GxE interaction in logistic regression under a multiplicative model (as per past studies; e.g., Caspi et al., 2003, Karwautz et al., 2012, Steiger et al., 2012, also see Munafò et al., 2009), three additional statistics were computed to quantify the interaction from an additive perspective: the relative excess risk due to interaction (RERI), the attributable portion due to the interaction (AP), and the synergy index (S). When an interaction is present in the data, RERI and AP will be greater than 0, whereas S will be
greater than 1. These additive models were conducted using Stata version 13. Estimates of these interaction effects were derived from relative risk ratios rather than odds ratios, as: (1) the formulae for RERI, AP, and S were designed to use with RR values, and (2) substituting OR values for RR values in these formula will over-estimate the interaction effects in cases where the baseline prevalence is not rare (e.g., greater than 10% prevalence; VanderWeele & Knol, 2014). To facilitate calculation of RR values, the two continuous predictors – traumatic experiences and impulsivity – were converted into categorical forms. Trauma history was split into no instances reported versus 1+ instances reported. As the appropriate cut-point for the impulsivity measure is unclear, several percentiles were trialled (5th, 10th, 15th, 20th, and 25th). Substantive conclusions did not change depending on the cut-off applied, and as such, results are reported for the lowest cut-off (5th percentile) to conceptually reflect those with lowest reported levels of impulsivity.

Results

Analysis 1: Traumatic Life Events

The sample comprised 909 individuals (65.7% female), from the following studies: two community samples, Stoltenberg et al. (2012; N = 436, 65.1% female), Akkermann et al. (2012; N = 369, 56.6% female), and a discordant sister-pair sample, the European Project (N = 104, 100% female).

Overall, 169 (18.6%) participants met criteria for an ED or equivalent. 5-HTTLPR frequencies (ll = 333, ls = 415, ss = 161) met the Hardy-Weinberg equilibrium, $\chi^2 = 2.55$, df = 1, $p > .05$. Traumatic Life Events were scored 0 to 17, ($M = 2.38$ events, $SD = 2.54$), and were positively skewed. Results of the logistic regression are displayed in Table 3.
As evident in Table 3, while there was no effect of traumatic events or genotype alone, presence of the s-allele was related to significantly greater likelihood of an ED for those who had experienced more traumatic life events compared to those with the l/l genotype ($OR = 1.23$, see Figure 2). A small but significant main effect of age was also noted. From an additive perspective however, none of the interaction indices reported significant findings to support an interaction effect: $RERI = -0.90$ (95% CIs: -4.17, 2.36), $p = .587$; $AP = -0.53$ (95% CIs: -2.85, 1.79), $p = .654$; and $S = 0.44$ (95% CIs: .01, 16.53), $p = .657$. 
Table 3

Main and Interaction Effects of 5-HTTLPR s-allele and Each Environmental Factor, Controlling for Age (and BMI where Possible) by Including all Covariate x Gene and Covariate x Environment Interaction Terms, in Predicting ED Status (Analyses 1 and 4) and BN Status (Analyses 2 and 3).

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Odds Ratio</th>
<th>Lower</th>
<th>Upper</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analysis 1: Traumatic life events (N= 909)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-4.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>1.01</td>
<td>2.75</td>
<td>.78</td>
<td>9.70</td>
<td>.115</td>
</tr>
<tr>
<td>Traumatic Events</td>
<td>.01</td>
<td>.91</td>
<td>.82</td>
<td>1.25</td>
<td>.906</td>
</tr>
<tr>
<td>5-HTTLPR x Traumatic Events</td>
<td>.21</td>
<td>1.23</td>
<td>1.06</td>
<td>1.44</td>
<td>.006</td>
</tr>
<tr>
<td>Age</td>
<td>.12</td>
<td>1.12</td>
<td>1.07</td>
<td>1.18</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Age x 5-HTTLPR</td>
<td>-.06</td>
<td>.94</td>
<td>.89</td>
<td>.99</td>
<td>.029</td>
</tr>
<tr>
<td>Age x Traumatic Events</td>
<td>-.002</td>
<td>1.00</td>
<td>.99</td>
<td>1.00</td>
<td>.432</td>
</tr>
</tbody>
</table>

**Analysis 2: Sexual/Physical abuse (N= 1097)**

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Odds Ratio</th>
<th>Lower</th>
<th>Upper</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual abuse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-4.77</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>.49</td>
<td>1.64</td>
<td>.50</td>
<td>5.32</td>
<td>.413</td>
</tr>
<tr>
<td>Sexual Abuse</td>
<td>2.15</td>
<td>8.56</td>
<td>2.16</td>
<td>33.92</td>
<td>.002</td>
</tr>
<tr>
<td>5-HTTLPR x Sexual Abuse</td>
<td>.53</td>
<td>1.69</td>
<td>.74</td>
<td>3.89</td>
<td>.214</td>
</tr>
<tr>
<td>Age</td>
<td>.14</td>
<td>1.15</td>
<td>1.10</td>
<td>1.19</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Age x 5-HTTLPR</td>
<td>-.02</td>
<td>.98</td>
<td>.94</td>
<td>1.03</td>
<td>.516</td>
</tr>
<tr>
<td>Age x Sexual Abuse</td>
<td>-.06</td>
<td>.94</td>
<td>.90</td>
<td>.99</td>
<td>.013</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Odds Ratio</th>
<th>Lower</th>
<th>Upper</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical abuse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-4.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>.45</td>
<td>1.57</td>
<td>.47</td>
<td>5.22</td>
<td>.461</td>
</tr>
<tr>
<td>Physical Abuse</td>
<td>1.57</td>
<td>4.79</td>
<td>1.37</td>
<td>16.70</td>
<td>.014</td>
</tr>
<tr>
<td>5-HTTLPR x Physical Abuse</td>
<td>.53</td>
<td>1.70</td>
<td>.85</td>
<td>3.41</td>
<td>.135</td>
</tr>
<tr>
<td>Age</td>
<td>.14</td>
<td>1.15</td>
<td>1.10</td>
<td>1.21</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Age x 5-HTTLPR</td>
<td>-.02</td>
<td>.99</td>
<td>.94</td>
<td>1.03</td>
<td>.536</td>
</tr>
<tr>
<td>Age x Physical Abuse</td>
<td>-.05</td>
<td>.95</td>
<td>.91</td>
<td>.99</td>
<td>.045</td>
</tr>
</tbody>
</table>
### Analysis 2: Sexual and/or Physical Abuse

The sample comprised 1097 individuals (71.8% female), from the following studies: two community samples, Stoltenberg et al. (2012; $N = 436$, 65.1% female), Akkermann et al. (2012; $N = 369$, 56.6% female), one clinical sample from Steiger et al. (2007) and Richardson...
et al. (2008), \((N = 127, 100\% \text{ female})\) and a discordant sister-pair sample, the European Project \((N = 168, 63\% \text{ controls, 100\% female})\).

Overall, 221 (20.1\%) participants met criteria for BN or equivalent. Three-hundred and fourteen (28.5\%) participants reported experiencing physical abuse, 165 (15\%) reported sexual abuse, and 85 (7.7\%) reported both physical and sexual abuse. 5-HTTLPR frequencies \((l/l = 407, l/s = 492, s/s = 201)\) deviated from the Hardy-Weinberg equilibrium, \(\chi^2 = 5.85, df = 1, p = .02\).

Results outlined in Table 3 show significant main effects for sexual abuse \((OR = 8.56)\), physical abuse \((OR = 4.79)\), and both sexual and physical abuse combined \((OR = 11.53)\). There was a significant GxE interaction \((OR = 3.15)\), whereby participants with the s-allele who experienced both sexual and physical abuse were more likely to endorse BN status compared to those with the l/l genotype (Figure 2). There was a main effect of age, and increased likelihood of BN was also significantly predicted by an interaction between (younger) age and each of the abuse variables.

From an additive perspective, a number of the interaction indices displayed significant findings to also support an interaction effect for physical abuse: \(RERI = 1.32 \ (95\% \text{ CIs: .06, 2.58}), p = .040; \ AP = .40 \ (95\% \text{ CIs: .09, .72}), p = .012; \) but not \(S = 2.40 \ (95\% \text{ CIs: .81, 7.13}), p = .116, \) and to support an interaction for sexual abuse \(RERI = 2.26 \ (95\% \text{ CIs: .05, 4.47}), p = .045; \ AP = .49 \ (95\% \text{ CIs: .13, .85}), p = .007; \) but not \(S = 2.70 \ (95\% \text{ CIs: .82, 8.90}), p = .102.\) All indices supported an interaction on additive scale for both sexual and physical abuse x 5-HTTLPR, \(RERI = 5.16 \ (95\% \text{ CIs: .73, 9.60}), p = .022; \ AP = .70 \ (95\% \text{ CIs: .43, .98}), p < .001; \ S = 5.41 \ (95\% \text{ CIs: 1.10, 26.71}), p = .038.\)
Figure 2. Significant GxE interactions between 5-HTTLPR and environmental factors 1) Traumatic life events, and 2) Sexual and physical abuse under the multiplicative model.

Analysis 3: Depression

The sample comprised 1254 individuals (62.5% female), from the following studies: two community samples, Akkermann et al. (2012; N = 369, 56.6% female), and van Strien et al. (2010; N = 623, 51.2% female), one clinical sample, Richardson et al. (2008; N = 89, 100% female) and a discordant sister-pair sample, the European Project (N = 168, 63% controls, 100% female).

Overall, 172 (13.7%) participants met criteria for BN or equivalent, while 184 (14.7%) participants met criteria for depressed mood. 5-HTTLPR frequencies (l/l = 438, l/s = 612, s/s = 205) met the Hardy-Weinberg equilibrium, $\chi^2 = .14$, $df = 1$, $p > .05$. Results of the logistic regression revealed no main or interaction effects of depression and 5-HTTLPR in predicting BN status (Table 3). Similar to the pattern observed in Analysis 2, younger age interacted with depressive status to predict greater likelihood of BN. There was also no support for an
interaction effect under an additive model, \( \text{RERI} = .15 \) (95% CIs: -0.95, 1.26), \( p = .785 \) and \( \text{AP} = .13 \) (95% CIs: -0.77, 1.03), \( p = .778 \). \( S \) could not be reliably computed for this interaction.

**Analysis 4: Impulsiveness**

The sample comprised 1122 individuals (72.2% female) from three community samples, Stoltenberg et al. (2012; \( N = 436 \), 65.1% female), Akkermann et al. (2012; \( N = 369 \), 56.6% female), and Racine et al. (2009; \( N = 317 \), 100% female).

Overall, 224 (20.0%) participants met ED criteria or equivalent. 5-HTTLPR frequencies (\( l/l = 384 \), \( l/s = 545 \), \( s/s = 193 \)) met the Hardy-Weinberg equilibrium, \( \chi^2 < .01 \), \( df = 1 \), \( p > .05 \). Impulsivity was measured using the Barratt Impulsiveness Scale Version 11 (BIS-11; Patton, et al., 1995), and was normally distributed. Data did not meet the assumption of linearity between continuous independent variables and the logit (\( p = .003 \)), suggesting that results may present an underestimation of the relationship (Hosmer & Lemeshow, 1989). Results of the logistic regression revealed no main or interaction effects of impulsiveness and 5-HTTLPR in predicting ED status (Table 3), which was supported by the indices measuring additive interaction, \( \text{RERI} = -1.18 \) (95% CIs: -4.22, 1.86), \( p = .448 \); \( \text{AP} = -0.85 \) (95% CIs: -2.49, .78), \( p = .307 \); and \( S = .24 \) (95% CIs: .03, 1.83), \( p = .170 \).

**Discussion**

To our knowledge, this is the first systematic review and secondary data meta-analysis investigating the role of 5-HTTLPR x environment and psychological factor interactions in risk for eating pathology. The aim was to summarise and re-analyse existing GxE research on eating disorder-related outcomes investigating the 5-HTTLPR polymorphism, in the largest sample tested to date, in order to elucidate the current state of knowledge and provide
guidance for future GxE studies in the field. Results of the secondary data meta-analysis revealed that when testing deviations from an additive model of interaction, the experience of sexual abuse, physical abuse, and both sexual and physical abuse each interacted with the s-allele of 5-HTTLPR to predict increased risk of bulimia-spectrum eating pathology. The significant interaction between 5-HTTLPR and both sexual and physical abuse (but not only one) was also supported from a multiplicative perspective, although there was no support for sexual abuse or physical abuse considered alone. In addition, there was a significant interaction between traumatic life events and 5-HTTLPR to predict an increased risk of eating pathology under the multiplicative model only. No effects were noted for the potential risk factors of depression and impulsiveness under either model.

Other noteworthy results include the large direct effects of sexual abuse and physical abuse on BN-spectrum disorders, an association demonstrated in previous meta-analyses (Chen et al., 2010; Norman et al., 2012; Smolak & Murnen, 2002). Conversely, there were no main effects of 5-HTTLPR genotype in any analyses, contrary to some past findings (Calati et al., 2011; Lee & Lin, 2010), although aligned with others (Castellini et al., 2012; Solmi et al., 2016).

The current GxE findings suggest that individuals with the ‘risky’ genotype may be relatively resilient to low levels of environmental risk, but disproportionately affected by greater environmental adversity (e.g., experiencing numerous types of abuse). From a biological perspective, it is plausible that this may function via the lowered serotonin transcription associated with the s-allele of 5-HTTLPR, leading to reduced availability of a key neurotransmitter in the stress response system (van Eekelen et al., 2012).

The present results demonstrate some links to findings in the depression field, where greater traumatic life events, including childhood abuse, have been found to interact with 5-
HTTLPR to predict depression (Karg et al., 2011; Nugent et al., 2011), although the interaction between life events and 5-HTTLPR is not undisputed (Munafò et al., 2009; Risch et al., 2009). One caveat is that ‘traumatic life events’ is a heterogonous concept. The types of events measured, scaling process, timing of events, age of participants, etc., may vary greatly, perhaps accounting for some inconsistency in GxE findings in the depression field (Uher & McGuffin, 2008). Careful consideration of these factors is encouraged for future analyses.

Aside from consistent measurement of environmental variables, another key issue affecting GxE research is low statistical power. Use of small sample sizes with insufficient power to detect GxE interactions has been a major point of criticism in GxE research for increasing risk of both false negative and false positive findings (Button et al., 2013). Sample sizes necessary to detect GxE effects are far bigger than typically involved in psychology (Luan et al., 2001), with one calculation of minimum sample size necessary to detect a large GxE interaction effect at 80% power, assuming no measurement error, $N = 600$ (Duncan & Keller, 2011). This increases substantially if only moderate effect sizes are involved. The median sample size of studies identified by the systematic review was 288, which is considered substantial in the ED field but lacking for genetic analyses. This is a particularly challenging limitation in light of the difficulty of obtaining genetic samples and highlights the immense value of the present collaboration, which has allowed us to utilise existing resources to maximize sample size and further knowledge regarding GxE effects in eating pathology.

One factor that may yet affect accuracy of the present findings is the possibility of publication bias amongst the studies identified by the systematic review. This has been noted in past GxE research, with one review reporting that significant findings were observed in 96% of initial GxE investigations but in only 27% of subsequent replication attempts (Duncan & Keller, 2011; Duncan et al., 2014). However, others argue that many instances of non-replication are related to methodological issues, including inadequate measurement of
traumatic life events (Caspi et al., 2010; Monroe & Reid, 2008). In any case, the tendency for positive findings to be more readily published, and null findings perhaps less likely to be initially submitted, can have a large effect on the accuracy of published studies by inflating false positive results (Dick et al., 2015; Ioannidis et al., 2014). It is therefore vital, for the success of future collaborative meta-analyses, for researchers to publish both significant and non-significant findings and for journals to support this initiative, while emphasising the use of reliable environmental measures.

Aside from the benefits of large sample sizes and resource efficiency in the present investigation, it further improved upon existing GxE research in eating pathology by correctly controlling for the potential effect of confounding variables (Keller, 2014). The inclusion of all necessary interaction terms was also facilitated by the large sample sizes investigated, and should be aimed for in future studies. Another strength of the present study was that it examined GxE interactions under both additive and multiplicative models of interaction. Most previous studies using a logistic regression model to assess their data have tested deviations from a multiplicative model. Conversely, studies of community samples with continuous outcome variables typically use linear regression models, which test deviations from an additive model. As the two methods produce somewhat different results, with the latter generally more conservative, caution should be taken in comparing the results of these models, and indeed, this may account for some of the discrepant findings in GxE research.

The present secondary data meta-analysis is not without limitations, primarily due to the need to harmonise heterogeneous datasets, which tested both community and clinical samples and contained varied measures of environmental and psychological factors and eating symptoms. The investigation of the 5-HTTLPR x depression interaction was in particular hindered by variability in the measurement of depression between studies. These
methodological issues may explain why this interaction was not found to be significant in the present analysis, contrary to findings in two of the initial studies (Mata & Gotlib, 2011; van Strien et al., 2010).

Nonetheless, the present study provides a detailed overview of current GxE findings involving 5-HTTLPR in the ED field, including studies assessing psychological variables. Subsequent research should focus on continued replication with large sample sizes, possibly best achieved through ongoing collaboration between researchers, given the resource-intensive nature of genetic research and scarcity of clinical ED samples. Such investigations would be best facilitated by researchers adopting standardised, or easily comparable, measures of environmental and psychological factors and eating symptoms that have excellent psychometric properties. Selection of measures should be carefully deliberated, both to maximise construct validity and to reduce measurement error, which can substantially increase statistical power (Bakermans-Kranenburg & van Ljzendorn, 2014). Polymorphisms and environmental or psychological factors selected should also be carefully justified, particularly in light of sample size restrictions (Dick et al., 2015).

Future studies may also benefit from adopting a differential susceptibility approach to GxE investigations. This hypothesis posits that certain alleles may be better conceptualised as conferring ‘plasticity’ in response to environmental stimuli, with alleles linked to poorer outcomes under negative environments conversely linked to better outcomes in positive environments (Belsky & Pluess, 2009). Such an analysis was not possible in the current paper due to lack of data assessing positive environments, however this pattern has been demonstrated for various polymorphisms, including 5-HTTLPR, in non-ED literature (see Bakermans-Kranenburg & van Ljzendorn, 2011, for a meta-analysis; Hankin et al., 2011). Accordingly, studies should include environmental measures that range from positive to negative in nature, such as parenting, peer relationships, or positive life events.
In sum, the present collaboration constitutes a large step forward in increasing knowledge of how genetics may moderate the manner in which environmental and psychological influences affect the likelihood of ED development. Given the ongoing uncertainty regarding why thus far identified risk factors appear to contribute towards ED development for some individuals but not others, genetics may be an important missing puzzle piece in identifying the source of individual variation in susceptibility to eating pathology.
References


5. CHAPTER 5

Extended Discussion of Studies Identified via Systematic Review in Study 1

This chapter will expand upon the findings of the systematic review of GxE interactions presented in Study 1. Study 1, in its published form, included a systematic review of the interactions between 5-HTTLPR and environmental or psychological factors in predicting ED-related outcomes. However, the review as conducted in its original form searched the literature for published studies of GxE interactions involving all genes, and was not restricted to 5-HTTLPR. This chapter will therefore discuss results of this ‘extended’ version of the systematic review, including all studies that examined an interaction between an environmental or psychological factor and any polymorphism on an ED-related outcome. In presenting this extended review, this chapter will address three main goals; 1, to present the findings of non-5-HTTLPR x environment interaction findings in the ED field and to further elaborate upon the findings of the studies examining 5-HTTLPR, including to evaluate the methodologies of these studies; 2, to discuss important limitations encountered by all GxE interaction studies and how these should be addressed in future research; and 3, to evaluate whether publication bias is present in this body of research.

5.1. Systematic review of gene x environment interactions in eating disorders

In Study 1, a systematic review was conducted to identify and summarise all published research that examined how the interaction between 5-HTTLPR and an environmental or psychological factor moderated an ED-related outcome (Rozenblat et al., 2017a). Study 1 contained an abbreviated version of the full review, which included published articles examining the interaction between an environmental or psychological factor and any gene that has been implicated in eating pathology (including BNDF, COMT, DAT1, DRD2, DRD4, NR3C1, see Table 7). The extended version of the review will be discussed in the present chapter, which will also include further discussion of studies that examined the 5-HTTLPR
polymorphism, given that exploration of the results and individual limitations of these studies was abridged in Study 1 due to word-count restrictions. The aim is to provide a complete and thorough overview of the current state of the knowledge and a clearer picture of the limitations facing studies of GxE interactions in the ED field.

5.1.1. Inclusion criteria

The inclusion criteria and search strategy for the systematic-review discussed in the current chapter were similar to that in Study 1, with minor differences reflecting the fact that the search strategy in the present chapter was designed to capture GxE interactions involving any polymorphism, unlike in Study 1, which focussed exclusively on GxE interactions investigating 5-HTTLPR. As such, the expanded inclusion criteria are presented below.

As in Study 1, the current definition of ‘environmental variable’ included both external factors, such as traumatic life events, and psychological factors, including personality traits and psychopathology. Although psychological factors are not strictly speaking ‘environmental’, they were included in the review under the premise that they may represent endophenotypes that moderate the relationship between a genetic variant and risk of an ED (e.g., impulsivity in BN; Steiger et al., 2005). Following similar reasoning, numerous studies have investigated psychological factors within a GxE framework both in the ED literature (Akkermann et al., 2011; Mata & Gotlib, 2011; Racine et al., 2009; van Strien et al., 2010b) and in the investigation of other psychopathologies (Lu et al., 2012; Mandelli et al., 2009; Wang et al., 2013).

ED-related outcomes included either a clinical ED diagnosis or disordered eating (e.g., emotional eating, drive for thinness, body dissatisfaction, dieting). Exclusion criteria included the use of Body Mass Index (BMI) or weight gain (e.g., measures of adipose tissue or increases in kilograms) as an outcome variable. This was in order to maintain focus on the psychological aspects of eating pathology and avoid capturing the medical literature on this
topic, which largely focusses on how GxE interactions affect metabolism and other physical processes in the body (e.g., Hung et al., 2014; Podolsky et al., 2007), as opposed to influencing cognition and behaviour. Studies that measured heritability through a twin design were also excluded, as the current search was strictly aimed at assessing the interaction between specific candidate genes and environmental or psychological factors. Twin studies do not involve collecting genetic samples from participants, but rather are aimed at providing rough measurements of what proportion of variation in individual phenotype is due to genetic factors, shared environments, and non-shared environments (Bulik, Sullivan, Wade, & Kendler, 2000; Slof-Op ‘t Landt et al., 2005; Thornton et al., 2011). However, these outcomes were not relevant for the purposes of the present review.

5.1.2. Search strategy

As in Study 1, the databases PsycINFO, PubMed, and EMBASE were searched through to January 2016 by two authors (V.R. and D.O) using the search terms (“eating disorder*” or “disordered eating” or “anorexi*” or “bulimi*” or “binge eating” or ”emotional eating” or “dietary restraint”) + (“gene environment interaction” or “gene” or “allele”), and limited to "human only" and English language. Results were limited to published studies. A total of 1353 papers were initially identified (701 duplicates), with 35 selected for closer reading. Of these, 13 papers met criteria for the systematic review, with a summary provided in Figure 1.

The systematic review identified a total of 14 studies across 13 papers (see Table 7, which is adapted from Study 1 with the addition of seven studies investigating non-5-HTTLPR polymorphisms). The seven studies examining 5-HTTLPR in Table 7 were presented in Study 1. The following segment will further discuss the findings and implications of the seven studies examining 5-HTTLPR that were briefly addressed in Study 1, as well as explore the seven additional studies that examined non-5-HTTLPR polymorphisms.
Figure 2  Flow chart depicting selection of papers for systematic review and secondary data meta-analyses based on analysis of candidate gene x environment interaction with an ED-related outcome variable.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Total No. of Participants (No. Women)</th>
<th>Mean age, yrs (SD)</th>
<th>Clinical sample</th>
<th>Genotype %</th>
<th>Outcome (measures)</th>
<th>Environmental factor</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5-HTTLPR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steiger et al., (2007)</td>
<td>92 (92)</td>
<td>25.4(6.4)</td>
<td>BN</td>
<td>34 47 20</td>
<td>BN (EDE, EAT-26, DSM-IV diagnosis)</td>
<td>Childhood sexual/physical abuse, (CTI)</td>
<td>No significant interactions</td>
</tr>
<tr>
<td>Racine et al., (2009)</td>
<td>344 (344)</td>
<td>19(1.4)</td>
<td>No</td>
<td>27 56 17</td>
<td>Binge eating (MEBS), Emotional eating (EDE-Q/DEBQ composite)</td>
<td>Impulsivity (Barratt Impulsiveness Scale), Dietary restraint (EDE-Q)</td>
<td>No significant interactions</td>
</tr>
<tr>
<td>Mata &amp; Gotlib (2011)</td>
<td>50(50)</td>
<td>13.9(1.9)</td>
<td>No</td>
<td>28 44 28</td>
<td>Overeating (EDI-C)</td>
<td>Depression (CDI)</td>
<td>Interaction between s/s (but not s/l) genotype and depression</td>
</tr>
<tr>
<td>van Strien et al., (2010b)</td>
<td>584 (295)</td>
<td>13.4(0.6)</td>
<td>No</td>
<td>32 50 18</td>
<td>Emotional, eating (DEQB)</td>
<td>Depression (Depressive Mood List)</td>
<td>Interaction b/w s-allele and depression on DEBQ scores</td>
</tr>
<tr>
<td>Karwautz et al., (2011)</td>
<td>256 (128 discordant sister-pairs)</td>
<td>25.6(8.4)</td>
<td>Half AN</td>
<td>38 43 18</td>
<td>AN (EATATE-I interview, DSM-IV diagnosis)</td>
<td>Life events (Oxford Risk Factor Inventory)</td>
<td>Interaction between s-allele and life events, specifically problematic parenting styles</td>
</tr>
<tr>
<td>Akkermann</td>
<td>252(252)</td>
<td>17.8(0.5)</td>
<td>No</td>
<td>103 (l/l)</td>
<td>Drive for thinness,</td>
<td>Life events (self-devised)</td>
<td>Interaction between</td>
</tr>
</tbody>
</table>
et al., (2012) & 136 (s/-) & Bulimia (EDI-2) scale), including sexual, physical, and emotional abuse & s-allele and life events on bulimia only (interaction with sexual abuse for both outcomes, none for physical abuse) \\
Stoltenberg et al., (2012) & 439 (284) & 22.5(6.2) & No & 33 46 21 & Disordered eating (EAT-26) & Life events (Traumatic Antecedent Questionnaire) \\
**Other genes** & **Gene (Alleles)** & **Measure** & **Interaction** \\
Racine et al., (2009) & 344 (344) & 19 (1.4) & No & 5TR2A – T102C (T, C) & Binge eating (MEBS), Emotional eating (EDE-Q/DEBQ composite) & Impulsivity (Barratt Impulsiveness Scale) \\
van Strien, et al., (2010a) & 279 (154) & 13.4(0.6) & No & DRD2 – Taq1A (A1, A2) & Emotional, external, restrained eating (DEBQ) & Parental psychological and behavioural control, parental support \\
Akkermann et al. (2011) & 765(426) & 16.1(1.5) & No & BDNF - Val66Met (Val, Met) & Bulimia, Drive for Thinness (EDI-2) & Self-induced food restriction \\
Steiger et al., (2011)* & 227(227) & 25.2(5.4) & 129 BN 98 controls & NR3C1 - Bcl1 (G, C) & BN Yes/No (EDE) & Childhood abuse (CTI) \\
Steiger et al., (2012)* & 304(304) & 26.0(6.4) & 174 BN 130 controls & Bcl1 (G, C) & BN Yes/No (EDE) & Childhood abuse (CTI)
<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Size</th>
<th>Mean Age</th>
<th>Diagnosis</th>
<th>Genotype</th>
<th>Phenotype</th>
<th>Environment</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groleau et al. (2012)</td>
<td>216 (216)</td>
<td>25.9</td>
<td>BN</td>
<td>DRD2 DAT1 COMT</td>
<td>Binge eating episodes (EDE)</td>
<td>Childhood physical/sexual/emotional abuse (CTI)</td>
<td>No significant interactions</td>
</tr>
<tr>
<td>van Strien et al., (2015)</td>
<td>93 (54)</td>
<td>20.7</td>
<td>No</td>
<td>DRD4 (7R/Non7R) &amp;</td>
<td>Emotional Eating (DEBQ)</td>
<td>Season of birth</td>
<td>No significant GxE interactions</td>
</tr>
<tr>
<td></td>
<td>586 (299)</td>
<td>44.9</td>
<td>No</td>
<td>(2R7R/Non 2R 7R)</td>
<td>Emotional Eating (DEBQ)</td>
<td>Season of birth</td>
<td>Interaction between 2R/7R risk allele and fall birth</td>
</tr>
</tbody>
</table>

**Notes.** GxE = gene x environment interaction; AN = anorexia nervosa; BN = bulimia nervosa; CDI = Children’s Depression Inventory (Kovacs, 1985) CTI = Childhood Trauma Interview (Fink et al., 1995); DEBQ = Dutch Eating Behavior Questionnaire (van Strien, 2002); EAT-26 = Eating Attitudes Test (Garner et al., 1982); EATATE-I = EATATE Lifetime Diagnostic Interview (Anderluh et al., 2009); EDE = Eating Disorders Examination (Fairburn & Cooper, 1993); EDE-Q = Eating Disorders Examination Questionnaire (adopted from Fairburn & Cooper, 1993); EDI-2 = Eating Disorders Inventory-2 (Garner, 1991a); EDI-C = Eating Disorders Inventory for Children and Adolescents (Garner, 1991b); MEBS = Minnesota Eating Behavior Survey (von Ranson et al., 2005). Results significant at $p < .05$ unless otherwise specified. *3/4 overlap in sample.
5.2. Findings of the systematic review

5.2.1. Overview of studies identified

The 14 studies comprised samples from European or North American countries, with $N$ varying from 50 to 765 ($M = 320$). Participants were mainly adolescents and young adults, with mean age spanning from 13.4 years to 26.0 years, with the exception of one study (44.9 years; van Strien, Levitan, Engels, & Homberg, 2015). Nine studies investigated community samples (total $N = 3392$, 73.7% female), with two-thirds of those studies investigating mixed gender samples. Five studies examined clinical ED patients ($N = 739$, 100% female), with three adopting a case-control design, including one discordant sister-pair sample ($N = 356$ controls, 100% female). Seven studies analysed 5-HTTLPR, with one also investigating HTR2A, and seven studies analysed other genes (DRD2, DRD4, BDNF, NR3C1, DAT1, and COMT). See Table 7 for an overview of the identified studies. Each study is discussed in detail below, with studies investigating 5-HTTLPR grouped into the following categories, as presented in the Study 1 meta-analysis, based on the main environmental variables analysed: 5-HTTLPR x Traumatic life events, 5-HTTLPR x Sexual and/or physical abuse, 5-HTTLPR x Depression, and 5-HTTLPR x Impulsivity.

5.2.2. 5-HTTLPR x traumatic life events

Three studies tested whether there was an interaction between the s-allele of 5-HTTLPR and greater number of traumatic life events to predict increased eating pathology. Of these studies, two found a significant GxE interaction (Akkermann et al., 2012; Stoltenberg et al., 2012), while the third (Karwautz et al., 2011), found a significant GxE interaction only when the measure of traumatic life events was
restricted to maladaptive parenting styles. These three studies are further discussed below.

Akkermann and colleagues (2012) aimed to determine whether traumatic life events at Time 1 interacted with the s-allele of 5-HTTLPR to predict higher levels of disordered eating three years later, in a population-representative sample of 252 adolescent females ($M = 17.8$ years, $SD = 0.5$ years). Traumatic life events before age 15 were assessed, as determined by a yes/no response to a list of 30 stressful events compiled by the authors, including sexual, physical, and emotional abuse. They found that compared to those who had experienced zero or one traumatic life events, participants who reported 6 – 18 traumatic events scored higher on the Bulimia scale of the EDI-2 (Garner, 1991) at Time 2 if they carried the s-allele ($p = .04$), but not if they were homogenous for the l-allele. The effect was not found for the EDI-2 Drive for Thinness scale. This effect remained significant when statistically controlling for depression and anxiety measured at Time 2, but not when impulsivity was controlled.

Stoltenberg et al. (2012) examined whether experiencing traumatic events in childhood (ages 0 – 12) interacted with low function 5-HTTLPR alleles to predict greater disordered eating in a college-based sample of 439 male and female young adults ($M = 22.5$ years, $SD = 6.2$). Disordered eating symptoms were measured via the EAT-26 (Garner et al., 1982), however were analysed according to a four-scale solution (self-perception of body shape, dieting, awareness of food contents, and food pre-occupation). Authors argued that this provided a better fit than the original three-factor EAT-26 solution (Ocker, Lam, Jensen, & Zhang, 2007), which included the scales dieting, bulimia and food pre-occupation, and oral control. Traumatic life events were measured retrospectively via the Traumatic Antecedents Questionnaire (Herman & van der Kolk, 1987), with participant scores classified as reflecting either a ‘low’ or a ‘high’
level of traumatic life events. The events assessed included physical abuse, sexual abuse, witnessing, and other traumas. To the knowledge of the writer, this measure does not have established psychometric properties and is not widely used in the assessment of childhood trauma. Unlike in the other studies identified by the systematic review, this study classified 5-HTTLPR as triallelic, with the lₐ/lₐ genotype considered to be the ‘high function’, while all other genotypes, including those with the lₐ allele of the long-allele, classified as ‘low function’ (i.e. lₐ/lₐ, lₐ/lₐ, lₐ/s, lₐ/s, s/s). This has been suggested to better reflect differences in in vitro 5-HTT expression (Uher & McGuffin, 2008), although it is unknown whether this should translate to differences in the GxE interaction, and findings from genetic association and GxE studies using the triallelic classification have been mixed (Parsey et al., 2006; Uher & McGuffin, 2008; Zalsman et al., 2006).

Stoltenberg et al. (2012) found that the low-expression genotype was associated with greater total EAT-26 scores in females who had experienced a ‘high’ level of abuse. Examining the four EAT-26 subscales separately (self-perception of body shape, dieting, awareness of food contents, and food pre-occupation) and using a Bonferroni corrected criterion value of $p = .012$, a similar pattern of results was reflected. For the self-perception of body shape subscale, an interaction effect was noted for the genotype x trauma interaction ($p = .012$), while for the dieting and food pre-occupation subscales, there were three-way interactions between genotype, abuse, and gender ($p = .002$ and $p = .005$). There were no significant GxE interactions on the awareness of food contents scale. A notable conclusion of this study was the clear gender difference, which found that the s-allele appeared to interact with environmental stressors to predict increased disordered eating for females only. Given the similar findings between Akkermann et al. (2012) and Stoltenberg et al. (2012), it would have been informative to know
whether the latter study would have shown a comparable pattern of results if the more common, biallelic, genotyping of the 5-HTTLPR had been assessed.

In a somewhat different study of traumatic life events, Karwautz et al. (2011) examined 128 sister pairs discordant for AN ($M = 25.55$ years, $SD = 8.4$ years), to determine whether there was an interaction between 5-HTTLPR and traumatic life events in predicting AN status. Traumatic life events were measured by the ORFI (Fairburn et al., 1998), a tool that included measures of parenting and childhood sexual and physical abuse. AN diagnosis was determined by a semi-structured interview based on DSM-IV criteria, the EATATE (Anderluh, Tchanturia, Rabe-Hesketh, Collier, & Treasure, 2009). Control sisters had no history of an ED. They found that ‘risky’ parenting styles increased likelihood of AN only for those with the s-allele ($p < .01$). However, the reporting of this result was somewhat misleading. In their publication, Karwautz et al. (2011) featured a figure displaying how risk of AN increases for those with the s-allele based on having experienced a greater number of ‘events’, while risk for those homogenous for the l-allele appears unchanged. However, a closer reading of the paper reveals that the GxE interaction was only present when problematic parenting was considered as the environmental variable, not other traumatic life events. Indeed, no other significant GxE interactions were found for the other environmental domains measured, including disruptive events (sexual and physical abuse), interpersonal problems, and dieting environment. The results of this study are therefore not strictly comparable to that of Akkermann et al. (2012) and Stoltenberg et al. (2012) as they show an interaction between 5-HTTLPR and maladaptive parenting, but not traumatic life events.

All three studies used well-established measures of eating pathology, with the Eating Disorder Inventory-2 (EDI-2; Garner, 1991) and EAT-26 (Garner et al., 1982)
showing good concurrent validity (Berland, Thompson, & Linton, 1986). However, neither Akkermann et al. (2012) nor Stoltenberg et al. (2012) used a well-established measure of trauma, such as the Childhood Trauma Interview (CTI; Fink, Bernstein, Handelsman, Foote, & Lovejoy, 1995) or Childhood Trauma Questionnaire (Bernstein & Fink, 1998), both which assess a range of inter-personal traumas during childhood, and have been demonstrated to have very good psychometric properties (Fink et al., 1995; Scher, Stein, Asmundson, McCreary, & Forde, 2001). In contrast, the two measures used in these studies did not have established psychometric properties, and indeed, the questionnaire used by Akkermann et al. (2012) was improvised by authors for the purpose of the study. However, these scales allowed the measurement of a broad range of traumatic life events throughout childhood that may have increased risk for disordered eating via an interaction with the stress-response systems in which 5-

HTTLPR is implicated.

### 5.2.2.1. Scaling of traumatic life events

One important consideration that may substantially influence the results of studies assessing traumatic life events is the scaling of this variable. A pre-requisite of testing a GxE in a scientifically rigorous manner is the need to justify the scaling of the environmental variable, and to ensure that it is robust to scale transformations (Dick et al., 2015; Uher & McGuffin, 2008). In Akkermann et al. (2012), individuals were assigned to either a ‘low’ traumatic life events group (0 – 1 events), or a ‘high’ traumatic life events group (6 – 18 events). Participants who reported 3 – 5 events appeared to be disregarded from the analysis. The justification provided in the study was that this reflected a comparison between the ‘bottom’ 25% and ‘top’ 25% of participants based on number of traumatic experiences. However, no theoretical basis is
described for this decision, nor is there any reference to past studies that may have used this cut off in analysing traumatic life events within a GxE framework. Furthermore, no justification was provided for why a dimensional scale for life events was not preferred. This is a pertinent question, given that dimensional scaling has been shown to be superior to using cut-offs and categorical variables when analysing GxE interactions (Eaves, 2006), and would have allowed for a greater sample size. Indeed, it is unclear whether this removal of the ‘middle’ 50% of participants prior to the GxE analysis is reflected in the overall sample size stated for this study. With such limited information, it is unclear whether the environmental variable is robust to scale transformation, and thus it is possible that the significant GxE finding (of borderline significance at $p = .04$) reflects a statistical phenomenon as opposed to an ecologically valid effect.

A similar issue arose in Stoltenberg et al. (2012), who required participants to respond to a series of questions (e.g., “I was beaten, kicked, or punched by someone close to me”) on a 4-point scale (from 0 = never or not at all to 3 = often or very much). This variable was then re-coded to group participants into either a ‘low’ or ‘high’ traumatic life events group, with roughly 85% and 15% of participants in the respective groups. The authors justified this cut off by stating that ‘high’ trauma is expected to be relatively uncommon, and that this distribution reflects the approximate prevalence of sexual and physical abuse identified in past research (Hussey, Chang, & Kotch, 2006). However, as in Akkermann et al. (2012), they did not explain why a categorical approach was preferred to maintaining the dimensional scaling, which is considered more desirable due to the reasons listed above.

In Karwautz et al. (2011), the four environmental domains were assessed separately, each on a dimensional scale (varying from three to six events). Three of the domains did not assess life events per se, rather assessing parenting, interpersonal
problems, and dieting environment. The one domain assessing stressful life events was on a scale from 0 to 4 (sexual abuse, physical abuse, and life event in the year prior to measurement), and therefore is not equivalent to the investigations of Akkermann et al. (2012) and Stoltenberg et al. (2012), or the traumatic life events x 5-HTTLPR line of research in the depression field, following the original study by Caspi and colleagues (2003).

5.2.3. 5-HTTLPR x Childhood sexual and physical abuse

Three studies tested whether childhood sexual, physical, or emotional abuse interacted with the s-allele of 5-HTTLPR to predict increased eating pathology (Akkermann et al., 2012; Karwautz et al., 2011; Steiger et al., 2007). One study (Akkermann et al., 2012) found a significant sexual abuse x 5-HTTLPR interaction for both the Bulimia ($p = .002$) and Drive for Thinness ($p = .03$) scales of the EDI-2 (Garner, 1991). The other two studies found no interaction between the experience of sexual abuse and presence of the s-allele in predicting AN status (Karwautz et al., 2011), as measured by the EATATE structured diagnostic interview (Anderluh et al., 2009), or in predicting disordered eating in a BN sample (Steiger et al., 2007), as measured by the EAT-26 (Garner et al., 1982). No study found a significant physical or emotional abuse x 5-HTTLPR interaction effect on eating outcomes.

Details of the studies by Akkermann et al. (2012) and Karwautz et al. (2011) are described above. In brief, both studies measured sexual, physical, and emotional abuse dichotomously, which as previously mentioned, is less favourable compared to a dimensional scale, as the latter provides the opportunity to measure abuse severity, frequency, and other relevant factors. A tool with these properties, the CTI (Fink et al., 1995), was used in Steiger et al. (2007). However, Steiger et al. (2007) included a
sample size of only 92 females ($M = 25.4$ years, $SD = 6.4$). This sample size is considered low for detecting GxE interactions (Duncan & Keller, 2011), and it is possible that the lack of significant findings may in fact reflect Type 2 error, as opposed to a true absence of a GxE interaction in the population.

5.2.4. **5-HTTLPR x Depression**

Two studies (Mata & Gotlib, 2011; van Strien et al., 2010b) assessed whether there was an interaction between depressed mood and 5-HTTLPR to predict disordered eating. Both studies reported a significant interaction. Van Strien and colleagues (2010b) assessed a population representative sample of 584 Dutch adolescent siblings, separately testing 286 younger siblings ($M = 13.4$ years, $SD = 0.6$) and 298 older siblings ($M = 15.2$ years, $SD = 0.5$) at two time points across four years. They assessed disordered eating using the emotional eating subscale of the Dutch Eating Behaviour Questionnaire (DEBQ; van Strien, Frijters, Bergers, & Defares, 1986), which is reported to have high reliability and convergent and discriminative validity (Dakanalis et al., 2013; Wardle, 1987). Depression was measured by the Depressive Mood List (Kandel & Davies, 1982). This tool measures the endorsement of symptoms of depression over the past 12 months (e.g., “How often did you feel too tired to do things?”), with answers to six questions coded on a 3-point scale in the present study ($1 = never/almost never, 2 = sometimes, 3 = often/always$).

They found that presence of the s-allele interacted with depressed mood to predict a change in emotional eating over the four years for both younger and older siblings. This interaction remained significant when controlling for the possible confounding effects of personality, parental emotional eating and overweight status, and restrained and external eating styles. Interestingly, the direction of the effect differed between boys and
girls amongst older siblings, with females with the s-allele displaying a positive association between depressive feelings and greater emotional eating, while this association was significant in a negative direction for boys.

Mata and Gotlib (2011) tested a convenience sample of 50 females ($M = 13.9$ years, $SD = 1.9$) of mixed background (including Caucasian, Hispanic, and Asian American) at two time points across six months. Disordered eating of a bulimic nature was quantified by the overeating subscale of the EDI for Children (Garner, 1991), which has been found to be a reliable and valid measure of childhood disordered eating (Eklund, Paavonen, & Almqvist, 2005; Gustafsson, Edlund, Kjellin, & Norring, 2010; Thurfjell, Edlund, Arinell, Hägglöf, & Engström, 2003). Depression was measured via the short form of the Children’s Depression Inventory (Kovacs, 1985), a 10 item tool measured on a 3-point scale, which asks participants to respond to questions assessing their feelings (e.g., sadness, irritability, loneliness) over the preceding two week period, and has solid psychometric properties as a screening tool for depressed mood (Allgaier et al., 2012).

Mata and Gotlib (2011) found a significant interaction between change in depressive symptoms and the s/s genotype to predict change in bulimic symptoms 6 months later ($p = .02$). Unlike in previous studies finding significant 5-HTTLPR x environment effects, and the results of van Strien et al. (2010b)’s 5-HTTLPR x depression analysis, there were no differences between the s/l and l/l genotypes. Given that the s-allele of 5-HTTLPR is believed to function in a dominant rather than additive manner (Lesch et al., 1996), this finding is unusual. Furthermore, it is unclear why the results of Mata and Gotlib (2011) and van Strien et al. (2010b) should differ in this manner, given that they tested the same polymorphism, the same psychological variable, and a very similar outcome (emotional eating and overeating). Indeed, given
that Mata and Gotlib (2011) contained a very small sample size (far beyond the desired \( N = 600 \) approximated by Duncan and Keller, 2011), it may be that the present interaction effect involving just the s/s but not the s/l genotype represents a statistical artefact, as opposed to a genuine reflection of the population trend. Indeed, numerous studies and published reviews have argued that many significant GxE interactions identified in the psychiatry literature may in fact represent false positives due to insufficient sample size (Duncan & Keller, 2011; Duncan et al., 2014; Luan, Wong, Day, & Wareham, 2001), an issue that will be discussed in further depth in the latter parts of this chapter. A final issue with the study of Mata and Gotlib (2011) is their failure to use a racially homogenous sample, or control for race as a possible confounding factor. This is a major limitation, due to possible population stratification, whereby the different allele frequencies present in individuals with diverse ancestry may confound the results of the GxE interaction analysis (Cardon & Palmer, 2003; Freedman et al., 2004).

### 5.2.5. 5-HTTLPR x Impulsiveness

One study, Racine et al. (2009), investigated whether the variables of impulsiveness or dietary restraint moderated the relationship between 5-HTTLPR and disordered eating in a college sample of 344 females (\( M = 19 \) years, \( SD = 1.4 \)). This study also tested the T102C polymorphism of 5HT2A, another genetic variant implicated in serotonin production that has been investigated in many GxE interaction studies in psychiatry (Antypa, Serretti, & Rujescu, 2013; Jokela et al., 2007; Mandelli & Serretti, 2013). Impulsiveness was assessed using the well-established Barratt’s Impulsiveness Scale (Patton et al., 1995), while dietary restraint was measured by a composite of the dietary restraint subscale of the DEBQ (van Strien et al., 1986) and the Eating Disorders
Examination - Questionnaire (EDE-Q; Fairburn & Cooper, 1993). The study assessed two disordered eating outcome variables, emotional eating, as measured via the emotional eating subscale of the DEBQ (van Strien et al., 1986), and binge eating, as measured by the binge eating subscale of the Minnesota Eating Behaviour Survey (MEBS; von Ranson, Klump, Iacono, & McGue, 2005). The psychometric properties of the DEBQ are discussed in the preceding segment, while the MEBS has been shown to be a reliable and valid measure (Klump, McGue, & Iacono, 2000; von Ranson et al., 2005).

There were no significant GxE interactions between any genotype and psychological factor in predicting scores on the DEBQ or the MEBS, including when participant BMI was included as a covariate. It should be noted that this analysis, along with the investigations of the 5-HTTLPR x depression interaction, is not strictly speaking, a pure ‘GxE’ interaction. This is because personality traits, and to a lesser extent, psychopathology, are heritable (Krueger, South, Johnson, & Iacono, 2008), and thus these analyses may also involve unmeasured GxG interactions (Trace et al., 2013). Nonetheless, a significant GxE interaction identified by a study measuring a personality trait or psychopathology provides valuable information regarding the potential modifiers of genetic risk for developing an ED or engaging in disordered eating behaviour.

5.2.6. Studies identified in the review measuring other genes (non-5-HTTLPR)

A total of six studies (Akkermann et al., 2011; Groleau et al., 2012; Steiger et al., 2011; Steiger et al., 2012; van Strien et al., 2015; van Strien, Snoek, van der Zwaluw, & Engels, 2010) investigated GxE interactions on an ED-related outcome assessing
polymorphisms other than those from the 5-HTT and 5-HT2A genes (these included DRD2, DRD4, BDNF, NR3C1, DAT1, and COMT). The results of each study and evaluation of the measures used is discussed below.

5.2.6.1. Dopamine-system polymorphisms

van Strien et al. (2010a) conducted a longitudinal investigation into whether the dopamine D2 receptor DRD2 gene Taq1A polymorphism (rs1800497) interacted with maternal and paternal psychological control to predict greater emotional eating four years later, in a cohort-based sample of 276 adolescents (55.2% female, M = 13.4 years, SD = 0.6). DRD2 is a gene involved in the dopamine system, and is hypothesised to be associated with emotional eating due to links with reward sensitivity, which is also implicated in various ED-related symptoms, such as binge eating (Davis et al., 2008; Volkow et al., 2003). The Taq1A polymorphism consists of the A1 and A2 alleles, with A1 considered the low-function variant (Jönsson et al., 1999). As in their study of the 5-HTTLPR polymorphism, emotional eating was measured via the emotional eating subscale of the DEBQ (van Strien et al., 1986). Parenting was measured by a scale devised by Steinberg, Lamborn, Darling, Mounts, and Dornbusch (1994), which authors described as common in Dutch studies (Van Zundert, Van Der Vorst, Vermulst, & Engels, 2006), with Cronbach’s alpha α = .65 for maternal psychological control and α = .70 for paternal psychological control. Both parenting and eating variables were measured at the initial baseline (T1) and at a four year follow-up (T2).

van Strien et al. (2010a) found that the A1 allele of DRD2 interacted with both maternal and paternal psychological control, as measured during T1, to predict increased emotional eating at T2 (p < .05). There were no differences noted according to gender. The interaction with both maternal and paternal psychological control
remained significant after controlling for an extensive list of confounders, including depressive feelings, education, BMI, personality, parental behavioural control, and parental emotional eating and overweight status.

One caveat of these findings is that Cronbach’s alpha was in the ‘questionable’ range for the measure of parental psychological control by Steinberg et al. (1994) used in the present study (α = .65 for maternal psychological control, and α = .70 for paternal psychological control). The high error variance implicit in this measure suggests that there may be a large discrepancy between participant scores on this scale and their ‘true score’ for the construct (Anastasi & Urbina, 1997). An implication of using such an unreliable measure of parental psychological control is that any significant results may be spurious. Given that the present study is already in the ‘underpowered’ range for detecting a GxE interaction (Duncan & Keller, 2011), the further addition of a high level of measurement error is particularly problematic (Burton et al., 2009; Wong, Day, Luan, Chan, & Wareham, 2003). This initial finding is nonetheless promising, particularly given its robustness in light of numerous control variables, although validation is necessary in an independent study using a larger sample size and a measure of parental psychological control with excellent psychometric properties.

Also investigating the dopamine system, Groleau et al. (2012) tested GxE interactions between a number of polymorphisms, including the *DRD2* Taq1A (rs1800497), dopamine D4 receptor (*DRD4*) 48 base-pair variable number tandem repeat (VNTR), dopamine transporter (*DAT1*) 40 base-pair VNTR, and a *COMT* polymorphism (rs4680) and the experience of childhood sexual, physical, or emotional abuse, in predicting average number of binge eating episodes per month in a sample of 216 women (*M* = 25.9 years, *SD* = 6.7) with BN-spectrum EDs. Like *DRD2*, the *DRD4*, *DAT1*, and *COMT* genes are also implicated in the dopamine system, which is
believed to be related to EDs via its effects on reward sensitivity (Davis et al., 2008; Volkow et al., 2003). Abuse was measured by the CTI, a semi-structured interview with good psychometric properties (Fink et al., 1995), and coded as present or absent for each trauma type. This measure was tested for inter-rater reliability in a sub-sample of participants and resulted in $\kappa = 0.77$ for emotional abuse, $\kappa = 0.84$ for physical abuse, and $\kappa = 0.93$ for sexual abuse. Binge eating episodes were measured via the EDE interview (Fairburn & Cooper, 1993), considered the ‘gold standard’ for assessing EDs. Within this measure, frequency of binge eating episodes is self-reported during the interview, with very good test-retest and inter-rater reliability (Rizvi, Peterson, Crow, & Agras, 2000). Each of the four polymorphisms were analysed separately with each type of abuse, and no significant interaction effects on binge eating were found.

A possible limitation of this study is that Groleau and colleagues (2012) tested only one element of bulimic symptomatology in an exclusively clinical BN sample. It may be the case that the examined dopamine polymorphisms do interact with the experience of abuse to predispose to bulimic symptoms, however the effect of this interaction may not be reflected solely through frequency of binge eating episodes. Assessing broader disordered eating symptoms and including a community control group would capture a wider spectrum of eating pathology and provide an outcome variable that may better capture any GxE interactions present. An example of an appropriate measurement tool is the bulimia scale of the EAT-26 (Garner et al., 1982), which measures the behavioural elements of bulimic-spectrum pathology that may also be associated with reward-sensitivity and the dopamine system.

In a final paper examining dopamine system polymorphisms, van Strien et al. (2015) investigated whether a 48 base-pair VNTR in \textit{DRD4} interacted with season of birth (spring or fall) to predict emotional eating in two different samples across two
studies. One sample consisted of 93 university students (58% female, $M = 20.7$ years, $SD = 2.6$) while the other featured 586 Dutch parents from a population-representative longitudinal study (51% female, $M = 44.9$, $SD = 3.9$). Emotional eating was measured using the emotional eating scale of the DEBQ (van Strien et al., 1986). Two ‘risk’ genotypes were tested separately: in a first analysis, presence of the 7-repeat (7R) allele was considered the ‘risky’ genotype, compared to no 7R allele, while in the second analysis, presence of the 7R or 2-repeat (2R) allele was considered ‘risky’, compared to not carrying the 7R or 2R allele. The authors describe that season of birth may be a risk factor for eating pathology as changes in sunlight and diet may influence early brain development and increase risk for later psychopathology. They also cited two initial studies identifying a gene x season of birth interaction in predicting differences in BMI (although the ‘risk’ season was spring birth in one study and fall birth in the other; Levitan, Kaplan, Davis, Lam, & Kennedy, 2010; Levitan et al., 2006). van Strien et al. (2015) defined the environmental factor in their present study as both spring birth versus birth in any other season, and fall birth versus any other season, based on the rationale that, in the Netherlands, the former involves a very large number of hours of sunlight (up to 17 hours per day) while the latter features very few hours of sunlight (as low as 4 hours per day).

In the first, smaller sample ($N = 93$) featuring university students, there were no significant GxE interactions between either of the genotype classifications and ‘risk’ seasons on emotional eating. There was, however, an interaction between sex and genotype, such that females with the 7R or 2R alleles were more likely to report disordered eating, an effect not present for males. In the second sample ($N = 586$) featuring parents, there was an interaction between presence of the 7R and/or 2R allele and birth during fall (October to December) to predict greater emotional eating in
females ($p = .04$). Interestingly, this interaction was also significant in males, however predicted lower levels of emotional eating ($p = .03$). These initial results show promise, however, as with van Strien et al. (2010a), replication is necessary in an independent sample. It is also desirable to clarify which season(s) of birth constitute a ‘risk’ factor, and to base this assertion upon sound theoretical and empirical grounding.

### 5.2.6.2. Other polymorphisms: Bcl1

Steiger et al. (2011) tested whether the c-allele of Bcl1 in *NR3C1* (rs41423247) and experiencing either sexual or physical abuse interacted to increase risk of BN. Their sample included a clinical BN group ($n = 129, M = 25.2$ years, $SD = 5.4$) and a control group ($n = 98, M = 24.2$ years, $SD = 5.7$), both comprising young adult women. The *NR3C1* gene is involved in the HPA axis, which plays a key role in human stress response (Wüst et al., 2004), with the low-function c-allele believed to result in increased cortisol responses to a psychological stressor (Kumsta et al., 2007). As in their previous studies, abuse was measured via the well-established structured interview, the CTI (Fink et al., 1995), and coded as present if participants endorsed either sexual or physical abuse, and as absent if no abuse was reported. BN status was confirmed using the EDE (Fairburn & Cooper, 1993). The authors identified a significant interaction between the c-allele and presence of abuse in predicting BN group membership ($p < .05$). This interaction was no longer significant when controlling for lifetime major depressive disorder, although controlling for anxiety disorders and post-traumatic stress disorder did not affect the significant GxE interaction.

In a sample containing approximately 75% of the participants featured in Steiger et al. (2011), Steiger et al. (2012) aimed to expand on this result by investigating how the
interaction between Bcl1 and abuse was moderated by impulsivity, sensation seeking, affective instability, and depression. These variables were selected as they represent traits that are associated with BN (Fischer et al., 2008), and due to early evidence that these traits may be amplified via a genetic predisposition (Paaver, Kurrikoff, Nordquist, Oreland, & Harro, 2008; Wagner, Baskaya, Lieb, Dahmen, & Tadić, 2009). The expanded sample consisted of 174 females with BN ($M = 26.0$ years, $SD = 6.4$) and 130 controls ($M = 24.8$, $SD = 6.3$), with the same measures of abuse and BN status used as in Steiger et al. (2011). Stimulus seeking and affective instability were measured via the Dimensional Assessment of Personality Pathology – Basic Questionnaire (DAPP-BQ; Livesley, Jackson, & Schroeder, 1992), while the motor impulsivity scale of BIS (Patton et al., 1995) was used to assess impulsivity. Depressed mood was assessed via the Centre for Epidemiological Studies Depression scale (Weissman, Sholomskas, Pottenger, Prusoff, & Locke, 1977). These measures all have very good to excellent reliability and construct validity (Stanford et al., 2009; Tromp & Koot, 2008).

As in their previous study, Steiger et al. (2012) reported a significant Bcl1 x abuse interaction in predicting BN status ($p < .05$), which became non-significant when controlling for depressed mood, but was not attenuated when controlling for motor impulsivity, affective instability, or sensation seeking. These results suggest that the genetic vulnerability conferred by the Bcl1 may also be associated with depression, which is not surprising given the role of the HPA axis in depression (Belmaker & Agam, 2008), but that it is largely independent of behavioural correlates of BN, including impulsivity, sensation seeking, and affective instability.
Lastly, Akkermann et al. (2011) examined whether the Met-allele of Val66Met on the Brain Derived Neurotrophic Factor (BDNF [rs6265]) gene is related to greater likelihood of disordered eating symptoms in individuals who exhibit higher levels of food restriction, compared to those with the Val/Val genotype. BDNF regulates synaptic activity and is abundant in regions of the brain believed to play a role in regulation of energy intake and eating behaviour (Kernie, Liebl, & Parada, 2000), with suggestion that mutations in the BDNF gene result in disturbed eating behaviour and obesity (Yeo et al., 2004). Akkermann et al. (2011)’s cohort-based sample consisted of 765 participants (55.7% female, $M = 16.1$ years, $SD = 1.5$), with disordered eating symptoms assessed via the Bulimia and Drive for Thinness scales of the EDI-2 (Garner, 1991). Food restriction was measured via a self-reported food record kept over two days followed by an interview with researchers to corroborate this record. They found the Met-allele interacted with both reduction of meal frequency ($p = .02$) and starvation ($p = .02$) to predict increased bulimic behaviours and attitudes in females only, an effect which remained significant when controlling for BMI.

A major strength of this study is the sample size used, which is the largest of any of the reviewed studies. This large sample sizes reduces the chances that the GxE effect identified may represent a spurious result, as is argued to be likely for many under-powered studies finding significant GxE results (Duncan & Keller, 2011). One caveat is that the measurement of food restriction was not ideal. The reliability of food diaries increases with the number of days a participant is required to maintain the diary, and the 48 hours period used in Akkermann et al. (2011) is considered below the adequate length of time for reliable measurement of nutrient intake (Schlundt, 1988). It should
also be noted that by measuring dietary restraint as the ‘environmental’ factor, results may in fact reflect a GxG interaction, as in some of the previous GxE analyses described.

Finally, since the publication of Study 1, one further study investigating a GxE interaction with an ED-related outcome has been published. Micali, Crous-Bou, Treasure, and Lawson (2017) investigated the interaction between maternal care and the oxytocin receptor gene (OXT-R) polymorphisms rs53576 and rs2254298 (which play a role in reproduction and offspring bonding; Gimpl & Fahrenholz, 2001) on disordered eating outcomes in the largest sample tested to date (N = 3,135 females, M = 29.6 years, SD = 4.4). Disordered eating behaviours included any lifetime history of purging, restrictive eating, and binge eating, as measured by the Structured Clinical Interview for DSM-IV-TR disorders (SCID-1; First, Spitzer, Robert, Gibbon, & Williams, 2002) and the ED version of the Longitudinal Interval Follow-up Evaluation interview (Anderluh, Tchanturia, Rabe-Hesketh, Collier, & Treasure, 2009; Keller et al., 1987), and was assessed to have high sensitivity (97.3%; Micali et al., 2017c). Maternal care was assessed via the PBI (Parker et al., 1979) maternal care subscale, which is discussed in depth in Chapter 2 of the present thesis and is a valid and reliable measure of paternal care (Wilhelm, Niven, Parker, & Hadzi-Pavlovic, 2005).

Results showed main effects of lower maternal care on greater likelihood of reporting binging and purging behaviours. Results overwhelmingly did not support an interaction with either OXT-R polymorphism in predicting disordered eating outcomes, with no evidence of a GxE between maternal care and the two polymorphisms identified across six separate regression models (examining the outcome variables restrictive eating, binging, and purging). However, authors then conducted post-hoc analyses by changing the definition of the outcome variables to create three new variables: binging
in the absence of purging, purging in the absence of binging, and both binge eating and lifetime purging. When adding these six additional regression models (12 in total), one model (rs2254298 x poor maternal care to predict lifetime binge eating and purging) returned a statistically significant finding ($p = .03$). Authors used this to argue a GxE interaction was identified in their study, however this finding is majorly limited by the post-hoc multiple testing with no Bonferonni correction (which would have changed the interpretation of their findings) or acknowledgment of ‘data mining’. Although the study by Micali et al. (2017a) constitutes the largest GxE interaction analysis in the ED field to date, which is an important strength, the inclusion of multiple GxE interactions and post-hoc testing is a key limitation. Authors also did not properly control for covariates as per Keller (2014).

5.3. Quality evaluation of studies identified in the systematic review

The following section will present the results of a systematic quality evaluation of the studies identified by the review. It will then discuss the numerous issues pertaining to study quality that are relevant to all studies identified by the review, with primary focus on problems related to insufficient sample sizes and inadequate statistical control of possible confounding variables.

Table 8 presents the results of the systematic quality evaluation using the Checklist for Methodological Quality, a structured tool by Downs and Black (1998). This table is replicated from Study 1 with the addition of seven papers investigating genes other than 5-HTT. As explained in Study 1, the criteria below were adapted to best assess GxE interaction research in EDs, as in its original form the Checklist for Methodological Quality was designed to assess systematic reviews of clinical trials. As a result, 14 non-applicable criteria were excluded. The items in the present assessment are as follows:
1) Clear description of the hypothesis/aim/objectives; 2) Clear description of main outcomes in introduction/method; 3) Characteristics of participants clearly described (to a level appropriate for GxE and ED research); 4) Clear description of main findings; 5) Characteristics of participants lost to follow-up described; 6) Exact probability values reported, e.g., $p = .035$ (or confidence intervals used as estimate of population parameter); 7) Participants representative of population (including clinical samples, but not convenience samples); 8) Any “data dredging” in results explicitly noted; 9) Appropriate statistical tests used; 10) Main outcome measures valid and reliable; 11) Participants in different groups (if case-control study) recruited over same time period; 12) Adequate adjustment for (potential) confounding variables (e.g., BMI; according to Keller [2014], this requires inclusion of all covariate x gene and covariate x environmental factor interaction terms in the model); and 13) Sufficient power (to detect a GxE interaction, as guided by Duncan and Keller, 2011).

Table 8

*Downs and Black (1998) Checklist for Methodological Quality, Adapted to Evaluate Studies Identified in a Systematic Review of the Role of Gene x Environment Interactions in Risk for Eating Pathology*

<table>
<thead>
<tr>
<th>Study</th>
<th>Gene</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steiger et al., 2007</td>
<td>5-HTT</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Racine et al., 2009</td>
<td>5-HTT/5HT2a</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>X</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mata &amp; Gotlib, 2012</td>
<td>5-HTT</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>van Strien, et al., 2010a*</td>
<td>DRD2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>van Strien, et</td>
<td>5-HTT</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Study</td>
<td>Gene</td>
<td>Criteria Achieved</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------</td>
<td>-------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karwautz et al., 2012</td>
<td>5-HTT</td>
<td>1 1 1 1 N/A 1 1 1 1 1 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steiger et al., 2011*</td>
<td>NR3C1</td>
<td>1 1 1 1 N/A 1 1 1 1 1 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akkermann et al., 2011*</td>
<td>BDNF</td>
<td>1 1 0 1 0 1 1 1 1 N/A 0 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akkermann et al., 2012</td>
<td>5-HTT</td>
<td>1 1 1 1 0 1 1 1 1 N/A 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steiger et al., 2012*</td>
<td>NR3C1</td>
<td>1 1 1 1 N/A 1 1 1 1 X 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grof et al., 2012*</td>
<td>DRD2, DRD4, DAT1, COMT</td>
<td>1 1 1 1 N/A 1 1 1 1 N/A 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stoltenberg et al., 2012</td>
<td>5-HTT</td>
<td>1 1 1 1 N/A 1 0 1 1 1 N/A 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Strien et al., 2015*</td>
<td>DRD4</td>
<td>1 1 1 1 N/A 1 0 1 1 1 N/A 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micali et al., 2017*</td>
<td>OXT-R</td>
<td>1 1 1 1 0 1 1 0 1 1 N/A 0 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Notes. 1 = Criteria met, 0 = Criteria not met. X = Unable to determine. N/A = Criteria not applicable. Analyses of van Strien et al. (2015) conducted separately for Study 1 and Study 2. *Not included in publication

Results of the systematic analysis reveal that the two biggest issues across all studies were their failure to adequately control for confounding variables, and use of insufficiently large sample sizes. These issues will be discussed in greater depth below. Otherwise, the studies in general used good measurement techniques and designs to assess effects of the GxE interaction on ED-related outcomes. Some exceptions were that a number of the studies did not describe characteristics of participants who were lost to follow-up (Akkermann et al., 2011; Akkermann et al., 2012; Mata & Gotlib, 2011; Micali et al., 2017a), thus increasing the possibility of a non-representative
sample. Similarly, a number of studies used convenience samples of college students (Mata & Gotlib, 2011; Racine et al., 2009), which somewhat limits the generalisability of results. Finally, it would have been prudent for van Strien et al. (2010a) and van Strien et al. (2010b) to include exact probability values for greater clarity in the reporting of their findings.

5.3.1. Sample size in gene x environment interaction studies

Across almost all studies identified in the review, low sample size was a problem. As discussed in Study 1, insufficient sample size is a key limitation of current GxE studies, in both the ED field and psychiatry more broadly. Current GxE interaction studies in psychology are believed to be statistically underpowered and thus not equipped to reliably detect significant GxE interactions. This issue has been suggested to increase risk of not only false negative findings (Type 2 error), but also to increase risk of false positive findings (McClelland & Judd, 1993; Burton et al., 2009; Button et al., 2013).

It is difficult to quantify the precise sample size required for sufficient power in a given analysis, which is determined by factors such as measurement error, correlation between variables, the level of variance in the outcome variable accounted for by the genetic and environmental variables, and the effect sizes believed to be involved (Duncan & Keller, 2011). Duncan and Keller (2011) provide a rough estimate that to detect a large interaction effect with 80% power, and assuming no measurement error is present, a minimum sample size of 600 is required. In the presence of measurement error, or small to moderate effect sizes, this estimate increases substantially. In the present systematic review, the mean sample size of all studies identified was 320. While this is considered a large sample size for a study in the ED field, it is insufficiently large
for genetic analyses. These sample size requirements are particularly challenging given the resource-heavy nature of obtaining genetic samples. They also require thorough planning from researchers in the ED field, who are accustomed to working with the far smaller sample sizes that typify research in psychology (Luan et al., 2001). Future studies should aim to prioritise available resources to maximise sample sizes before analysing GxE interactions, and studies with low numbers of participants should explicitly note this as a limitation, and regard any significant findings as tentative and requiring replication. Indeed, it may be the case that GxE interaction research predominantly falls to large-scale projects (e.g., heavily funded population-representative research initiatives), which are sufficiently well-equipped to conduct this type of investigation. Another feasible option could involve collaboration between research groups, with use of pre-specified common measures, in order to achieve the necessary sample sizes. Finally, for this type of progress to be achieved, it is vital for journals to favour studies using large samples and possibly enforce minimum sample sizes.

5.3.2. Controlling for the effect of confounders

As outlined in Study 1, another major limitation of all studies included in the review is that they did not include adequate statistical control of potential confounding variables. According to Keller (2014), including the variable one wishes to control for (e.g., age) as a covariate in a general linear model (e.g., a regression equation) controls for the effects of this variable only on the model’s main effects. To adequately control for the effect of this variable on the interaction term, it is also necessary to include the covariate x gene and covariate x environment terms. In the case of controlling for multiple variables, one must also include the covariate x covariate contrast term. No
study included in this review controlled for possible confounds in this manner, thus suggesting that none of the studies properly accounted for the effect of confounding variables.

Failing to control for covariates according to this method leaves open the possibility that there are alternative explanations for the observed GxE interaction effects identified by the aforementioned studies. This problem appears to be widespread amongst research investigating candidate GxE effects, with Keller (2014) finding that out of 47 novel GxE interaction studies identified in a review by Duncan and Keller (2011), 45 studies reported statistically significant findings, however no study properly controlled for potential confounders. This alarming finding suggests that very few studies published prior to 2014, if any at all, properly controlled for the effects of variables such as age, gender, socioeconomic status, and so on, on the GxE interaction. As of July 2017, Keller’s paper has been cited 165 times since its publication in 2014, suggesting that candidate GxE studies are beginning to adopt this corrected manner of controlling for covariates. It would also be prudent for studies published prior to Keller (2014) to re-assess their results using this corrected model, in order to demonstrate whether the GxE interactions reported in their papers remain significant upon proper statistical control of possible confounding factors.

### 5.3.3. Further statistical limitations

A number of other common limitations of the studies identified in the review pertain to their use of statistical methods. As mentioned in Study 1, numerous studies (Karwautz et al., 2011; Mata & Gotlib, 2011; Racine et al., 2009) examined 5-HTTLPR according to genotype (i.e., comparing the effects of s/s genotype, s/l genotype, l/l genotype in the same analysis) rather than comparing those with the ‘risky’ s-allele (s/s
genotype or s/l genotype) to those without this allele (l/l genotype). These studies utilised contrast terms (i.e. genotype x environmental factor) in general linear models (i.e., regression or analysis of variance [ANOVA]) to test whether the GxE significantly predicts a change in the ED-related outcome variable). However, Aliev, Latendresse, Bacanu, Neale, and Dick (2014) have recently suggested this is a statistically flawed method of analysing GxE interactions where the genotype does not have a binary coding. The inclusion of a GxE contrast term where the gene has more than two groups is likely to result in either false positive or false negative findings. Furthermore, evidence suggests that the s-allele acts in a functionally dominant, rather than additive manner, with the presence of one s-allele enough to reduce 5-HTT mRNA transcription and serotonin reuptake compared to those with the l/l genotype (Lesch et al., 1996). Together, these studies suggest that it is preferable to analyse 5-HTTLPR only using the two-group, ‘s-allele present’ versus ‘s-allele absent’ coding, which many of the studies identified in the review did not adhere to (e.g., Mata & Gotlib, 2011; Steiger & Bruce, 2007).

Another statistical consideration pertained to whether the analyses selected by authors measured an additive or multiplicative model of statistical interaction (Rothman, Greenland, & Walker, 1980). Testing an ‘additive’ model of interaction occurs when authors use, for example, a multiple linear regression model with a continuous outcome variable, or, complete additional data analysis (as conducted in Study 1). This model assumes that interaction effects may be modelled in an cumulative manner (e.g., outcome = G risk + E risk). Meanwhile, assessing a multiplicative model of statistical interaction (as commonly tested in epidemiology due to case-control designs involving binary outcomes; Schwartz, 2006) involves the use of logistic regression models. Under the multiplicative approach, risk is modelled as a product
(e.g., outcome = G risk x E risk). Both models are considered plausible means of testing GxE interactions (Ottman, 1996), although can produce somewhat varied findings in the same data sets. It is therefore beneficial for authors to include data analysed under both models, in order to increase transparency and gather the most complete evidence available regarding the GxE interaction under examination.

The issue of multiple testing is another common limitation of many of the studies in the present review. This was evident in numerous studies, which tested several different genetic variants with several different environmental and/or outcome variables (e.g., Racine et al., 2009, who tested the interaction between two polymorphisms with two psychological factors on two disordered eating measures; Groleau et al., 2012, who tested the interaction between four polymorphisms and three environmental factors; and van Strien et al., 2015, who tested two separate groupings of the polymorphism under investigation and two environmental factors). These numerous, repeated analyses, increase the risk of obtaining a false positive result, which is a prevalent issue in many GxE interaction studies (Munafò & Flint, 2009). Indeed, testing randomly-generated data sets, Sullivan (2007) simulated candidate gene association analyses using various genotype groupings and multiple polymorphisms, and found that in 90% of cases a nominally significant, and potentially publishable, result was found. To minimise such spurious results, it is important for GxE studies to perform only a limited number of pre-planned analyses, with use of genetic variants and environmental factors with strong theoretical grounding.

5.4. Discussion of publication bias

A factor vital to consider during the interpretation of systematic reviews is the possibility that findings are affected by publication bias. Publication bias refers to the
greater tendency for positive findings to be published compared to null results (i.e., the file draw problem; Rosenthal, 1979). Publication bias can act both at the level of the researcher (Easterbrook, Gopalan, Berlin, & Matthews, 1991), who may be more likely to submit positive results while disregarding null findings, and at the level of journals, which are often more likely to accept papers containing significant findings and reject those that do not (Higgins & Green, 2008). As a result of this preference for positive findings, researchers may be more inclined to mine data for significant findings in favour of conducting only planned analyses using pre-determined statistical methods (Ioannidis, Munafo, Fusar-Poli, Nosek, & David, 2014). This is because positive results are viewed more favourably than null results, and studies believed to contribute ‘novel’ findings are preferred over studies that ‘just’ aim to replicate previous research (Dick et al., 2015). Indeed, in a highly-cited but somewhat controversial paper, Ioannidis (2005) argues that the majority of published research does not reflect true findings, due to biasing factors such as multiple testing, reliance on $p$-values, undue weight given to small effect sizes, use of insufficiently large samples, large flexibility in a study design, variables, or outcome measures, financial interests or prejudice of researchers, and whether the research is considered a ‘hot’ topic.

Some researchers argue that publication bias plays a large role in the genetic literature, with a substantial number of initial findings failing to replicate (Duncan et al., 2014; Ioannidis, Ntzani, Trikalinos, & Contopoulos-Ioannidis, 2001). In a review by Duncan and Keller (2011), authors reported that while 96% of initial published GxE interaction findings in psychiatry were significant, this was true of only 27% of studies that attempted to replicate those initial findings. Importantly, it is likely that these replication attempts were also affected by publication bias.
A pertinent example of the wide impact of this issue involves a seminal study of GxE interactions in psychiatry by Caspi et al. (2003), who reported a significant interaction effect between stressful life events and 5-HTTLPR in predicting depressive symptoms. This paper has been heavily cited (7675 citations as of July 2017), and dozens of replication attempts have ensued, which due to the genetic component of the research is a resource-heavy endeavour. Five meta-analyses on this topic have been published to date. Three of these meta-analyses (Culverhouse et al., 2017; Munafo et al., 2009; Risch et al., 2009) failed to find a significant interaction, while two meta-analyses reported significant findings (Karg et al., 2011; Sharpley et al., 2014). However, the latter two meta-analyses have been criticised for being too liberal in their inclusion criteria (e.g., including studies with outcome measures other than depression, and studies with incompatible statistical and genetic models; Culverhouse et al., 2017), with one study by Duncan et al. (2014) showing evidence of substantial publication bias that may account for the positive findings identified in Karg et al. (2011).

The vast popularity of this line of investigation, despite continued mixed findings, illustrates how a research question may receive extensive attention and ‘hype’ on the basis of a small number of early significant results. If these particular early papers were more readily published due to their positive or ‘interesting’ findings, as is argued to be the case in molecular genetic research (Ioannidis & Trikalinos, 2005), while similar early studies with null findings were not published or not submitted for publication, it may be the case that the ‘force’ of publication bias has insidiously lead to the expenditure of a large quantity of resources in exploring a topic where, ultimately, the GxE interaction is tenuous. With a lower level of initial publication bias, it is possible that these resources could have been directed to develop other, possibly more fruitful, lines of inquiry. While the view that publication bias accounts for the vast majority of
significant GxE findings remains controversial (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Monroe & Reid, 2008), it is worthwhile taking note of these criticisms and learning from possible mistakes in the depression field, in order to avoid repeating such errors and misallocating finite resources in future ED research.

5.4.1. Publication bias in the present systematic review

It is thus important to consider to what extent the results of the present systematic review are influenced by publication bias. This is typically assessed via funnel plots (Light, Pillemer, & Wilkinson, 1984), which plot each study according to sample size and effect size, using the logic that studies with the largest sample size will form the top of the funnel, with smaller studies symmetrically distributed at the base, creating an inverted ‘v’ shape plot. An asymmetrical distribution would indicate publication bias, although judging asymmetry is considered a subjective task (Duval & Tweedie, 2000).

A barrier to this conventional method of assessing publication bias in the present combined samples meta-analyses is that the use of funnel plots is not recommended when the number of published studies is low (Sterne et al., 2011). The present chapter will therefore examine publication bias with guidance from a number of informal strategies described in Ioannidis et al. (2014) and used in Duncan and Keller (2011), which provide indirect evidence of publication bias.

Firstly, in light of the assertion that researchers are more inclined to submit positive findings, and journals more likely to accept them, it is worthwhile to compare the proportion of published positive findings compared to null results in the 14 studies identified through the review and the subsequently published paper by Micali et al. (2017a). Unfortunately, none of these studies sought to replicate previously identified significant GxE interactions, so it was not possible to compare the number of significant
GxE interactions in initial reports compared to replication attempts, as was done in Duncan and Keller (2011).

As evident in Table 9, from a total of 15 studies, 12 reported at least one significant GxE interaction with an ED-related outcome, and a 13th study (Steiger et al., 2007), reported significant GxE interactions on non-ED outcome variables. Thus, only two (Groleau et al., 2012; Racine et al., 2009) of the 15 identified published studies did not contain ‘positive’ findings.

There was also evidence of a high number of GxE interactions tested per study ($M = 6.4$ interactions), with a small portion of these reported as significant ($M = 1.6$ interactions). Importantly, the total number of GxE interactions tested refers to only ‘unique’ GxE combinations, however many of these were tested a number of times with the exclusion/inclusion of numerous potential confounding variables. The large number of interactions tested in each study indicates that their findings may have been influenced by multiple testing, whereby numerous analyses are conducted in search of ‘significant’ findings. Indeed, most studies drew conclusions based on one or two significant GxE interactions from a longer list of non-significant findings. It is not possible to measure how many further non-significant analyses were conducted but not reported in the final published manuscripts. Furthermore, of the 24 identified significant interactions identified across the 15 studies, only five were significant at $p < .01$ (three from the same study; Stoltenberg et al., 2012), with most reported at $p < .05$. Effect sizes were small to medium at best.
Table 9

*Number of Gene x Environment Interactions Tested in Each Study Identified in the Review and the Number of Significant Interactions Identified.*

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample size</th>
<th>No. GxE interactions tested</th>
<th>No. GxE interactions significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micali et al. (2017)</td>
<td>3135</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Akkermann et al. (2011)</td>
<td>765</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>van Strien (2015), Study 2</td>
<td>586</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>van Strien et al. (2010b)</td>
<td>584</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Stoltenberg et al. (2012)</td>
<td>439</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Racine et al. (2009)</td>
<td>344</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Steiger et al. (2012)*</td>
<td>304</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>van Strien et al. (2010a)</td>
<td>279</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Karwautz et al. (2011)</td>
<td>256</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Akkermann et al. (2012)</td>
<td>252</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Steiger et al. (2011)*</td>
<td>227</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Groleau et al. (2012)</td>
<td>216</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>van Strien (2015), Study 1</td>
<td>93</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Steiger et al. (2007)</td>
<td>92</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Mata &amp; Gotlib (2011)</td>
<td>50</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>96</strong></td>
<td><strong>24</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Notes.* List of GxE interactions excludes additional models testing covariates, with the exception of gender. Number of interactions tested is based according to both the GxE analyses present in the results section, as well as those interactions planned according to the method. *Steiger et al. (2011) and (2012) include a participant overlap of approximately 75%.*

Another sign that would indicate possible publication bias is if studies reporting positive findings had smaller sample sizes than studies reporting negative findings. This would suggest that studies with null results would be required to be of ‘higher quality’ to be accepted for publication (Duncan et al., 2014). This was not found in the present case, with a mean sample of $n = 327.5$ in studies containing significant findings.
compared to $n = 280$ in studies with null results (the study by Micali et al., 2017a was excluded from this calculation at it was deemed a substantial outlier).

5.4.2. Summary of publication bias

While it was not possible to formally assess for publication bias in the present systematic review, the brief qualitative review undertaken in the present chapter illustrated an alarming number of GxE analyses conducted in some of the papers (e.g., 12 in Groleau et al., 2012, and in Micali et al., 2017a), and revealed that 12 out of 14 studies reported at least one significant GxE interaction. However, there did not appear to be a tendency on behalf of journals to require studies with null results only to test larger samples compared to studies reporting significant findings. One interpretation is that these smaller studies may have in fact not found GxE interactions as they were underpowered compared to the studies using larger sample sizes. Overall, any conclusions must naturally be drawn with a high level of caution, given the small number of studies involved in the review. As the number of studies investigating GxE interaction in the ED field grows, it is of paramount importance that researchers include results of all planned GxE analyses in their final manuscripts, not only those that are statistically significant or deemed most ‘interesting’.

5.5. Chapter summary

The present chapter provided a detailed summary of the studies identified in a systematic review of GxE interactions in predicting an ED-related outcome variable. Out of the 14 studies identified in the review, GxE interactions with significant findings in two or more studies include: Interaction between 5-HTTLPR and traumatic life events to predict disordered eating and bulimia (Akkermann et al., 2012; Stoltenberg et al.,
interaction between 5-HTTLPR and depressive mood to predict emotional eating/over-eating (Mata & Gotlib, 2011; van Strien et al., 2010b); and, interaction between Bcl1 and childhood abuse to predict BN (Steiger et al., 2011; Steiger et al., 2012). There was consistently no interaction between 5-HTTLPR and physical abuse, as well as 5-HTTLPR and emotional abuse, to predict an ED-related outcome (Akkermann et al., 2012; Karwautz et al., 2011; Steiger et al., 2007). All other interactions were either only examined in one sample, or returned conflicting findings (e.g., the 5-HTTLPR x sexual abuse interaction; Akkermann et al., 2012; Karwautz et al., 2011; Steiger et al., 2007).

A quantitative quality review (Downs & Black, 1998) and a qualitative assessment of the studies identified by the review were both conducted. The main issues identified included use of small samples, inadequate control of potential confounders of the GxE interaction, and a number of limitations in the statistical analyses techniques used, including possible issues relating to multiple testing. Finally, the importance of conducting planned analyses using polymorphisms and environmental and psychological factors with a strong theoretical background was discussed. This approach, along with submitting for publication both null and positive findings, and journals supporting this initiative, is vital for the ongoing development of the GxE interactions investigations in the ED field.
6. CHAPTER 6

Extended Discussion of Meta-Analysis and Methodology from Study 1

This chapter will expand upon the methodological details of the combined samples meta-analysis featured in Study 1. It will describe in greater detail the processes involved in identifying suitable studies, selecting the genetic, environmental, and psychological variables for the four combined-samples, and ensuring that the separate data sets were converted into a compatible format in order to perform the secondary-data analyses. It will also expand upon discussion of the meta-analytic results and their implications. For further analyses of the combined-samples data, including closer examination of gender differences and main effects, please see Appendix B.

6.1. Identifying studies suitable for meta-analysis

The results of the present systematic review identified 14 studies that assessed how the interaction between a gene and an environmental or psychological factor influenced an ED-related outcome. In order to perform the meta-analysis, it was necessary to identify and combine studies that analysed the same polymorphism and environmental or psychological factor, and that also contained compatible ED-related outcome variables. This task was achieved through a number of steps. Firstly, the author thoroughly investigated the method and results sections of each paper and created a comprehensive list of variables measured in each study. This list of variables included polymorphisms, environmental measures, psychological measures, and ED-related outcome measures. It also included any other variable measured by the study, even those only used as covariates in the original study and not analysed with a GxE framework (for example, a number of studies controlled for depression or personality traits; e.g., Stoltenberg et al., 2012). This decision was driven by the logic that if full
data sets were obtained from authors of the original studies, these variables could be re-analysed within a GxE interaction framework (e.g., testing the interaction between 5-HTTLPR and depression).

The next step involved grouping studies that examined the same polymorphism and at least one of the same ‘environmental’ or ‘psychological’ variables (i.e., physical abuse, depression). From the seven studies investigating polymorphisms other than 5-HTTLPR (Akkermann et al., 2011; Groleau et al., 2012; Steiger et al., 2011; Steiger et al., 2012; van Strien et al., 2015; van Strien et al., 2010a), no two studies examined a common potential environmental variable. These studies were therefore ineligible for meta-analysis. From the remaining seven studies, all investigating 5-HTTLPR, three examined traumatic life events (Akkermann et al., 2012; Karwautz et al., 2011; Stoltenberg et al., 2012), four contained measures of sexual and physical abuse (Akkermann et al., 2012; Karwautz et al., 2011; Stoltenberg et al., 2012; Steiger et al., 2007), four contained a measure of depression (Akkermann et al., 2012; Karwautz et al., 2011; Mata & Gotlib, 2011; van Strien et al., 2010b), and three examined impulsivity (Akkermann et al., 2012; Racine et al., 2009; Stoltenberg et al., 2012).

6.2. Contacting authors

Corresponding and first authors of each of the aforementioned studies were sent an email invitation to partake in the meta-analysis. The email explained the aims of the study, requested that authors contribute their data (specifying the variables being sought), and invited the author, along with two other colleagues involved in the production of the original paper, to join the authorship for the meta-analysis in recognition of the time and resources involved in collecting the original genetic data sets. All authors responded to the email and agreed to contribute their data sets.
Data from one additional study, Richardson et al. (2008), were also included in Analysis 2 (sexual and physical abuse), and Analysis 3 (depression). While this study did not explicitly analyse the GxE interaction with an ED-specific outcome in their publication, it drew from the same larger sample used by Steiger et al. (2007) and thus was considered eligible. Overlapping participant data between these two studies \((n = 54)\) were removed by authors prior to sending their data files.

Similarly, Karwautz et al. (2011) was part of a European multi-centre collaboration (henceforth The European Project) and data for the meta-analysis were drawn from the larger unpublished sample, including additional data from clinical BN patients. Participant data from The European Project were only included if item-level responses to the ORFI (Fairburn et al., 1998) were available, in order to ensure consistent measurement of the environmental factor ‘traumatic life events’ across studies. Item-level data were not available from all research centres involved in the project and therefore the present sample size does not match that of Karwautz et al. (2011), but includes additional participants with a BN diagnosis. Data from one study (Mata & Gotlib, 2011) initially identified as compatible for Analysis 3 (depression) was not included, because although the authors were willing to contribute their data, they had trouble locating the item-level scores on their measure of depression. Furthermore, as their study contained only 50 participants it was deemed that the increase in sample heterogeneity that would result from including this study was not justified by such a small sample size contribution. Data from Mata and Gotlib (2011) was therefore ineligible for the meta-analysis for these reasons.
6.3. Integrating data sets

Based on the data received from authors, the final four combined-sample analyses included in the meta-analysis were:

1. Traumatic life events x 5-HTTLPR to predict likelihood of an ED or severe disordered eating
2. Sexual and/or physical abuse x 5-HTTLPR to predict bulimic symptoms or BN diagnosis
3. Depression x 5-HTTLPR to predict bulimic symptoms or BN diagnosis
4. Impulsivity x 5-HTTLPR to predict likelihood of severe disordered eating

In order to conduct the four combined-samples meta-analyses, it was necessary to convert the data provided by contributing authors into a format that was compatible for combined analyses. This required the environmental, psychological, and ED-related variables in each data set to be re-scaled for equivalence. This was achieved via varying methods in each of the analyses, depending upon the particular variables that required integration. For Analysis 1, there was substantial overlap in traumatic life events, and thus a dimensional scale was retained. In Analyses 2 and 3, the environmental variables sexual and/or physical abuse and depression were low on compatibility and thus required dichotomisation into yes/no categories. All studies included in Analysis 4 used the same measure of impulsiveness, the BIS-11 (Patton et al., 1995), which was thus retained in its original form.

6.4. Re-coding of data included in meta-analysis

The ED and disordered eating outcome variables were dichotomised in all four analyses due to the fact that the included studies used a wide assortment of
measurement tools to assess disordered eating/EDs (e.g., both dimensional measures of disordered eating and diagnostic interviews for diagnosis of clinical EDs). Finally, the genetic variable, \(5\text{-HTTLPR}\), was coded, with l/l genotype = 0 and s/l or s/s genotypes = 1. Further details regarding how measures were combined in each analysis are contained below.

### 6.4.1. Coding of genotype

As previously mentioned, all genotype information provided by authors was coded into two categories; \(0 = \text{s-allele absent (l/l)}\); and \(1 = \text{s-allele present (l/s or s/s)}\). \(5\text{-HTTLPR}\) allelic composition in Stoltenberg et al. (2012) was originally coded using the tri-allelic grouping, with the l-allele further divided into Lₐ and Lₑ alleles. The study classified the Lₐ/Lₐ genotype as ‘high function’, while the Lₑ allele was coded as ‘low function’ along with the s-allele. In the present meta-analysis the Lₑ and Lₑ alleles were re-coded into the l-allele, as per the bi-allelic classification, to ensure consistency with the other studies in the secondary data analysis. All studies collected genotype information through analysis of blood or saliva samples using standard best-practice techniques for genotyping.

### 6.5. Coding of variables in Analysis 1: Traumatic life events x \(5\text{-HTTLPR}\) to predict eating disorder status

Analysis 1 included data from 909 individuals (65.7% female), from the following three studies: Two community samples, Stoltenberg et al. (2012; \(N = 436, M = 22.5\) years, 65.1% female), Akkermann et al. (2012; \(N = 369, M = 17.8\) years, 56.6% female), and a discordant clinical sister-pair sample, the European Project (\(N = 104, M = 27.8\) years, 100% female).
6.5.1. Coding eating disorder status in Analysis 1

Disordered eating was measured via the EAT-26 (Garner et al., 1982) in Stoltenberg et al. (2012), via the Drive for Thinness and Bulimia scales of the EDI-2 (Garner, 1991) in Akkermann et al. (2012), while ED diagnosis was established via a semi-structured interview, the EATATE-I (Anderluh et al., 2009), in The European Project. As ED status was determined categorically in the latter study, to ensure compatibility, cut-offs were applied to participant EAT-26 and EDI-2 scores to create an ‘ED status’ variable, consisting of participants who met criteria for an ED or experienced substantially elevated levels of disordered eating based on their EAT-26 or EDI-2 scores.

The cut-off score selected in Stoltenberg et al. (2012) for the EAT-26 (Garner et al., 1982) was 20+, which is the established cut-off for a possible ED. For data provided by Akkermann et al. (2012), those with a score above 5 and 3 on the EDI-2 (Garner, 1991) Drive for Thinness and Bulimia subscales, respectively, were classified as having ‘ED’ status. These cut-offs were based on past findings examining discriminant validity (Nevonen & Broberg, 2001; Norring & Sohlberg, 1988), as the EDI-2 scales do not have ‘official’ cut-off scores. It was determined that individuals scoring above the specified cut-offs on these measures of disordered eating were experiencing levels of disordered eating atypical for the general population, and were thus classified as having ‘ED status’ for the purposes of the analysis. A limitation of this method is that neither the EAT-26 or the EDI-2 are intended for use as diagnostic tools, and thus these cut-off scores, although supported by psychometric investigations, lack the precision of a semi-structured interview, such as that used in The European Project (the EATATE; Anderluh et al., 2009). Nonetheless, the cut-off scores selected resulted in a similar portion of individuals identified as meeting ‘ED status’ in each of the two community
samples (15.7% in Stoltenberg et al., 2012; 13.1% in Akkermann et al., 2012), suggesting that the two cut-off values in the EAT-26 and EDI-2 likely reflected a similar ED severity level.

Epidemiological, population-based studies estimate that lifetime ED prevalence in young women, using strict DSM-5 criteria (APA, 2013), is around 1-5%, with AN least prevalent and BED most prevalent (Allen et al., 2013; Cossrow et al., 2016; Smink et al., 2014). However, studies of disordered eating in community samples generally indicate that between 10-50% of young female adults experience disordered eating (Fear, Bulik, & Sullivan, 1996; Neumark-Sztainer et al., 2011; Solmi et al., 2014), with one longitudinal study (Neumark-Sztainer et al., 2011) showing that from early adolescence to mid-young adulthood, over 50% of women engaged in dieting or unhealthy weight control behaviours, while at ages 23 to 26, around 20% of females reported use of extreme weight control behaviours (e.g., vomiting, laxative use, diuretic use, and so on). Another study indicated that between 10-29% of women engaged in binge eating and reported loss of control while eating, with 7.8% reporting binges occurring more than once a week (Striegel-Moore et al., 2009). These later findings more broadly reflect the eating pathology present in the general population. Therefore, the proportion of participants categorised as comprising the ‘ED’ group through use of the cut-off scores in Akkermann et al. (2012) and Stoltenberg et al. (2012) appears to be aligned with the expected population rates of women experiencing substantial eating disturbances in this age range.

6.5.2. Measuring traumatic life events

Each of the three studies (Akkermann et al., 2012; Stoltenberg et al., 2012; The European Project) assessing traumatic life events used separate measures of traumatic
life events: A self-devised scale in Akkermann et al. (2012) assessing 30 events; the Traumatic Antecedent Questionnaire (Herman & van der Kolk, 1987) in Stoltenberg et al. (2012), which included 41 questions, with some assessing positive life events; and a structured interview, the ORFI (Fairburn et al., 1998) in The European Project. The first step in evaluating compatibility involved inspecting the items in each measure to assess for overlap. Altogether, there were 17 life events measured in Akkermann et al. (2012) and Stoltenberg et al. (2012) that were deemed comparable. These items covered a broad range of traumatic experiences during adolescence, such as abuse, accidents, poor parental care, etc. Fourteen of these events overlapped with participant data from The European Project, which was scaled to match the 18-item (0-17 events) solution for equivalence. For a full list of items, please see Table 10.

Participant scores on each item in Akkermann et al. (2012) and The European Project were coded with dichotomous (Yes/No) responses in the original data sets. It was therefore necessary to also dichotomise the items from Stoltenberg et al. (2012), which were measured on a 4-point Likert scale, and assessed two life periods, age 0 – 12 and 13 - present. The responses ‘Never’ or ‘Rarely’ were coded as ‘No’ while the responses ‘Occasionally/Moderately’ or ‘Often’ coded as ‘Yes’, with a response of ‘Yes’ in either age period resulting in an overall ‘Yes’ response for that life event.
## Table 10

*Final Seventeen Life Events Included in Analysis 1 from each of the Three Studies*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Parental death</td>
<td>Someone close to me died</td>
<td>Parental death</td>
</tr>
<tr>
<td>2</td>
<td>Living in institution/foster care</td>
<td>Living with different people at different times</td>
<td>Separation from parents for more than three months</td>
</tr>
<tr>
<td>3</td>
<td>Parent severe alcoholism/lack of care</td>
<td>Caregivers so into alcohol/drugs they couldn’t take care of me</td>
<td>No equivalent question</td>
</tr>
<tr>
<td>4</td>
<td>Poor parental care</td>
<td>High family problems resulted in low care</td>
<td>Parental neglect</td>
</tr>
<tr>
<td>5</td>
<td>Severe health problem or inpatient treatment</td>
<td>Serious illness or hospitalisation</td>
<td>Significant health problem(s) as a child</td>
</tr>
<tr>
<td>6</td>
<td>Accidents and traumas</td>
<td>Serious accident</td>
<td>Significant accidents</td>
</tr>
<tr>
<td>7</td>
<td>Physical abuse inside family (i.e., domestic violence)</td>
<td>Witnessing physical violence in family</td>
<td>Parents physical violence</td>
</tr>
<tr>
<td>8</td>
<td>Physical abuse towards child (in family)</td>
<td>Beaten/kicked/punched by someone close to me</td>
<td>Physical abuse = Yes + perpetrator in family</td>
</tr>
<tr>
<td>9</td>
<td>Physical abuse outside family environment</td>
<td>Medical att. in family due to violence*</td>
<td>No equivalent question</td>
</tr>
<tr>
<td>10</td>
<td>Physical abuse outside family to the child</td>
<td>Someone outside family attacked me</td>
<td>Physical abuse = Yes + perpetrator not in family</td>
</tr>
<tr>
<td>11-14</td>
<td>Sexual abuse by family member</td>
<td>• Someone threatened me with physical harm unless I did something sexual</td>
<td>Five questions assessing sexual abuse:</td>
</tr>
<tr>
<td></td>
<td>• Sexual abuse by non-family member</td>
<td>• I saw sexual things that scared me</td>
<td>• Exposure</td>
</tr>
<tr>
<td></td>
<td>• Rape</td>
<td>• Someone forced me to have sex against my will</td>
<td>• Touching</td>
</tr>
<tr>
<td></td>
<td>• Sexual assault</td>
<td>• Someone older touched me sexually against my wishes</td>
<td>• Oral Sex</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Intercourse</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Other</td>
</tr>
<tr>
<td>15</td>
<td>Emotional abuse inside family</td>
<td>People in my family called me insulting names</td>
<td>No equivalent question</td>
</tr>
<tr>
<td>16</td>
<td>Severe burden/serious concerns*</td>
<td>Some close very sick/in accident</td>
<td>Serious health problem in family</td>
</tr>
<tr>
<td>17</td>
<td>Running away from home</td>
<td>Running away from home</td>
<td>No equivalent question</td>
</tr>
</tbody>
</table>

*Note.* *Taken as roughly equivalent*
6.6. Coding of variables in Analysis 2: Sexual and/or physical abuse x 5-HTTLPR to predict bulimic status

The sample for Analysis 2, investigating the interaction between 5-HTTLPR and sexual and physical abuse, comprised 1097 individuals (71.8% female) from the following five studies: Two community samples, Stoltenberg et al. (2012; N = 436, M = 22.5 years, 65.1% female), Akkermann et al. (2012; N = 369, M = 17.8 years, 56.6% female), one clinical sample from the papers Steiger et al. (2007) and Richardson et al. (2008), (N = 127, M = 26.1 years, 100% female) and a discordant clinical sister-pair sample, The European Project (N = 168, M = 27.1 years, 63% controls, 100% female).

6.6.1. Coding bulimic status in Analysis 2

As mentioned in Analysis 1, disordered eating was measured via the EAT-26 (Garner et al., 1982) in Stoltenberg et al. (2012) and via the Drive for Thinness and Bulimia scales of the EDI-2 (Garner, 1991) in Akkermann et al. (2012), while ED diagnosis was established via a semi-structured interview, the EATATE-I (Anderluh et al., 2009), or via DSM-IV criteria (APA, 1994) in The European Project. Only participants with bulimia-spectrum pathology were included in the present analysis, thus accounting for the different number of participants drawn from The European Project in Analyses 1 and 2. In Steiger et al. (2007) and Richardson et al. (2008), bulimia diagnosis was measured according to the EDE (Fairburn & Cooper, 1993) using DSM-IV criteria (APA, 1994), and included individuals with BN binge-purge, BN non-purge, EDNOS binge-purge, EDNOS BN non-purge, and EDNOS BED.

As in Analysis 1, given that the three studies (Richardson et al., 2008; Steiger et al., 2007; The European Project) examining clinical ED samples measured bulimia-spectrum EDs using a categorical approach, it was necessary to also dichotomise
participant scores on the disordered eating outcome variables in the two community samples (Akkermann et al., 2012; Stoltenberg et al., 2012). Use of the previously described cut-offs was no longer appropriate, as these did not pertain exclusively to bulimia-spectrum eating pathology. A solution was reached whereby participant responses to particular items in the EDI-2 (Garner, 1991) in Akkermann et al. (2012) and EAT-26 (Garner et al., 1982) in Stoltenberg et al. (2012) which directly addressed BN criteria were used to determine BN status. Participants endorsing the following items on either the EAT-26 or EDI-2 were allocated to the bulimia group: 1, Engage in regular episodes of binge eating; 2, Experience loss of control while binge eating; and 3, Engage in compensatory behaviour following binges. In addition, to account for participants who may not have endorsed all three items but still reported substantially elevated bulimia-spectrum disordered eating, participants scoring a summed score of 7 or greater on the bulimia scale of the EDI-2 or EAT-26 were also classified in the BN group. This approach resulted in a similar proportion of each community sample being identified as belonging in the BN group (3.8% in Akkermann et al., 2012, and 4.1% in Stoltenberg et al., 2012).

6.6.2. Coding sexual and physical abuse

As sexual and physical abuse were assessed using different measurement tools in each study, it was necessary to recode these variables as either present or absent to ensure cross-study compatibility. Table 11 describes how each of the measures used was dichotomised, with the exception of data from The European Project, which was originally coded in yes/no format. This resulted in the following proportion of individuals classified as having experienced physical abuse: 29% in Akkermann et al. (2012), 23.9% in Stoltenberg et al. (2012), 21.4% in The European Project, and 52.8%
in Steiger et al. (2007) and Richardson et al. (2008). A slightly lower proportion of participants in each study reported sexual abuse, with 11.9% in Akkermann et al. (2012), 10.1% in Stoltenberg et al. (2012), 26.2% in The European Project, and 35.4% in Steiger et al. (2007) and Richardson et al. (2008).

These rates mostly correspond to the prevalence of physical and sexual abuse identified in the general population (Briere & Elliott, 2003; Fergusson, Lyskey, & Horwood, 1996; Pereda, Guilera, Forns, & Gómez-Benito, 2009), although the rates reported by participants in Steiger et al. (2007) and Richardson et al. (2008) were somewhat more elevated. This may be due to the fact that their participants were recruited through an inpatient hospital setting, where history of child sexual abuse has been reported to be higher than in community samples (Hall, Tice, Beresford, Wooley, & Hall, 1989; Wurr & Partridge, 1996). Another possibility is that the tool used by these studies, the CTI (Fink et al., 1995), is more sensitive than the measures employed in the other studies, or that the cut-off applied to the CTI was too liberal. In any case, the rates identified in Steiger et al. (2007) still fall within a reasonably normative range, and the cut-off selected was the most logical choice based on both equivalence with the cut-offs applied in the other data sets, and the fact that a more stringent cut-off would have resulted in excluding participants reporting ‘moderate’ levels of abuse, which does not match the manner in which abuse is classified in the broader literature (Chen et al., 2010). The present solution is therefore believed to be the most appropriate way of determining the abuse status of participants in Steiger et al. (2007).
Table 11

Criteria to Determine Presence of Lifetime Physical and/or Sexual Abuse in each of the Five Studies Included in Analysis 2.

<table>
<thead>
<tr>
<th>Study and Tool</th>
<th>Physical Abuse</th>
<th>Sexual Abuse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stoltenberg et al. (2012), TAQ</td>
<td>Reporting ‘occasional’ or greater abuse for any of the three questions, at either ages 0-12 or 13-present (e.g., “I was beaten, kicked, or punched by someone close to me”).</td>
<td>Reporting ‘occasional’ or greater abuse, for any of the three questions, at either ages 0-12 or 13-present, or, reporting ‘rarely or a little bit’ in response to two or more questions (e.g., “Someone older touched me sexually against my wishes or tried to make me touch them”).</td>
</tr>
<tr>
<td>Akkermann et al. (2012), Self-devised scale</td>
<td>Reporting ‘Yes’ to any of three questions assessing physical abuse by family or non-family member</td>
<td>Reporting ‘Yes’ to any of four questions assessing sexual abuse by family or non-family member</td>
</tr>
<tr>
<td>European Sample, ORFI</td>
<td>Reporting occurrence of physical abuse</td>
<td>Reporting occurrence of sexual abuse</td>
</tr>
<tr>
<td>Steiger et al. (2007) &amp; Richardson et al. (2008), CTI</td>
<td>Reporting any physical abuse of ‘moderate’ severity or greater</td>
<td>Reporting any sexual abuse of ‘moderate’ severity or greater</td>
</tr>
</tbody>
</table>

Notes. CTI = Children’s Trauma Inventory (Fink et al., 1995); ORFI = Oxford Risk Factor Inventory (Fairburn et al., 1998); TAQ = Traumatic Antecedents Questionnaire (Herman & van der Kolk, 1987).

6.7. Coding of variables in Analysis 3: Depression x 5-HTTLPR to predict bulimic status

The sample for Analysis 3, investigating the interaction between 5-HTTLPR and depression, comprised 1254 individuals (62.5% female) from four studies: Two
community samples, Akkermann et al. (2012; $N = 369$, $M = 17.8$ years, 56.6% female), and van Strien et al. (2010b; $N = 623$, $M = 14.3$ years, 51.2% female), one clinical ED sample, Richardson et al. (2008; $N = 89$, $M = 26.3$ years, 100% female) and a discordant sister-pair clinical ED sample, The European Project ($N = 168$, 63% controls, $M = 27.1$ years, 100% female).

6.7.1. Coding the depression variable

Depression was measured using separate tools in each of the four studies. In the two clinical samples, The European Project and Richardson et al. (2008), history of depression was determined via established diagnostic tools based on DSM-IV criteria (APA, 1994), both which entailed a semi-structured clinical interview. Participants were then coded ‘Yes’ or ‘No’ to denote presence or absence of past depression. In contrast, the two community samples, van Strien et al. (2010b) and Mata and Gotlib (2011) measured depressive symptoms dimensionally, via use of self-report measures. In order to ensure that participant scores on depression were comparable between the data sets, it was necessary to dichotomise participant responses from the two community samples into a ‘present’ or ‘absent’ category. The measures used in each study, as well as the cut-off criteria applied to participant scores in Akkermann et al. (2012) and van Strien et al. (2010b), are presented in Table 12.

Studies of criterion validity of the MADRS-S (Montgomery & Asberg, 1979) suggest that a score of 15 or greater indicates likely depression (Svanborg & Åsberg, 2001; Svanborg & Ekselius, 2003), while there are no studies examining criterion validity in the Depressive Mood List (Kandel & Davies, 1982). However, the items that comprise the two measures both draw upon DSM-IV (APA, 1994) criteria, and all 6 items on the Depressive Mood List are also assessed by the MADRS-S. These items
assessed various features of depressed mood, including low energy/lack of motivation, disrupted sleep, low mood, lack of future-directedness, irritability, and rumination. Due to this complete overlap, participant scores on the Depressive Mood List were rescaled to be equivalent to MADRS-S scores and a cut-off score of 15 was also applied.

Table 12

Criteria to Determine Presence or Absence of Depressed Mood

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Scale</th>
<th>Criteria for Depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akkermann et al. (2012)</td>
<td>MADRS-S</td>
<td>Score above 15 (Svanborg &amp; Åsberg, 2001; Svanborg &amp; Ekselius, 2003)</td>
</tr>
<tr>
<td>van Strien et al. (2010b)</td>
<td>Depressive Mood List</td>
<td>Score above 15 (items scaled to be compatible with MADRS-S)</td>
</tr>
<tr>
<td>The European Project</td>
<td>ORFI</td>
<td>DSM-IV criteria</td>
</tr>
<tr>
<td>Richardson et al. (2008)</td>
<td>SCID-1</td>
<td>DSM-IV criteria</td>
</tr>
</tbody>
</table>


6.7.2. Coding bulimic status in Analysis 3

Bulimia diagnosis was measured via semi-structured diagnostic interviews based on DSM-IV (APA, 1994) criteria in The European Project (EATATE-I; Anderluh et al., 2009) and in Richardson et al. (2008; via the EDE, by Fairburn & Cooper, 1993). The two community samples used dimensional measures of disordered eating (EDI-2; Garner, 1991, in Akkermann et al., 2012; and DEBQ, van Strien et al., 1986, in van Strien et al., 2010b) to assess bulimic symptoms, so it was once again necessary to
categorise participants into ‘bulimic status’ and ‘non-bulimic status’ groups according to their responses on these measures. In addition to the DEBQ, participants in van Strien et al. 2010b also responded to a series of questions assessing bulimic symptoms. As in Analysis 2 (Sexual and Physical Abuse), it was therefore possible to determine bulimic status based on participant responses to questions directly relating to bulimic symptomatology (binge eating behaviour, loss of control during binges, and engagement in compensatory behaviour). This resulted in of 3.8% of participants from Akkermann et al. (2012) and 2.4% of participants from van Strien et al. (2010b) being classified as having ‘bulimia status’.

6.7.3. Limitations of the data integration process in Analysis 3

A major limitation of Analysis 3 is the fact that there was variation in the duration over which depression was (retrospectively) assessed by each study. The European Project and Richardson et al. (2008) assessed lifetime history of depression, while the questionnaire in van Strien et al. (2010b) measured depressive mood over the past one year only. It is unclear which time period participant results on the MARDS-S (Montgomery & Asberg, 1979) reflected, as this was not reported by Akkermann et al. (2012) and this tool does not specify a time frame for which patients should retrospectively rate their symptoms. However, it is typically used to assess state-level depressive mood and is considered sensitive to change (Fantino & Moore, 2009; Montgomery & Asberg, 1979). It is therefore likely that this captured a notably different group of participants compared to those reporting one year and lifetime depressive mood.

A further possible limitation of Analysis 3 was some disparity in age amongst the collated samples. Mean age in the two community samples, drawn from Akkermann et
al. (2012) and van Strien et al. (2010b), was 17.8 years ($SD = 0.54$) and 14.3 years ($SD = 1.1$ years), respectively. In comparison, mean age in the clinical samples was 26.3 years ($SD = 6.7$) in Richardson et al. (2008) and 27.1 years ($SD = 9.3$) in The European Project. This is an issue because it is possible that the relationship between depression and ED symptoms may differ in adolescence, when onset of EDs is more common and most disruptive (Fairburn & Harrison, 2003; Hudson et al., 2007), compared to early adulthood. This was addressed in the analysis by controlling for the effects of age, and indeed, a small but significant age x depression interaction was noted in the model, with a stronger relationship between depression and ED status for the younger participants. However, all older participants were by default classified with an ED (as they were drawn from the two ED clinical samples, Richardson et al., 2008, and The European Project). Ideally, rather than testing a sample with a bimodal distribution of age, this analysis would best be completed in the two groups separately, if sample size allowed.

6.8. Coding of variables in Analysis 4: Impulsivity x 5-HTTLPR to predict disordered eating

The sample for Analysis 4, investigating the interaction between 5-HTTLPR and impulsivity, comprised 1122 individuals (72.2% female) from three community-based samples, Stoltenberg et al. (2012; $N = 436$, $M = 22.5$ years, 65.1% female), Akkermann et al. (2012; $N = 369$, $M = 17.8$ years, 56.6% female), and Racine et al. (2009; $N = 317$, $M = 19.1$ years, 100% female). All four studies used the BIS-11 (Patton et al., 1995) to assess impulsivity, and thus no transformation of participant scores was necessary for this variable. The dichotomisation of the disordered eating variable is discussed below.
6.8.1. Coding the disordered eating variable in Analysis 4

Disordered eating status was based on established cut-offs. The cut-off criteria applied in Analysis 1 were utilised for data from Akkermann et al. (2011) and Stoltenberg et al. (2012), while a cut-off score of 2.3 was selected for participant responses on the EDE-Q (Fairburn & Cooper, 1993) in Racine et al. (2009). This cut-off has been proposed in an investigation of criterion validity of the EDE-Q in a community sample (Mond, Hay, Rodgers, Owen, & Beumont, 2004). Application of these cut-offs resulted in a much higher proportion of participants in Racine et al. (2009) being classified in the disordered eating group (37.9%) compared to Akkermann et al. (2012; 13.1%) and Stoltenberg et al. (2012; 15.7%). Given that all the cut-offs selected were based on published psychometric analyses of criterion validity, and that all three scales assess comparable symptomatology (Engelsen & Laberg, 2001), this difference is likely to reflect a genuinely higher level of disordered eating in the former sample, possibly due to the fact that it tested a female-only sample.

6.9. Summary of data integration process and general limitations

The process required to combine the various data sets provided by differing authors in order to perform the four meta-analyses was extensive and carefully thought out. All analyses required dichotomisation of the ED-related outcome variable, and the environmental and psychological variables were re-coded for all but Analysis 4 (Impulsivity). Specific details and limitations have been discussed in depth in the preceding sections.

One issue that remains to be considered is whether a dichotomised ‘ED’ variable was able to appropriately serve as an outcome measure that reflected the consequences of a given environmental exposure or psychological factor combined with a genetic
vulnerability. In each of the four analyses, participants were classified according to the presence or absence of clinical-level eating pathology, based on a variety of criteria, including purpose-specific diagnostic tools, established cut-offs applied to scales measuring disordered eating, and endorsement of DSM-IV (APA, 1994) criteria for particular EDs.

One argument may be that this dichotomisation provides a somewhat blunted outcome variable. Indeed, even if participants in each study were classified according to DSM diagnosis using a gold-standard diagnostic tool such as the EDE (Fairburn & Cooper, 1993), it is likely that there would be substantial heterogeneity in traits and etiological factors between two individuals with the same disorder. For example, one set of individuals with BN may be predominantly classified as ‘dysregulated’, with elevated levels of impulsivity or emotional regulation difficulties (Cassin & von Ranson, 2005). Conversely, others may experience BN symptomatology as a consequence of extensive dieting, or over-regulation, which may precede a binge-eating episode (Keel et al., 2004; Stice, 2001). It is therefore likely that the genetic correlates of these different sub-phenotypes may also vary substantially.

Indeed, Steiger and Bruce (2007) state that individuals with BN characterised by a ‘dysregulated’ presentation show greater abnormalities in serotonin production compared to those lower on impulsivity, hostility, or recklessness (Steiger, 2004). In terms of environmental factors, the impact of sexual abuse has been shown to be associated more strongly with impulsivity and bulimic symptomatology than with restrictive behaviours (Everill & Waller, 1995; Molendijk et al., 2017). These findings suggest that a DSM diagnosis of BN may not be a sensitive outcome measure given the different endophenotypes that may be involved (Steiger & Bruce, 2007).
Conversely, using a dimensional measure of a particular trait may better identify and isolate particular ED symptoms or phenotypes that may be associated with particular genetic and environmental influences. Measures of disordered eating, such as the EDI-2 (Garner, 1991), which examine individual levels of disordered eating traits, such as drive for thinness, body dissatisfaction, and binge/purging behaviour, may provide a more nuanced and trans-diagnostic measure of difficulties experienced by individuals with disordered eating patterns. For these reasons, future studies of GxE interactions in EDs, including those examining clinical ED samples, should prioritise carefully selected dimensional measures of disordered eating in favour of over-arching diagnostic categories, in order to increase the accuracy of detecting specific GxE risk factors for specific types of eating pathology.

6.10. Discussion of findings from the combined-samples meta-analysis

The following section of this chapter is dedicated to discussion of the main findings of the meta-analysis featured in Study 1 that were beyond the scope of the publication. Discussion will first centre on Analysis 1, traumatic life events (including additional analysis of main effects and the GxE interaction), followed by Analysis 2 (sexual and physical abuse), Analysis 3 (depression, including additional analysis of main effects), and Analysis 4 (impulsivity, also including additional discussion of main effects).

6.10.1. Study 1, Analysis 1: Traumatic life events x 5-HTTLPR to predict eating disorder status

In the first analysis in Study 1, there was evidence of a significant interaction between traumatic life events and 5-HTTLPR in predicting ED status (OR: 1.23, 95% CI: 1.06, 1.44), corresponding to findings in two of the initial studies included in the combined-samples analysis (Akkermann et al., 2012; Stoltenberg et al., 2012). While
the logistic regression model, assessing a multiplicative form of interaction, indicated a
significant GxE interaction effect, this was not the case under the additive model. The
two models test a somewhat different form of statistical interaction, with some
argument that the additive approach may better correspond to the biological realities of
GxE interactions (Rothman & Greenland, 1998). There is reason therefore to exercise
some caution in interpreting these findings.

A significant GxE interaction between 5-HTTLPR and traumatic life events may be
understood from a biological perspective, with the s-allele associated with reduced
production of serotonin, a key neurotransmitter in the stress response system (van
Eekelen et al., 2012). It is possible that this plays a role in regulating individual
responses to stress, such that individuals with the s-allele who experience traumatic life
events may be somewhat more poorly equipped to cope with these events, compared to
those homogenous for the l-allele (Caspi et al., 2003). This greater environmental
susceptibility may lead to disordered eating behaviour for a number of reasons, for
example binge-eating to increase emotional regulation or restrictive eating behaviours
as a method of increasing positive affect by re-gaining control in difficult environments
(Polviv & Herman, 2002; Whiteside et al., 2007).

The significant GxE interaction identified in Study 1 reflects the results of Caspi et
al. (2003), whose seminal study found that the s-allele of 5-HTTLPR interacted with
traumatic life events to predict an increase in depressive symptoms. In the depression
field, this prompted dozens of follow-up studies and five meta-analyses, although many
replication attempts were not successful and the meta-analyses have returned conflicting
findings (Culverhouse et al., 2017; Karg et al., 2011; Munafò et al., 2009; Risch et al.,
2009; Sharpley et al., 2014). As discussed in the previous chapter, many have criticised
GxE research in psychiatry, arguing that a substantial portion of findings may in fact
represent false-positive results, and that issues such as multiple testing, publication bias, and inadequate sample sizes plague this area of research (Duncan & Keller, 2011; Ioannidis et al., 2001). These issues have all been raised as criticisms of the 5-HTTLPR x stressful life events line of investigation in the depression field (Dick et al., 2015). It is also proposed by some that the experience of traumatic life events itself may be in part genetically influenced or have a genetic association with mental health outcomes (Kendler, Karkowski, & Prescott, 1999), and thus could also possibly account for some of the GxE effect noted in the present study. It is thus imperative that the significant GxE interaction between traumatic life events and 5-HTTLPR identified in Study 1 is treated cautiously, particularly due to failure to replicate under an additive model, and requires independent follow-up studies to corroborate this finding.

6.10.2. Study 1, Analysis 2: Sexual and/or physical abuse x 5-HTTLPR to predict bulimic status

In Analysis 2 of Study 1, under a multiplicative model there was no significant interaction between experiencing sexual abuse and having the s-allele of 5-HTTLPR on bulimia-spectrum eating pathology, and no significant interaction between physical abuse and 5-HTTLPR in predicting bulimia. However, there was an interaction between 5-HTTLPR and physical and sexual abuse considered together, such that participants who reported experiencing both physical and sexual abuse, and also carried one or more copies of the s-allele, were significantly more likely to endorse bulimic symptoms than those who had experienced both types of abuse but were homogenous for the l-allele. Under the additive model, all three interactions, 5-HTTLPR with physical abuse, sexual abuse, and both physical and sexual abuse, returned significant results, although the effect size was largest for the latter environmental variable. It may thus be concluded
that there is some evidence for a possible 5-HTTLPR x sexual abuse and 5-HTTLPR x physical abuse interaction, whereas evidence for the 5-HTTLPR x sexual and physical abuse interaction is stronger.

Considering again how reduced serotonin transcription associated with the 5-HTTLPR s-allele may alter functioning of the stress-response system, it is possible that s-allele carriers are relatively resilient to low levels of environmental risk (e.g., a single event or ‘type’ of abuse), but that they may be disproportionally affected by greater levels of abuse (e.g., both physical and sexual abuse). This is corroborated by the findings of Analysis 1 in Study 1, whereby likelihood of an ED increased with each additional traumatic life event for those with the ‘risky’ genotypes, while experiencing a higher number of traumatic events was unrelated to ED status for those with the l/l genotype.

The results of Analysis 2 in Study 1 also revealed significant main effects of abuse, whereby experiencing sexual abuse (OR: 8.56, 95% CI: 2.16, 33.92), physical abuse (OR: 4.79, 95% CI: 1.37, 16.70), and both sexual and physical abuse (OR: 11.53, 95% CI: 2.26, 58.68) predicted an increased likelihood of bulimic status. While caution should be taken in directly interpreting effect sizes of this main effect given the inclusion of the GxE interaction term in the regression model, this corresponds to past findings that have found main effects of sexual and physical abuse on EDs (Chen et al., 2010; Munafò et al., 2009; Norman et al., 2012; Smolak & Murnen, 2002). As discussed in Chapter 2, the relationship between sexual and physical abuse and eating pathology may be attributable to a number of factors, such as lowered self-esteem following abuse leading to increased risk of body dissatisfaction and disordered eating behaviours (Cervera et al., 2003), dissociation following abuse increasing risk of engaging in binge eating episodes (Engelberg et al., 2007), or the desire to regain
control following abuse resulting in attempts at overly restrictive eating behaviours (Slade, 1982).

6.10.2.1. Significant interaction with age

Due to issues relating to multiple testing, it is important to be cautious when interpreting significant findings that fall outside the scope of the main research question (Benjamini & Hochberg, 1995). One such finding in Analysis 2 of Study 1 is worthy of (cautious) mention. Across all three regression models investigating sexual, physical, and both sexual and physical abuse, there was an inverse relationship between age and abuse, such that younger participants who had experienced abuse were more likely to be classified with BN status than older participants. This was significant in the overall sample for the age x sexual abuse ($p = .013$), age x physical abuse ($p = .045$), and age x sexual and physical abuse ($p = .003$) interactions. Results in a sample comprised of only the female participants (not included in Study 1, featured in Appendix B) reflected a similar pattern, with the interaction significant for the age x physical abuse analysis ($p = .019$), and approaching significance for the sexual abuse x age ($p = .065$) and sexual and physical abuse x age ($p = .070$) analyses. It is likely that the differences between the overall ($N = 1097$) sample included in Study 1 and the female-only ($N = 788$) sample re-analysed in Appendix B arose due to the greater power in the former sample, although the significant interactions in the overall sample may also be driven by the presence of male participants. One interpretation of these results is that younger adolescents may be more likely to develop eating pathology in response to experiencing childhood abuse compared to older adolescence (e.g., as in Romans et al., 2001), although these results require investigation in longitudinal, purpose-specific studies examining how particular environmental factors may differentially predispose disordered eating at various ages.
6.10.3. Study 1, Analysis 3: Depression x 5-HTTLPR to predict bulimic status

Analysis 3 in Study 1, investigating whether depressed mood interacted with 5-HTTLPR to predict likelihood of endorsing BN symptoms, did not reveal any main or interaction effects. This is contrary to two of the original studies (Mata & Gotlib, 2011; van Strien et al., 2010b), which both found a significant depression x 5-HTTLPR interaction to predict overeating and emotional eating, respectively. The two other studies involved in the present analysis, Richardson et al. (2008) and The European Project, did not publish results pertaining to the depression x 5-HTTLPR interaction in their publications.

As discussed in previously, there were a number of limitations in these studies that may have resulted in spurious GxE findings, in particular the small sample size ($N = 50$) used by Mata and Gotlib (2011). However, it is equally likely that the limitations of Analysis 3 in Study 1 obscured any significant GxE interaction effects. In particular, each of the studies measured depression over a different duration (e.g., ranging from current depressive mood to history of lifetime depression), thus decreasing the compatibility between data sets and resulting in a less precise environmental variable (see previous section for further discussion). Furthermore, the outcome variable in the Study 1 was bulimic status, which does not precisely match the dimensional overeating and emotional eating outcome variables used in Mata and Gotlib (2011) and van Strien et al. (2010b). The extent to which these constructs are related is contested, with some studies clearly differentiating between emotional eating and bulimic/overeating symptoms (Stark, 2001), and others treating them as interchangeable (Racine et al., 2009).
Another point to consider is that unlike traumatic life events and the experience of sexual and physical abuse, depressed mood constitutes a ‘psychological’, rather than ‘environmental’ variable. Depressed mood, and impulsivity (discussed below), were both included in Study 1 as these variables were analysed within the GxE framework by previous studies identified by the systematic review (e.g., Mata & Gotlib, 2011; van Strien et al., 2010b). It is nonetheless important to note that these psychological variables may interact in different ways with the ‘genetic predispositions’ under investigation in GxE interaction models. For example, it is possible that other genetic factors may simultaneously play a role in depressed mood and onset of an ED, such that any GxE interaction identified may be a result of these unmeasured genetic influences. One avenue of research that may more specifically address this issue could be to investigate whether the environments sought by individuals who are experiencing depressed mood (e.g., social withdrawal; Girard et al., 2014) may themselves be related to greater likelihood of developing an ED for individuals with a particular genetic predisposition.

6.10.4. Study 1, Analysis 4: Impulsivity x 5-HTTLPR to predict disordered eating

The final analysis in Study 1 examined the interaction between impulsivity and 5-HTTLPR in predicting clinical-level eating pathology, and did not return any significant main or interaction effects. There are a few considerations that may have influenced this the relative lack of association between impulsivity and disordered eating, as well as lack of interaction with 5-HTTLPR in these results. Firstly, the ED outcome variable was constructed based on three different dimensional measures of disordered eating (EDI-2, EAT-26, & EDE-Q). While all measures were dichotomised based on
established cut-off scores indicating likely clinical-level eating pathology, it is likely that these cut-offs are not entirely equivalent and thus may have to some extent ‘diluted’ the ED outcome variable.

Another possibility is that while the construct of impulsivity as a whole may not be related to the ED outcome variable, specific subscales analysed independently may be associated with disordered eating behaviours. Subscale data was unavailable for the present analysis. Impulsivity is typically divided into four distinct sub-types, negative urgency, lack of premeditation, lack of perseverance, and sensation seeking, as assessed by tools such as the UPPS Impulsive Behaviour Scale (Whiteside & Lynam, 2001). As described in Chapter 2, the facet of negative urgency has most heavily been associated with ED behaviours, in particular, bulimic symptoms such as binge eating (Racine et al., 2017). The present analysis however was not able to differentiate different types of impulsivity. Moreover, the present study included data from the BIS-11 (Patton & Stanford, 1995), whose subscales include motor-impulsivity, attentional impulsivity, and non-planning impulsivity. The BIS-11 has been found to correlate strongly with the UPPS Impulsive Behaviour Scale \(r = .67\), however correspondence between the subscales of the two measures is low (Meule, Vögele, & Kübler, 2011). The use of the overall score for the BIS-11 (although a common practice in studies of eating pathology; Meule, 2013), and inability to measure negative urgency specifically, may have thus this may have obscured any possible relationships between disordered eating and sub-types of impulsivity that exist in this sample.

6.11. **Conclusions and strengths of the combined-samples meta-analysis**

The present secondary data meta-analysis (Study 1) constitutes a large step forward in GxE research in the eating pathology field. Firstly, it integrated and summarised
existing GxE findings and provided the highest level of evidence to date regarding current GxE interaction effects involving 5-HTTLPR. The results showed early evidence of interactions between 5-HTTLPR and traumatic life events, and 5-HTTLPR and sexual and physical abuse, and that the interactions between this polymorphism and the psychological variables of depression and impulsivity were not present. The same pattern of results was also found in a female-only sub-sample, suggesting absence of clear gender differences, although the proportion of males in the overall sample was low (32.0%).

The large sample sizes involved in the four analyses (ranging from 909 to 1254) are a particular strength of the current meta-analysis. Inadequate sample size is a major issue in existing GxE research, with some arguing that the minimal sample size required to adequately power a GxE investigation is approximately N = 600 (Duncan & Keller, 2011). The largest sample size of studies identified in the ED field was N = 765 (Akkermann et al., 2011), with the average sample size N = 320 (although one recently published study investigating an oxytocin receptor polymorphism included a sample of N = 3135; Micali et al., 2017a). Understandably, collecting genetic samples is resource intensive, and given the relativity scarcity of clinical ED patients collecting data from ED samples is difficult and time-consuming. This highlights the immense value of the collaboration in Study 1, which has allowed for utilisation of existing resources to maximise sample size and further knowledge regarding GxE interaction effects in eating pathology.

In addition to testing GxE interactions in EDs in the largest sample to date at the time of publication, the present meta-analysis also improved upon a range of other issues present in previous GxE research. In particular, it was the first study in the ED field to include all covariate x gene and covariate x environment interaction terms in the
regression model during statistical analysis, thus properly controlling for the effects of potential confounding variables on the GxE interaction (Keller, 2014). The only major consideration that was unable to be addressed in the Study 1 was to quantitatively assess the extent to which publication bias may have influenced findings. This is a factor that remains to be improved upon in subsequent large-scale studies of GxE interactions in eating pathology.

6.12. Recommendations for future meta-analyses

Future meta-analyses of this type are strongly encouraged. This would require continued GxE interaction research in eating pathology, with a focus on examining the same, established environmental risk factors (e.g., sexual and physical abuse), and using compatible ED outcome variables. Such an initiative, containing a larger number of studies than available at the present time, would also carry the benefit of examining a variety of additional factors in further depth, such as age, cohort effects, precision of timing of stressful life events or experiences of abuse, and more.

With a greater number of studies included in a combined-samples meta-analysis, the main limitation would be the introduction of greater heterogeneity, due to studies using different measures and samples taken from varied populations. One possibility suggested by Culverhouse et al. (2013) to optimise the power versus heterogeneity trade-off is to only include studies with large samples (e.g., N > 300). Culverhouse et al. (2013) also argues that this is preferable as smaller samples are more likely to be affected by publication bias (Duncan & Keller, 2011).

However, this approach has been contested by Moffitt and Caspi (2014) who argue that a cut-off sample size of N > 300 is arbitrary in nature. They also claim that smaller studies often included higher quality measures (e.g., interview-based) compared to
larger studies, and provide a more accurate reflection of the constructs in question, including the precise temporal relationship of life events, childhood abuse, and other factors. Reduction in measurement error has been argued to be equally, if not more important, to statistical power than increase in sample size (Luan et al., 2001; Wong et al., 2003). Thus, the inclusion of measures with high reliability and validity may present an alternative means of increasing study power where large sample sizes are unavailable. Overall, it is important for individual studies to prioritise both large samples and the use of measures with excellent psychometric properties in order to propel the success of future meta-analytic findings and to continue to shed light on the gene-environment interplay in psychology.
CHAPTER 7

Introducing a Follow-up Study of the Interactions Between 5-HTTLPR and Environmental and Psychological Factors in Eating Pathology

As discussed in depth across the previous chapters of the present thesis, there is an important role for replication of results in GxE interaction research. A major issue plaguing current GxE interaction research is that the early excitement following studies of novel candidate GxE interactions is often followed by failures to replicate and controversy regarding the initial finding (Dick et al., 2015; Ioannidis & Trikalinos, 2005). This is clearly exemplified by the initial finding of a significant 5-HTTLPR x stressful life events interaction to predict depression by Caspi et al. (2003), which was followed by numerous studies and five meta-analyses reporting conflicting findings (Culverhouse et al., 2017; Karg et al., 2011; Munafò et al., 2009; Risch et al., 2009; Sharpley et al., 2014).

In light of continued reports of a ‘replication crisis’ in psychology and, more specifically, in genetic association studies (Ioannidis et al., 2014; Ioannidis et al., 2001) a key step in validating the findings of the current meta-analysis is to test their replication in an independent sample. As such, the second study of this thesis (Study 2) aimed to follow-up on Study 1 by attempting to conceptually replicate some of the analyses and extend upon others, using a large independent sample from the Australian Temperament Project (ATP). The ATP is a 33-year longitudinal study from Victoria, Australia, that has followed an ethnically homogenous cohort of $N > 2,000$ individuals from infancy to adulthood, with the aim to examine which factors play key roles in social-emotional development across this timespan. At present, the ATP has completed 16 ‘Waves’ of data collection (from 1983 to 2015), using surveys, interviews, and
observational tasks, with respondents including parents, healthcare providers, teachers, and the participants themselves.

This rich data set provides an ideal opportunity to examine the relationship between EDs, 5-HTTLPR genotype, and numerous psychological and environmental factors. Various factors were measured by the ATP throughout the past 30 years, including those that overlap conceptually with the variables utilised in Study 1, such as disordered eating (EDI; Garner, 1991), sexual and physical abuse (ATP-devised questionnaire), depression (Short Mood and Feelings Questionnaire; Angold, Costello, Messer, & Pickles, 1995) and impulsivity (with individual facets such as sensation seeking [Thrill and Adventure Seeking sub-scale of the Sensation Seeking Scale; Zuckerman, Kolin, Price, & Zoob, 1964] and emotional control [ATP-devised scale] measured independently). Participants of the ATP have also provided genetic data for genotyping a number of polymorphisms, including 5-HTTLPR, with saliva samples (N = 567) collected in 1998 and a further 83 samples collected by V.R. in 2015 for the purposes of Study 3 of the present thesis (more details are viewable in Chapter 12).

In order to minimise multiple testing and false-positives results, only environmental and psychological factors with a strong theoretical rationale should be analysed within a GxE framework (Dick et al., 2015). As such, Study 2 included analysis of only the environmental and psychological variables that were already examined in Study 1, with the aim to collect further evidence to support the presence or absence of the GxE interactions identified by the review. Study 2 also expanded on the Study 1 review in a number of ways. Firstly, it compared the effects of mild-to-moderate versus severe parental physical punishment to analyse how severity of the environmental factor influences ED outcomes and whether this has implications for any related GxE interactions. This is was included to examine whether the severity-dependent GxE
interactions identified in Study 1 (e.g., greater evidence of a GxE when both sexual and physical abuse were experienced) might generalise to other environmental factors. Study 2 also assessed the depression x 5-HTTLPR interaction in one large homogenous sample, overcoming the limitation in Study 1 whereby four samples in which depression was assessed using disparate methods were combined for analysis. Finally, rather than analysing impulsivity per se, Study 2 examined emotional control, a variable closely associated with negative urgency, the specific facet of impulsivity most closely related to ED symptomatology. Only the traumatic life events analysis was not examined using this data set due to lack of overlapping events measured by the ATP surveys, although it was possible to analyse self-reported childhood sexual abuse and (parental) physical punishment. Overall, Study 2 built upon Study 1 by addressing the various methodological and theoretical issues discussed thus far throughout this thesis and constitutes a high-quality contribution to the growing GxE literature in the ED field.
This chapter features the original manuscript for the following published study, Study 2 of the present thesis:


The only changes to the manuscript presented in the current chapter include formatting for consistency with the present thesis. To view the published version of Study 2, please see Appendix C.

**Author contributions**

VR was responsible for conducting all analyses and preparing all sections of the manuscript (90%). IK, JR, EW, RK, and CO were involved in collecting data and revising the manuscript for important intellectual content. All authors contributed to and approved the final manuscript.
Investigating direct links between depression, emotional control, & physical punishment with adolescent Drive for Thinness and Bulimic behaviours, including possible moderation by the serotonin transporter 5-HTTLPR polymorphism

Vanja Rozenblat\textsuperscript{a}, Joanne Ryan\textsuperscript{b}, Eleanor Wertheim\textsuperscript{c}, Ross King\textsuperscript{e}, Craig A. Olsson\textsuperscript{e,d,b}, and Isabel Krug\textsuperscript{a}.

\textsuperscript{a} The University of Melbourne, Psychological Sciences, Faculty of Medicine, Dentistry and Health Sciences, Parkville, 3010 Victoria, Australia

\textsuperscript{b} Murdoch Children’s Research Institute, Royal Children’s Hospital Melbourne, Parkville 3010, Victoria, Australia

\textsuperscript{c} La Trobe University, School of Psychology and Public Health, Faculty of Health, Bundoora 3086, Victoria, Australia

\textsuperscript{d} The University of Melbourne, Department of Paediatrics, The Royal Children’s Hospital Melbourne, Faculty of Medicine, Dentistry and Health Sciences, Parkville 3010, Victoria, Australia

\textsuperscript{e} Deakin University Geelong, Centre for Social and Early Emotional Development, School of Psychology, Faculty of Health, 3220, Victoria, Australia

*Corresponding author. School of Psychological Sciences, The University of Melbourne, Level 12 Redmond Barry Building, Parkville 3010, Australia. \textit{E-mail address}: vanja@rozenblat.net (V.Rozenblat)

Keywords: 5-HTTLPR; gene-environment interactions; disordered eating; parental physical punishment; depression; emotional control
Abstract

Objectives: To examine the relationship between psychological and social factors (depression, emotional control, sexual abuse, and parental physical punishment) and adolescent Drive for Thinness and Bulimic behaviours in a large community sample, and to investigate possible genetic moderation. Method: Data were drawn from the Australian Temperament Project (ATP), a population-based cohort study that has followed a representative sample of 2443 participants from infancy to adulthood across 16 waves since 1983. A subsample of 650 participants (50.2% female) of Caucasian descent who provided DNA were genotyped for a serotonin transporter promoter polymorphism (5-HTTLPR). Adolescent disordered eating attitudes and behaviours were assessed using the Bulimia and Drive for Thinness scales of the Eating Disorder Inventory-2 (15-16 years). Depression and emotional control were examined at the same age using the Short Mood and Feelings Questionnaire, and an ATP-devised measure of emotional control. History of sexual abuse and physical punishment were assessed retrospectively (23-24 years) in a subsample of 467 of those providing DNA. Results: EDI-2 scores were associated with depression, emotional control, and retrospectively-reported parental physical punishment. Although there was statistically significant moderation of the relationship between parental physical punishment and bulimic behaviours by 5-HTTLPR (p = .0048), genotypes in this subsample were not in Hardy-Weinberg Equilibrium. No other GxE interactions were significant. Conclusion: Findings from this study affirm the central importance of psychosocial processes in disordered eating patterns in adolescence. Evidence of moderation by 5-HTTLPR was not conclusive; however, genetic moderation observed in a subsample not in Hardy-Weinberg Equilibrium warrants further investigation.
Eating disorders (EDs) are believed to have a substantial heritable component (Bulik et al., 2016), with estimates from twin studies ranging from 40 to 60% (Yilmaz et al., 2015). Thus far, research examining molecular genetic mechanisms that may increase risk for eating pathology has largely investigated whether certain genetic polymorphisms (e.g., serotonin transporter linked polymorphism, 5-HTTLPR) are found in different frequency in those with a clinical ED compared to controls. These studies have largely produced inconsistent findings (Calati et al., 2011; Solmi et al., 2016), supporting the notion that genetic risk operates in a manner more complex than simple association. One line of research receiving increasing attention is the possibility that certain polymorphisms may induce differential risk, depending upon exposure to certain environmental factors, via gene by environment (GxE) interaction.

Studies examining whether GxE interactions play a role in ED aetiology have largely focussed on 5-HTTLPR, with the short (s) allele associated with lower serotonin transcription activity compared to the long (l) allele (Heils et al., 1996). Serotonin plays a role in mood regulation, appetite, and weight (Blundell, 1984; Kalra et al., 1999; Leibowitz & Alexander, 1998; Ruhé et al., 2007), all known to be involved in eating pathology. Serotonin is also involved in the stress-response system (Gotlib et al., 2008; van Eekelen et al., 2012), and as 5-HTTLPR is a functional polymorphism it may conceivably play a role in EDs, directly or indirectly through interaction with other environmental stressors. However, in other fields of psychiatry, the role of 5-HTTLPR in moderating the effects of environmental stressors remains controversial; for example, in depression, where independently conducted meta-analysis continue to contradict one another (Karg et al., 2011; cf. Munafò et al., 2009; Risch et al., 2009). Lack of consensus stems from a range of methodological limitations, such as insufficient sample size, inappropriate statistical techniques and multiple testing, as well as substantial
publication bias favouring significant GxE findings (de Vries et al., 2016; Dick et al., 2015; Duncan & Keller, 2011; Duncan et al., 2014).

To date, seven publications have investigated the role of GxE interactions in the ED field involving 5-HTTLPR (Rozenblat et al., 2017). Systematic review and meta-analysis of these studies suggested that 5-HTTLPR may moderate the risk relationship between experiencing both sexual and physical abuse and bulimic symptomatology (combined $N = 1,096$), and traumatic life events and ED symptomatology ($N = 909$). This was not the case, however, for risk relationships between depressive and bulimic symptomatology ($N = 1254$) or impulsivity and disordered eating ($N = 1122$). Findings from this review suggest that risk associated with 5-HTTLPR may be intensified under increasingly severe social stress, but not psychological distress.

However, findings from this work were based on a combined sample that was derived by summing across small highly heterogeneous samples (e.g., two community samples, $N = 369$, Akkermann et al., 2012; $N = 623$, van Strien et al., 2010; a clinical sample, $N = 89$, Richardson et al., 2008; and a discordant sister-pair sample, $N = 168$ from European cross-institutional data set used in Karwautz et al., 2011). Testing interactions in one large, homogenous sample would be preferable (Cochran, 1954). This, for example, may explain why 5-HTTLPR was found to moderate the effects of depression on eating outcomes in two of the original studies (Mata & Gotlib, 2011; van Strien et al., 2010) but not in the combined data analysis (Rozenblat et al., 2017). Furthermore, the lack of significant interaction between impulsivity and 5-HTTLPR in the combined-sample may be partly due to the analysis of impulsivity as an overall construct, rather than separately testing the particular facets of impulsivity that have previously been associated with EDs, such as negative urgency (Racine et al., 2009;
Negative urgency refers to the tendency to act rashly or feel strong impulses when experiencing negative affect (Whiteside & Lynam, 2001), and, along with the broader ability to regulate one’s emotions, has wide empirical support for a role in EDs, particularly bulimia nervosa symptomatology (Claes, et al., 2005; Fischer et al., 2003), with some evidence linking emotional regulation and 5-HTTLPR function (Hariri & Holmes, 2006). From a theoretical perspective, lowered emotional control may lead to greater eating pathology, as individuals may attempt to control their emotional states via altered food intake (e.g. binge eating or restricted intake; Haynos & Fruzzetti, 2011; Pearson, Wonderlich, & Smith, 2015). Meanwhile, the other psychological factor that has been analysed in a GxE framework, depressed mood, is believed to precipitate bulimic behaviours under a number of key ED models (e.g. Dual Pathway Model; Stice, 2001) with support from longitudinal investigations of high-risk samples (Stice et al., 2017), although there is evidence suggesting depressed mood may also arise as a consequence of eating pathology (Puccio et al., 2016).

While many prior studies investigating GxE interactions have focussed on patients with clinical EDs (Rozenblat et al., 2017), analysis of disordered eating in community samples is of equal, if not greater importance. Developing a better understanding of the correlates and risk factors for pre-clinical eating pathology, which may later develop into a ‘full blown’ eating disorder (Herpertz-Dahlmann et al., 2013), can help promote prevention at the earliest possible opportunity to reduce ED incidence (Stice et al., 2007). From a practical perspective, this also allows for the collection of far larger samples compared to studies using case-control designs, which is a key consideration in genetic association research (Duncan & Keller, 2011).

To further test preliminary findings from Rozenblat et al. (2017) in a homogenous sample, the present study used data from 650 participants who provided
DNA in the Australian Temperament Project (ATP), a population based cohort study that has followed a representative sample of around 2000 participants from infancy to adulthood since 1983. The first aim was to assess the direct effects of depressed mood, emotional control, sexual abuse, and parental physical punishment on adolescent drive for thinness and bulimic behaviours. The second aim was to examine the extent to which the relationships between these factors and eating pathology were moderated by 5-HTTLPR. Results of this study constitute an important step towards accumulating evidence regarding whether genetic factors may moderate the influence of psychological and environmental risk factors on EDs.

Method

Participants

Australian Temperament Project participants were initially recruited in infancy (4-8 months) in Victoria in 1983, using a stratified random sampling framework, via maternal and child health centres in urban and rural locations. The first survey included 2,443 infants (48.0% female), with 16 surveys completed to date. The present study involved a sub-set of 650 participants (50.2% female) who had completed the 11th survey at age 15-16 years, providing information on drive for thinness and bulimic behaviours, depressive symptoms and emotional control, and who also provided a saliva sample for genotyping at this time (N = 567), or in their early 30s (N = 83, in 2015 as part of a separate sub-study). Of the 650 participants, 467 participants also provided retrospective information regarding sexual abuse and parental physical punishment in the 14th survey at age 23-24, and were included in the respective analyses. To avoid issues related to genetic heterogeneity, 23 participants who self-identified as non-Caucasian were excluded prior to forming the sample. The final analysable sample had
a higher proportion of participants from the highest SES quartile than the original sample (for full socio-demographic information, see Table 1). Due to missing data, the final sample comprised 643 participants for the depression analyses and 649 for the emotional control analyses. Parents and adolescents provided written informed consent for each survey wave and for the collection of saliva samples. The data collection was approved by the Australian Institute of Family Studies Ethics Review and carried out in accordance with the latest version of the Declaration of Helsinki.

Measures

**Disordered Eating**

Drive for thinness and bulimic behaviours were assessed at age 15-16 via the Eating Disorder Inventory-2 (EDI-2; Garner, 1991) Drive for Thinness and Bulimia scales. The Drive for Thinness scale consists of 7-items measuring participants’ desire to lose weight or fear of weight gain (e.g., “I am preoccupied with the desire to be thinner”). Internal consistency in the current sample was α = .92. The Bulimia scale consists of 7 items measuring bulimic behaviours, including binging and purging (e.g., “I stuff myself with food”), with Cronbach’s α = .74 in the current sample. For details of scoring and some minor modifications made for an Australian context, refer to Krug et al., (2016).

**Psychological Stress Exposures**

Depression was assessed at the same time-point as disordered eating via the Short Mood and Feelings Questionnaire (SMFQ) (Angold et al., 1995), a 13-item subscale derived from the original 33-item questionnaire. The SMFQ is intended as a screening measure for children and adolescents that queries depressive symptoms
according to DSM-III criteria (APA, 1980; e.g., “I feel miserable or unhappy”), with responses provided on 3-point scale (*rarely/never, sometimes, often/always*). Participants with missing data on five or more items were excluded from analyses (*α* = .83 in current sample).

Emotional control measured participants’ capacity to control their emotions and was also assessed at age 15-16 using an ATP-devised measure consisting of 10-items (e.g., “I am able to keep my feelings under control” and “I am able to calm down if I am feeling nervous”) rated on a 6-point scale from *never to always*. This measure has been previously used in studies examining internalising problems (Toumbourou et al., 2011), with *α* = .70 in the present sample.

**Sexual and Physical Stress Exposures (Retrospective)**

A number of retrospective indicators were used at age 23-24 to assess sexual abuse and parental physical punishment during childhood and adolescence. Sexual abuse was based on a ‘yes’ response to the questions: “You had a sexual experience with a person who was not a family member prior to 16” and a follow up ‘no’ response to the question “Was this consensual?”, or, a ‘yes’ response to the question “A family member did, or tried to do, sexual things to you”.

Mild-to-moderate parental physical punishment was based on a ‘yes’ response to the question “Your parent/s used harsh physical treatment (e.g., smacking, hitting) to discipline you”, and severe parental physical punishment was based on an additional ‘yes’ response to a follow up question, “Did you ever suffer effects that lasted to the next day or longer (e.g., bruising, marking, pain, soreness)?”, creating two distinct severity categories.
Table 1.

**Sociodemographic Details of Participants Included in the Present Sample**

<table>
<thead>
<tr>
<th></th>
<th>Full Sample</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>312</td>
<td>116</td>
<td>36.6</td>
</tr>
<tr>
<td>Females</td>
<td>338</td>
<td>274</td>
<td>81.5</td>
</tr>
<tr>
<td><strong>SES Quartile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest</td>
<td>231</td>
<td>116</td>
<td>36.6</td>
</tr>
<tr>
<td>Medium-High</td>
<td>197</td>
<td>103</td>
<td>32.5</td>
</tr>
<tr>
<td>Medium-Low</td>
<td>129</td>
<td>59</td>
<td>18.6</td>
</tr>
<tr>
<td>Lowest</td>
<td>75</td>
<td>39</td>
<td>12.3</td>
</tr>
<tr>
<td><strong>Parent Marital Status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married/Defacto</td>
<td>538</td>
<td>264</td>
<td>49.8</td>
</tr>
<tr>
<td>Separated/Divorced</td>
<td>73</td>
<td>42</td>
<td>56</td>
</tr>
<tr>
<td>Single/Widowed</td>
<td>22</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>Remarried</td>
<td>12</td>
<td>8</td>
<td>66</td>
</tr>
<tr>
<td><strong>Father’s Occupation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Professional</td>
<td>172</td>
<td>85</td>
<td>49.8</td>
</tr>
<tr>
<td>Managerial</td>
<td>126</td>
<td>61</td>
<td>48</td>
</tr>
<tr>
<td>Semi-Skilled</td>
<td>200</td>
<td>105</td>
<td>52</td>
</tr>
<tr>
<td>Unemployed/Pensioner/Houseduties</td>
<td>114</td>
<td>41</td>
<td>36</td>
</tr>
<tr>
<td><strong>Mother’s Occupation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Professional</td>
<td>175</td>
<td>80</td>
<td>46</td>
</tr>
<tr>
<td>Managerial</td>
<td>54</td>
<td>31</td>
<td>57</td>
</tr>
<tr>
<td>Semi-Skilled</td>
<td>193</td>
<td>104</td>
<td>54</td>
</tr>
<tr>
<td>Unemployed/Pensioner/Houseduties</td>
<td>215</td>
<td>100</td>
<td>47</td>
</tr>
<tr>
<td><strong>Father’s Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>176</td>
<td>86</td>
<td>49</td>
</tr>
<tr>
<td>Diploma/Apprenticeship</td>
<td>103</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>Year 11/12</td>
<td>167</td>
<td>87</td>
<td>52</td>
</tr>
<tr>
<td>Year 10 or less</td>
<td>148</td>
<td>78</td>
<td>53</td>
</tr>
<tr>
<td><strong>Mother’s Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>133</td>
<td>66</td>
<td>21</td>
</tr>
<tr>
<td>Diploma/Apprenticeship</td>
<td>102</td>
<td>55</td>
<td>16</td>
</tr>
<tr>
<td>Year 11/12</td>
<td>227</td>
<td>116</td>
<td>35</td>
</tr>
<tr>
<td>Year 10 or less</td>
<td>171</td>
<td>78</td>
<td>21</td>
</tr>
</tbody>
</table>
**5-HTTLPR Genotyping (Moderation Variable)**

Following the 11th survey, DNA for 567 participants was isolated using Qiagen QIAamp kits from buccal epithelial cells via cotton swabs, with further details described in Jorm et al. (2000). Saliva samples for an additional 83 participants were collected following the 16th survey in 2015 using Oragene saliva pots or tubes and analysed at the Australian Genomics Research Facility (AGRF), Adelaide, Australia. Genotype frequencies were similar in the original and 2015 samples. For all samples, 5-HTTLPR genotype was coded as per the di-allelic model into s-present (s/s or s/l genotype) or s-absent (l/l genotype) groups, as the s-allele is believed to operate in a genetically dominant manner (Lesch et al., 1996).

**Potential Confounding Factors**

Age, height, and weight were self-reported at age 15-16, with the latter two figures used to calculate participant BMI. SES status was measured according to maternal and paternal education and occupation as reported by parents in the first survey in 1983.

**Data Analysis**

The main and interaction effects of 5-HTTLPR and the two psychological stress exposures (depression and emotional control), as well as the three social stress exposures (sexual abuse, mild-to-moderate parental punishment, and severe parental physical punishment), were assessed using separate linear regression models. Outcome variables were Drive for Thinness and Bulimia scores. GxE models were adjusted for sex and BMI, as per Keller (2014), by including all the covariate x gene and covariate x environment interaction terms in the regression models. Prior to analyses, missing data (23.5%) for the BMI variable were imputed using multiple imputation in IBM SPSS Version 21, with no systematic patterns of missingness observed. A total of 10 tests
were conducted with p-values adjusted accordingly (adjusted p-value = 0.05/10, corrected $p = 0.005$), to correct for multiple-testing, a frequent limitation of genetic association studies (Munafò & Flint, 2009). Standardised effect sizes are reported.

**Results**

5-**HTTLPR** genotype distribution ($l/l = 197$, $s/l = 341$, and $s/s = 112$) for the overall sample met the Hardy-Weinberg Equilibrium, $\chi^2 = 2.96$, $df = 1$, $p > .05$. 5-**HTTLPR** genotype distribution was not in Hardy-Weinberg Equilibrium for the subsample providing retrospective reports of sexual and physical stress ($l/l = 139$, $s/l = 255$, and $s/s = 73$; $\chi^2 = 6.11$, $df = 1$, $p = .014$). Further descriptive statistics are presented in Table 2. Across all regression models, female sex predicted Drive for Thinness and Bulimia scores, while BMI predicted Drive for Thinness (all $p < .001$). Tables pertaining to each regression model discussed below are contained in the Supplementary Materials.

Table 2

*Descriptive Statistics for Mean Values of Continuous Predictor and Outcome Variables in the Overall Sample (N = 650), in Females (N = 326), and in Males (N = 324)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Full sample</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.72 (.16)</td>
<td>15.74 (.14)</td>
<td>15.72 (.17)</td>
</tr>
<tr>
<td>EDI-2 Bulimia</td>
<td>1.78 (.65)</td>
<td>1.94 (.73)</td>
<td>1.62 (.53)</td>
</tr>
<tr>
<td>EDI-2 Drive for Thinness</td>
<td>2.23 (1.15)</td>
<td>2.81 (1.22)</td>
<td>1.64 (.70)</td>
</tr>
<tr>
<td>Emotional control</td>
<td>3.74 (.63)</td>
<td>3.62 (.64)</td>
<td>3.85 (.59)</td>
</tr>
<tr>
<td>Depression</td>
<td>.49 (.34)</td>
<td>.59 (.36)</td>
<td>.39 (.28)</td>
</tr>
<tr>
<td>BMI</td>
<td>21.27 (3.28)</td>
<td>21.20 (3.10)</td>
<td>21.34 (3.44)</td>
</tr>
</tbody>
</table>
Depression

There was a significant positive association between depressive symptoms and Drive for Thinness ($\beta = .24, p < .001$), as well as Bulimia scores ($\beta = .41, p < .001$); however, there was no evidence of genetic moderation by 5-HTTLPR. There was a significant interaction between depression and sex, with depression associated with greater Drive for Thinness ($\beta = .46, p = .001$), and to a lesser extent, Bulimia ($\beta = .36, p = .018$), for females only. There were no other significant effects.

Emotional Control

Lower emotional control was significantly associated with greater Drive for Thinness ($\beta = -.22, p < .001$) and Bulimia ($\beta = -.29, p < .001$) scores; however, there was no evidence of genetic moderation by 5-HTTLPR. There was a significant interaction between sex and emotional control, with lower emotional control associated with greater Drive for Thinness ($\beta = -.45, p = .001$) and Bulimia ($\beta = -.67, p < .001$) for females to a greater extent than for males. However, amongst those with the highest levels of emotional control, females displayed lower levels of Bulimia than did males.

Sexual Abuse and Parental Physical Punishment

Of the 467 participants (59.1% female) who provided data on sexual abuse and parental physical punishment in the 14th survey, 22 (4.7%) reported sexual abuse, 180 (38.5%) reported mild to moderate parental physical punishment, and 27 (5.8%) reported severed parental physical punishment. See Table 3 for further descriptive statistics. Predictor and outcome variables for this sub-sample did not differ from the overall sample (t-tests all $p > .05$).
Table 3.
Descriptive Statistics for Mean Values of Continuous Predictor and Outcome Variables in the Subsample (N = 467), in Females (N = 199), and in Males (N = 157)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Full sample</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.72 (.15)</td>
<td>15.73 (.15)</td>
<td>15.71 (.14)</td>
</tr>
<tr>
<td>EDI-2 Bulimia</td>
<td>1.81 (.67)</td>
<td>1.92 (.68)</td>
<td>1.62 (.51)</td>
</tr>
<tr>
<td>EDI-2 Drive for Thinness</td>
<td>2.37 (1.20)</td>
<td>2.79 (1.22)</td>
<td>1.69 (.74)</td>
</tr>
<tr>
<td>Emotional control</td>
<td>3.74 (.64)</td>
<td>3.64 (.65)</td>
<td>3.88 (.59)</td>
</tr>
<tr>
<td>Depression</td>
<td>.51 (.34)</td>
<td>.58 (.02)</td>
<td>.40 (.28)</td>
</tr>
<tr>
<td>BMI</td>
<td>21.19 (3.13)</td>
<td>21.17 (2.97)</td>
<td>21.21 (3.37)</td>
</tr>
</tbody>
</table>

There was a direct effect of severe parental physical punishment in predicting Bulimia ($\beta = .14, p = .001$), but not Drive for Thinness scores. There were no direct effects of sexual abuse or mild-to-moderate parental physical punishment on either disordered eating outcome, although there was an interaction between sexual abuse and sex ($\beta = .28, p = .039$), with males who reported experiencing sexual abuse tending to display lower Drive for Thinness than those who did not report sexual abuse. This pattern was not evident in females. However, this result did not withstand $p$-value adjustment for multiple testing.

There was also statistically significant moderation of the relationship between severe parental punishment and bulimia scores by 5-HTTLPR, with greater punishment related to higher Bulimia scores for those with the s-allele only ($\beta = .22, p = .0048$; see Figure 1). This finding remained after Bonferroni correction for multiple testing was
applied. However, this result was based on a sample where genotypic frequencies were not in the Hardy-Weinberg Equilibrium, $\chi^2 = 6.11$, $df = 1$, $p = .014$. No other GxE interactions were significant.

Figure 1. Interaction between 5-HTTLPR short (s) allele and the experience of severe parental physical punishment in predicting EDI-2 Bulimia scores.

Discussion

This study affirmed the central relationship between depression, emotional control, and physical abuse and adolescent bulimic behaviours and attitudes regarding thinness. Conversely, 5-HTTLPR did not directly predict any pattern of disordered eating, nor was there conclusive evidence that 5-HTTLPR moderated any risk factor for disordered eating. A statistically significant interaction between 5-HTTLPR and
retrospectively-reported parental physical punishment was observed; however, genotypes in this subsample were not in Hardy-Weinberg Equilibrium so cautious interpretation and independent replication is needed.

Findings from this study support a key role for depression and compromised emotional control in adolescent drive for thinness and bulimic behaviours. The relationships differed by sex in most cases. Female sex predicted greater overall drive for thinness and bulimia, and sex differences were present in the relationships between depression, emotional control, and sexual abuse with disordered eating symptoms. Parental physical punishment was the only variable that showed no sex differences in its relationship to eating pathology. Results support the notion that correlates of and risk factors for disordered eating symptoms show substantial variation between males and females (Lewinsohn et al., 2002; Striegel-Moore et al., 2009). They also support past studies linking depression (Puccio et al., 2016) and emotional control (Svaldi et al., 2012) to eating disorder symptomatology, and are aligned with theories proposing that individuals may engage in disordered eating in attempt to better regulate negative affect or other undesirable emotions (Haynos & Fruzzetti, 2011; Pearson, Wonderlich, & Smith, 2015, Stice, 2001). Overall, results highlight the importance of psychological factors and the role of the social environment in eating pathology.

The tentative suggestion that 5-HTTLPR may moderate the relationship between severe, but not mild-to-moderate parental physical punishment and bulimic behaviours is reflected in findings from Rozenblat et al. (2017), which reported moderation of both sexual and physical abuse by 5-HTTLPR in predicting bulimia-spectrum pathology, with strongest effects when both types of abuse were experienced. This suggests that further investigation in larger independent samples in Hardy-Weinberg Equilibrium (the expected allele distribution in a given population, deviation from which may
compromise validity of results) may well support moderation of more extreme forms of adversity by 5-HTTLPR. Further support for the idea that 5-HTTLPR might moderate more severe forms of risk for disordered eating come from past studies in the ED field that found traumatic life events were associated with bulimic symptoms and disordered eating for individuals with the 5-HTTLPR s-allele (Akkermann et al., 2012; Stoltenberg et al., 2012), and is reflected by the focus on traumatic life events and sexual abuse in the depression field (Nugent et al., 2011).

Lack of genetic moderation of depression or emotional control in predicting drive for thinness and bulimic tendencies is mostly consistent with previous findings (Rozenblat et al., 2017). These results align with the secondary data analysis in Rozenblat et al. (2017), and suggest that the null findings for depression and impulsiveness reported in the secondary data analysis were likely not due to sample heterogeneity or use of the broad impulsiveness variable as opposed to examining a personality construct that is more closely associated with eating pathology, such as emotional control. However, sample size limitations mean that the presence of small effects cannot be entirely ruled out and further investigation in larger samples remains important.

The lack of genetic moderation reported for depression does, however, contradict the significant GxE interactions between depression and 5-HTTLPR identified in two past studies (Mata & Gotlib, 2011; van Strien et al., 2010); however, the sample of Mata and Gotlib (2011)(N = 50) was very low for investigation of genetic association (Duncan & Keller, 2011), while van Strien et al. (2010) examined emotional eating, which differed somewhat from the eating constructs measured in the present study. One possibility is that as psychological factors appear to be strong direct predictors of eating pathology, they may function as risk factors irrespective of 5-
HTTLPR genotype. In contrast, certain environmental factors may have a more tenuous association with ED symptoms and thus plausibly could increase risk primarily for individuals with a genetic susceptibility.

The absence of direct genetic association in this study also partly conflicts with previous findings (Calati et al., 2011; Chen et al., 2015). Direct genetic prediction of ED has been investigated in several past studies examining clinical populations with mixed results. Two meta-analyses identified a direct association between 5-HTTLPR and eating pathology (Odds Ratio: 1.35, 95%CI: 1.07 – 1.71, Calati et al., 2011; Chen et al., 2015), although they examined almost entirely the same group of studies, while the largest and most recent meta-analysis on this topic reported no association (Solmi et al., 2016). Notably, these meta-analyses were limited by substantial heterogeneity, the inclusion of studies with very small sample sizes (N < 100), and omission of tests for publication bias. Publication bias is noted to be a major problem affecting studies of GxE interactions and contributing to false-positive findings (de Vries et al., 2016; Duncan & Keller, 2011), with such issues argued to most strongly affect studies with small sample sizes (Ioannidis et al., 2014).

Strengths and Limitations

Strengths of the present study include use of a homogenous, high-quality data set, with measurement of drive for thinness and bulimic tendencies in a community sample. Results are therefore of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample investigating GxE interactions in eating pathology, following Akkermann et al. (2011)(N = 767), with mean sample size of existing ED GxE studies N = 288 (Rozenblat et al., 2017). This study was powered to
detect direct and interaction effects of moderate size, which would be of clinical
significance if detected. In light of growing evidence that genetic effect sizes involved
in psychiatric disorders are exceedingly small, even larger samples are desirable.
Methodological issues include use of the di-allelic model of 5-HTTLPR, with some
evidence that the tri-allelic may better represent activity of this polymorphism
(Wendland et al., 2006), as well as the use of self-report questionnaires to measure most
constructs, with accounts of sexual abuse and parental physical punishment measured
retrospectively. Accordingly, the measure of emotional control used in the present study
does not have published psychometric properties, although it has been used in previous
research (O’Connor et al., 2011; Toumbourou et al., 2011). Finally, 5-HTTLPR is just
one of numerous genetic factors that may be involved in the aetiology of disordered
eating.

**Implications and Future Directions**

Findings from this study suggest that psychological and environmental variables
remain central in eating pathology, while evidence for specific candidate genes
continues to be tentative at best. Although a statistically significant genetic interaction
effect was identified in this study, evidence remains inconclusive because the subsample
on which it was based was not in Hardy-Weinberg Equilibrium. It is important to note,
however, that the null results reported in this study sit in contrast to the substantial
genetic contribution to most psychiatric outcomes estimated in twin study designs
(Trace et al., 2013). This suggests that there is still much work to do in the area of
eating pathology to adequately explain the variation reported in twin studies. Null
findings from this study suggest a more complex picture of genetic determination, one
that would benefit from a move to genome-wide approaches, with an emphasis on
identifying polygenic effects that emerge from networks of genes, which may better
reflect the genetic foundations of complex diseases. Future studies of candidate genes should prioritise increasing statistical power, which may be achieved via data sharing across consortiums of life-course studies. Studies such as the present investigation provide a valuable contribution that should form part of future meta-analytic investigations, and constitute an important step forward in progressing investigation of how psychosocial and genetic factors may be related to eating pathology.

Acknowledgements: The ATP study is located at The Royal Children’s Hospital Melbourne and is a collaboration between Deakin University, The University of Melbourne, the Australian Institute of Family Studies, The University of New South Wales, The University of Otago (NZ), and the Royal Children's Hospital; further information available at www.aifs.gov.au/atp. The views expressed in this paper are those of the authors and may not reflect those of their organisational affiliations, nor of other collaborating individuals or organisations. We acknowledge all collaborators who have contributed to the Australian Temperament Project, especially Professors Ann Sanson, Margot Prior, Frank Oberklaid, John Toumbourou and Ms Diana Smart. We would also like to sincerely thank the participating families for their time and invaluable contribution to the study. This paper forms part of Vanja Rozenblat’s PhD with publication undertaken at The University of Melbourne.

Role of the funding source: This work was supported by an Early Career Researcher Grant (1350035), an Australian Research Council Senior Research Fellowship (DP 130101459), and the Australian Postgraduate Award. None of these institutions had any role in the study design, collection, analysis and interpretation of data, preparation of the manuscript, or decision to submit the manuscript for publication.

Conflicts of interest: The authors declare no conflicts of interest.
Author Contributions: VR was responsible for conducting all analyses and preparing all sections of the manuscript. All other authors were involved in collecting data and revising the manuscript for important intellectual content. All authors contributed to and approved the final manuscript.
References


de Vries, Y., Roest, A., Franzen, M., Munafò, M., & Bastiaansen, J. (2016). Citation bias and selective focus on positive findings in the literature on the serotonin transporter gene (5-HTTLPR), life stress and depression. *Psychol Med*, 46(14), 2971.


9. CHAPTER 9

Methodological Details and Further Discussion of Study 2

This chapter provides further information regarding the methodology of Study 2, followed by continued discussion of the strengths, limitations, and implications of this study. Firstly, it describes in detail the data collection process, including background of the ATP, participant selection and involvement, and procedural elements related to the saliva collection conducted for the purposes of this thesis. The chapter then includes further discussion of the methodological strengths and weaknesses of Study 2, concluding with an elaborated discussion of the implications of the current findings.

9.1. Methodological details of Study 2

9.1.1. Background to the Australian Temperament Project

The ATP is a longitudinal study that has followed a large cohort of participants from infancy to adulthood, with the goal of exploring socio-emotional outcomes during human development. The ATP comprises a representative sample originally recruited using a random stratified sampling framework devised with the assistance of the Australian Bureau of Statistics. Sixty-seven local government areas in Victoria, Australia, were selected: 20 urban (1604 infants) and 47 rural (839 infants). Families were recruited to the study via Maternal and Child Health centres if their 4 – 8 month old infant attended a centre in one of the stipulated local government areas within a specified two week period in 1983 (between 22\textsuperscript{nd} April and 6\textsuperscript{th} May). Approximately 3000 questionnaires were distributed to mothers along with reply-paid envelopes, with 2443 usable questionnaires (52% male, 48% female) returned to form the original ATP sample.
Since the first survey in 1983, a further 16 surveys have been issued to parents, usually completed by mothers, primary school teachers (three surveys), and from 1994, the participants themselves (see Table 13 for details). Surveys were originally distributed via mail with reply-paid envelopes provided, while later waves included the option of an online questionnaire. The questionnaires have measured a broad range of demographic and psychosocial information, including temperament, personality, risk-taking behaviours, peer relationships, social competence, family relationships, behavioural and emotional problems, interests and hobbies, physical health, school adjustment, major life events, civic mindedness, and more. As of 2012, an additional sub-study focusing on participants who have had children (‘Generation 3 Study’) has also been underway.
Table 13

*Year of Data Collection, Sample Size, and Response Rate for Each Survey Wave in the Australian Temperament Project*

<table>
<thead>
<tr>
<th>Wave</th>
<th>Year</th>
<th>Age</th>
<th>Sample</th>
<th>Parent</th>
<th>Child/Teen</th>
<th>Teacher</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1983</td>
<td>4 – 8 months</td>
<td>2443</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1984</td>
<td>1 – 2 years</td>
<td>2226</td>
<td>88%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1985</td>
<td>2 – 3 years</td>
<td>2234</td>
<td>76%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1986</td>
<td>3 – 4 years</td>
<td>2286</td>
<td>75%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1988</td>
<td>5 – 6 years</td>
<td>1785</td>
<td>89%</td>
<td>80%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1990</td>
<td>7 – 8 years</td>
<td>1874</td>
<td>92%</td>
<td>67%</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1992</td>
<td>9 – 10 years</td>
<td>1799</td>
<td>85%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1994</td>
<td>11 – 12 years</td>
<td>1743</td>
<td>84%</td>
<td>83%</td>
<td>71%</td>
</tr>
<tr>
<td>9</td>
<td>1995</td>
<td>12 – 13 years</td>
<td>1661</td>
<td>77%</td>
<td>74%</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1996</td>
<td>13 – 14 years</td>
<td>1670</td>
<td>83%</td>
<td>81%</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1998</td>
<td>15 – 16 years</td>
<td>1666</td>
<td>79%</td>
<td>78%</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2000</td>
<td>17 – 18 years</td>
<td>1650</td>
<td>79%</td>
<td>78%</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>2002</td>
<td>19 – 20 years</td>
<td>1580</td>
<td>70%</td>
<td>73%</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>2006</td>
<td>23 – 24 years</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>2010</td>
<td>27 – 28 years</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>2015</td>
<td>32 – 33 years</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Home Visit Study** 1998

**Generation 3 Study** 2012 – onwards

**Genes and Family Processes Study** 2015 (Data collection for this thesis)

*Notes.* Two-thirds of the original sample were sampled from 1988. Data from later years are unavailable.
9.1.2. Data included in Study 2

To recap, data used in Study 2 of the present thesis includes information collected at age 15/16 (Wave 11) and 23/24 (Wave 14). At age 15/16, participants completed the EDI-2 (Garner, 1991) Bulimia, Drive for Thinness, and Body Dissatisfaction scales. The EDI-2 was also completed at one other time point (Wave 9, age 12/13). Due to negative parental feedback from the EDI-2 survey completed at age 12/13, the Body Dissatisfaction scale included in the age 15/16 survey contained modified items (e.g., “I think I am too fat”, “I think I am too skinny”). Consequently, the Body Dissatisfaction scale showed low internal consistency (α = .52) and was thus excluded from consideration in the studies of the present thesis. The Drive for Thinness and Bulimia scales have shown to map well onto disordered eating behaviour in non-clinical samples in previous research (Klemchuk, Hutchinson, & Frank, 1990).

In the survey completed at 15/16 years, participants also reported on depressed mood (Short Mood and Feelings Questionnaire; Angold et al., 1995) and an ATP-devised measure of emotional control. In addition, data from the 14th survey at age 23/24, during which participants retrospectively reported on childhood sexual abuse and parental physical punishment (coded as mild-to-moderate or severe) via a number of dichotomous questions, was also included in Study 2. Missing data for EDI-2 variables and emotional control was less than 5% and were substituted using the median value. Participants with missing data on the dichotomous questions in the 14th survey were excluded from analysis. For further information on these variables, please refer to the methodology section of Study 2. Participant genotype was also included in the present study, collected either following the survey at age 15/16, or specifically for the purposes of the present thesis in 2015 (with the collection process labelled as the ‘Genes and Family Processes Study’). An in-depth description of this process is described below.
Genetic data collection was funded by an Early Career Research Grant (1350035) awarded to Dr Isabel Krug for the purposes of genetic data collection.

As stated in the published manuscript, multiple imputation was used to impute missing data for the BMI variable, with Little’s MCAR test revealing that data was missing at random, $\chi^2 = 13.74$, $df = 8$, $p < .05$.

### 9.1.3. Procedure for collection of saliva samples in 2015: Genes and Family Processes Study

In 1998 following the 12th survey, participants were invited to provide saliva samples for the genotyping of a number of polymorphisms (e.g., 5-HTTLPR, DAT1, COMT). A second collection wave was completed in 2015 as part of the ATP Genes and Family Processes Study. This sub-study involved contacting participants who had taken part in a Home Study Visit in 1998 and completed an observational Family Interaction Task. The Home Visit Study was an earlier ATP sub-study involving 435 participants who engaged in a video-recorded discussion task with a parent, for the purposes of recording observationally-measured family interactions. The Home Visit Study is discussed in depth in Chapter 12. From the 435 individuals who had participated in the Home Visit Study, 239 had provided genetic data following Wave 11. The second DNA collection undertaken in 2015 involved inviting the additional 196 participants who had not yet provided a saliva sample to do so. Collection of saliva samples in 2015 was restricted to these 196 participants, as participant DNA was initially sought to measure possible genetic associations between disordered eating and observationally-measured parenting (with the latter variable measured only in the 435 individuals who completed the Home Visit Study). The title ‘ATP Genes and Family Processes Study’ was assigned for this additional saliva collection project.
During the 2015 collection of saliva samples, participants were initially contacted via email or mail to participate in the ‘ATP Genes and Family Processes Study’. The invitation included an introduction to the study, an information sheet, and a form seeking participants to indicate whether they were interested in participating. The information sheet contained details regarding the study background, funding, benefits and potential risks of participating, explained why participants were being asked for a DNA sample, and included details regarding confidentiality, along with a reminder that participation was strictly voluntary and that participants could withdraw their consent at any stage. The email also explained that participants would be sent a $20 Coles Myer gift card to thank them for their participation in the study, if they chose to accept this.

Participants replied via email or returned a completed ‘Expression of Interest’ form via a reply-paid envelope provided in the initial mail-out. Approximately two weeks subsequent to the initial invitation, reminder emails or letters were sent to participants who did not respond to the initial contact. These emails or letters were framed as a ‘friendly reminder’ and contained the same information provided in the original invitation. Finally, participants who did not respond to the first or second contact attempt were then contacted via their mobile or home phone (depending upon preference indicated during earlier contact) to invite them to participate in the ATP Genes and Family Processes study. For details regarding participation rates, including individuals who did not provide saliva samples (categorised according to reason), please see Table 14. Many participants who did not respond to the initial or reminder email or mail invitations were nonetheless happy to participate once phone contact was established, with non-response to the original contact due to a number of reasons, including being busy, not seeing the email, or not using an email account regularly. Participants who responded to the original email or mail by declining to participate, or
declined to participate following phone contact, were thanked for their ongoing support of the project and were not further contacted for the purposes of the Genes and Family Processes study. Finally, a number of participants from the original sample were not contacted for a variety of reasons, including to avoid potential participation overburden due to ongoing participation in an ATP study that was running concurrently (the ‘Generation 3 Study’), due to ‘red flag’ status (indicating participants who were at risk of withdrawing from the ATP, based on earlier phone contact), or participants who were still involved in the study but indicated they did not wish to actively participate at the present time. Participants who lived overseas were also unable to participate due to customs restrictions regarding transporting genetic material.

Table 14

*Participation Outcomes for ATP Participants Eligible to Provide Saliva Samples Based on Participation in the Home Visit Study.*

<table>
<thead>
<tr>
<th>Participation outcome</th>
<th>Number of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total targeted sample</td>
<td>196</td>
</tr>
<tr>
<td>Provided saliva sample</td>
<td>84</td>
</tr>
<tr>
<td>Unable to contact/Overseas</td>
<td>59</td>
</tr>
<tr>
<td>Declined to participate</td>
<td>18</td>
</tr>
<tr>
<td>Excluded from contact</td>
<td>35</td>
</tr>
</tbody>
</table>

*Notes.* The ‘total targeted sample’ refers to participants who had completed the family interaction task but who had not provided a saliva sample at an earlier date.

Once participants replied by email or mail indicating they were interested in participating, or if participants did not respond to email or mail contact, participants were then phoned for the purposes of providing more information about the saliva collection process and potentially scheduling an appointment time. Participants were
phoned up to 6 times, with a maximum of two voice mail messages, and in cases where contact was not established, parents of participants or a listed alternate contact were contacted via telephone, up to 4 times. This contact plan followed the standard practice at the ATP. If no successful communication had been established by this point, the participant was considered ‘unable to be contacted’ and removed from the participant list. See Figure 3 for a visual representation of the aforementioned saliva collection process.

It was necessary to coordinate the ATP Genes and Family Processes study along with two other ATP studies running concurrently, the 16th survey of the main ATP study, and the Generation 3 Study. The Generation 3 Study included a sub-set of ATP participants who indicated they were pregnant during a biannual participant ‘check-in’, while the 16th survey was also conducted in 2015 and involved all individuals still participating in the ATP. To minimise participant burden, a careful schedule for participant contact was devised to incorporate the saliva collection into existing ATP (bi-annual) participant contact. To comply with the requirement that ATP participants be contacted at maximum once every 6 months (unless otherwise specified), each participant was allocated a ‘contact month’ during which they were able to be invited to the Genes and Family Processes Study. During this invitation process, participants were also sent information pertaining to the ATP 16th survey and invited to complete this survey online using an individual access code, or via mail if requested. At this time, the researcher also sought to update participant contact details as part of the standard bi-annual check-in and inquired regarding any new pregnancies, for the purposes of recruitment to the Generation 3 Study.
Figure 3. Overview of saliva sample collection process undertaken by V.R. for the ATP Genes and Family Processes Study.

During the initial phone call to participants who indicated they wished to participate, or who did not reply to the initial or follow-up invitations, the researcher (V.R.) explained the background of the study and how the saliva collection process would operate. If participants were happy to proceed, a mutual time was scheduled for an appointment during which participants provided a saliva sample via guidance from V.R. over the telephone. Participants were asked to refrain from eating, drinking (excluding water), smoking, or chewing gum for at least 30 minutes prior to the scheduled conversation.

Following the phone call during which the telephone appointment was set, each participant was sent a package containing the following materials: The information
sheet, a consent form, a reply-paid envelope for returning the completed consent form, an appointment letter detailing the day and time of the saliva collection appointment, an OG-500 saliva collection tube marked only with the participant ID code and no other identifying information, instructions for the OQ-500 saliva collection tube, and a reply-paid padded bag in which to return the saliva tube. A reminder SMS was sent to participants on the morning of their appointment, to confirm whether the scheduled time was still suitable. Participants were then called at the scheduled time, and if they were still available and willing to proceed the appointment was carried out. In some cases, the appointment was re-scheduled if the pre-arranged time no longer suited the participant. During the appointment, the content of the consent form was first explained to the participant and then completed by the participant if they were willing to do so. This consent form was then sealed in a separate envelope to that which contained the saliva sample, in order to maintain confidentiality. The participant was then required to rinse their mouth with water and was guided through the process of depositing saliva into the OG-500 tube. This tube was placed by participants in the reply-paid padded bag and participants were asked to post the two envelopes containing the consent form and saliva sample at their earliest convenience. Once both the consent form and saliva sample were received, participants were sent a $20 Coles Myer gift card, if they chose to accept this, along with a letter thanking them for their participation.

Received samples were stored in a secure location in the Royal Childrens’ Hospital, Melbourne, marked only with a confidential identifying number for each participant. When all samples were received they were sent to the Australian Genomics Research Facility (AGRF) in Adelaide, Australia, via registered express post, and genotyped for the 5-HTTLPR polymorphism. Results of the genotyping were sent in an Excel document containing each participant’s unique identifier, and 5-HTTLPR polymorphism
with both the biallelic and triallelic classifications included (although the latter was unavailable for the originally collected DNA in 1998).

As the ATP Genes and Family Processes study was part of the ongoing ATP operation, it was necessary to learn a substantial number of detailed processes and regulations and to adhere to these during planning for data collection and when conducting the data collection process itself. Prior to contacting participants, V.R. thus underwent a number of training sessions. This included learning a structured set of processes for contacting and communicating with participants (e.g., rules for contact times, appropriate contact methods, etc.), as well as how to best respond to a range of participant queries or comments, with specific training regarding how to sensitively address participants’ concerns and expressions of a reluctance to continue participating in the ATP. Training also included detailed instruction on participant confidentiality procedures, and relatedly, how to use the IT system ‘REDcap’, in which all participant information and contact details (including phone, email, and mail) were stored during the course of the study. To ensure the success and smooth progress of the saliva collection process, V.R. was inducted to the broader ATP duties and activities and attended a range of meetings and progress updates throughout 2014 and 2015.

9.2. Further discussion of Study 2: Strengths and limitations

A number of primarily methodological strengths and limitations raised in the discussion of the Study 2 warrant further attention, in order to better contextualise results of the study. Strengths discussed include use of a dimensional disordered eating outcome measure, the EDI-2 (Garner, 1991), the selection of 5-HTTLPR for inclusion in the GxE interaction model, sample size (a relative strength) and the testing of a limited number of GxE interactions coupled with the use of Bonferroni correction for multiple
testing. Limitations include deviation from the Hardy-Weinberg Equilibrium in the sub-sample in which sexual abuse and parental physical punishment was assessed, the use of a retrospective measure of sexual abuse and parental physical punishment, the use of a normative sample, and sample size (also a relative weakness).

9.2.1. Dimensional measurement of eating pathology

The use of dimensional measures to quantify disordered eating is a strength of Study 2. Use of a dimensional scale, such as the EDI-2 (Garner, 1991), provides a more nuanced measure of eating pathology compared to the use of a diagnostic dichotomy, as in Study 1. This allows for the identification of subtler genetic effects that may be related to underlying symptoms or patterns of symptoms (Mazzeo et al., 2009; Mitchell et al., 2010), and may not be well reflected by an overall DSM-5 diagnosis (Fairburn & Cooper, 2011). Indeed, eating pathology is argued to be of a transdiagnostic nature best conceptualised with a combination of categorical and dimensional models (Wade, Bergin, Martin, Gillespie, & Fairburn, 2006), due to underlying pathology and risk/maintaining factors that are common across a variety of diagnoses (Fairburn et al., 2003). Case-control designs comparing ‘ED cases’ to controls or comparing across ED diagnoses are likely ‘too blunt’ to sufficiently represent the spectrum of underlying pathology that may be affected by GxE interactions. Moreover, examining specific disordered eating behaviours or attitudes is informative for clinical ED populations, and presents an opportunity to understand some of the possible risk factors to developing clinical-level EDs (Rohde et al., 2015). Finally, the use of a dimensional tool facilitates recruitment of a far larger sample, an important consideration in studies of genetic association (Duncan & Keller, 2011).
While measuring disordered eating attitudes and behaviours, as opposed to presence or absence of a clinical diagnosis, was a strength of Study 2, the use of a normative sample rather than a clinical population does present some possible drawbacks. The use of a normative sample results in lower numbers of individuals returning high scores on the disordered eating scales, and consequently, less variation in the outcome variable compared to studies that use a case-control design. For example, the finding that lower emotional control was related to greater EDI-2 (Garner, 1991) Drive for Thinness for females but not males may have been related to the fact that there was greater variability in the Drive for Thinness outcome variable amongst females compared to males ($SD = 1.22$ in females, $SD = .70$ in males). On the other hand, a gender difference in emotional control was also evident when inspecting EDI-2 Bulimia as the outcome variable, where there was substantially less difference in the variance of scores between genders ($SD = .73$ in females, $SD = .53$ in males). This suggests that gender differences in emotional control may indeed be ‘genuine’, as opposed to a reflection of gender differences in variation of scores on the outcome variables. However, it remains that variation in the disordered eating outcomes was lower than that of clinical samples. It is also possible that had the sample included a larger number of individuals with very high scores on the two EDI-2 scales, this data may have been more conducive to providing evidence for a direct genetic effect or GxE interaction.

The use of a non-clinical sample in the present study also presents a point of divergence from a substantial portion of previous research examining genetic association in the ED field. For example, the present study did not report a main effect of 5-HTTLPR on eating pathology. This conflicts with a number of past meta-analyses that did find an effect (Calati et al., 2011; Lee & Lin, 2010). Study 2 cannot be said to directly challenge these findings, as these meta-analyses used exclusively clinical
samples which may show a relationship with 5-HTTLPR that cannot be detected in subclinical populations. However, the most recent meta-analysis on this topic, by Solmi et al. (2016) who also analysed exclusively clinical samples, reflects the pattern of null findings obtained in the present sample. The most current and complete meta-analytic evidence therefore suggests no direct association between 5-HTTLPR and EDs.

9.2.2. Sample size

Sample size in the present study was both a relative strength and weakness. The study constitutes the second largest independent sample at the time to investigate GxE interactions in the ED field (following Akkermann et al., 2011, and more recently, Cardi et al. 2017a). According to Duncan and Keller (2011), this sample is powered to identify large genetic effects, and the present data suggested an absence of effects of this magnitude. However, it remains possible that smaller effects related to 5-HTTLPR are present but that the current sample size was not sufficiently large to identify these effects. While there is no ‘one-size-fits-all’ sample size point-estimate to determine whether a sample is indeed large enough to test hypotheses involving genetic effects, it is increasingly recognised that samples into the thousands are ideal for most complex diseases (Sham & Purcell, 2014). This size is often very difficult to collect and impractical outside of a population-wide endeavour. Nonetheless, the present sample was powered to identify effect sizes that would be of clinical significance if detected.

A number of other factors can be taken into account to maximise the power of a given sample. These include investigating common polymorphisms, such as 5-HTTLPR in the present study, and commonly occurring environmental factors (Sham & Purcell, 2014), as well as the use of measures with excellent psychometric properties (Dick et al., 2015). Methodological vigour can also be improved by examining genes and
environments with a strong theoretical rationale, to account for issues related to multiple testing and reducing the chances of spurious findings (Duncan et al., 2014). This can be achieved in the ED field to an extent, for example, by testing established polymorphisms such as 5-HTTLPR and environmental factors that are known ED risks. Both these considerations were taken into account in Study 2. However, we remain limited by the uncommon nature of the environmental conditions under investigation, such as severe physical punishment. This is particularly relevant in light of the conclusions drawn in Studies 1 and 2 suggesting that it may indeed be these more ‘extreme’ events that interact with a genetic predisposition to moderate risk of EDs, and suggests that continuing to increase sample sizes is vital for future investigations.

9.2.3. Multiple testing

Study 2 also addressed the problem of multiple testing, another critical issue previously identified as limiting GxE interaction research (Duncan et al., 2014) and discussed in previous chapters of this thesis (e.g., Chapter 5). Common criticisms of GxE interaction research include the observation that many existing studies test a large number of GxE interactions, and generally focus attention on the few ‘significant’ findings identified (Sham & Purcell, 2014). In many instances in the ED field, these interactions have been at a borderline significance level (e.g., Akkermann et al., 2012; van Strien et al., 2010b). It is unknown how many further analyses have been conducted but not reported due to null findings. This poses a risk when the alpha level is set at .05 (for example), as in the long run, 5 out of 100 analyses will return a ‘significant’ finding where the alternative hypothesis is in fact untrue. Moreover, this issue is compounded by the ‘file drawer problem’, whereby researchers are less likely to submit findings that do not meet a significance threshold for publication (and those submitted may be less
likely to be accepted; Rosenthal, 1979). Assessing a collection of GxE interactions according to whether any of these analyses result in a p-value below .05 therefore presents a high chance of proliferating false-positive conclusions throughout the literature (Sham & Purcell, 2014).

To address this issue of multiple testing, Study 2 comprised a set of pre-defined GxE analyses based on prior empirical research from Study 1, rather than ‘fishing around’ for possible ‘novel’ GxE interactions. Despite this, the number of interactions tested in the Study 2 was large, with ten regression models in total: five environmental or psychological variables coupled with two outcome measures of disordered eating. To limit the likelihood of false positives, the stringent Bonferroni correction was applied to the original alpha level (p < .05), leading to a corrected alpha level of p < .005 (p < .05/10 analyses). It can therefore be concluded that the significant interaction between 5-HTTLPR and severe parental physical punishment (p = .0048) is unlikely to be due to chance.

9.2.4. Properly adjusting for covariates and use of a homogenous sample

As in the first study of the present thesis, but not in any other GxE investigation in the disordered eating field, the present study adjusted for covariates as per (Keller, 2014). This involved including all gene x covariate and environment x covariate interactions in the regression model, which is the only way to adequately account for the influence of these covariates on the GxE interaction term. As mentioned previously, it is alarming that all previous studies identified in the Study 1 systematic review had omitted this step and consequently, their findings must be interpreted with the knowledge that covariates (e.g., age, BMI, ethnicity) have not been statistically controlled. Finally, Study 2 measured an ethnically homogenous sample, an important
consideration given the possibility of systematic differences in genotype across individuals from diverse ethnic backgrounds (Tang et al., 2005). This required the exclusion of only a small number of participants ($N = 23$), with the original sample reflecting the largely Caucasian population of Victoria in 1983. An additional, related limitation was that due to the longitudinal nature of the study, and resource limitations, genetic data was only collected for less than half of the ATP participants, and full data necessary for the present thesis was only available for approximately 650 individuals. These individuals tended to under-represent the lowest SES bracket, which is a common pattern in such longitudinal endeavours (e.g., Dickinson et al., 2012), although it is argued that this type of drop-out minimally affects the validity of regression models (Wolke et al., 2009).

### 9.2.5. Deviation from the Hardy-Weinberg Equilibrium

While this study presented many strengths and improvements on previous research, it also contained a number of limitations that are important to note. Firstly, while a GxE interaction was identified using the Bonferroni corrected alpha value, this analyses was limited by the fact that the sub-sample which it tested deviated from the Hardy-Weinberg Equilibrium (Hardy, 1908; Weinberg, 1908). This equilibrium represents the expected distribution of alleles in a given population that is under no evolutionary pressure. Populations that meet this equilibrium are theorised to adhere to the following conditions: Presence of random mating, lack of selective pressure on the genotype, absence of mutation and gene flow (i.e. inbreeding), and a homogenous population (Rodriguez, Gaunt, & Day, 2009; Wigginton, Cutler, & Abecasis, 2005). However, populations are invariably subject to some form of evolutionary pressure and this strict adherence to Hardy-Weinberg Equilibrium is rare (Shoemaker, Painter, & Weir, 1998).
Rather, a certain level of deviation from this equilibrium is expected, with allele frequencies examined for deviations of a statistically significant nature. In cases where significant deviations from the Hardy-Weinberg Equilibrium are identified, possible causes may include presence of the aforementioned evolutionary pressures or homogeneity in a population, but may also be a result of insufficient sample size, genotyping error, or chance (Hosking et al., 2004; Rodriguez et al., 2009). Genotyping error is unlikely to be the case in the present sample given that genotyping was conducted at a reputable genomics facility in Australia. Indeed, some argue that deviations from the Hardy-Weinberg Equilibrium are not appropriate for detecting genotyping errors and may be more likely to reflect other influences (e.g., evolutionary pressures; Little et al., 2009; Yong Zou & Donner, 2006). Therefore there is no clear conclusion regarding why deviation from this equilibrium was observed in Study 2, with the most likely reasons including the lower sample size of the sub-sample in question, or perhaps a mild level of genetic heterogeneity. Only participants who self-identified as Caucasian were included in the study, however Australia is a multi-ethnic community and thus even the Caucasian-only sample represented a number of European background (e.g., United Kingdom, Italy/Greece, and Yugoslavia).

Deviation from the Hardy-Weinberg equilibrium has been addressed in a number of ways in previous studies of GxE interactions. A review by Salanti, Amountza, Ntzani, and Ioannidis (2005) suggests that some studies have reported deviations from the equilibrium and not commented further on the implications for their results, others have omitted these tests altogether, while a not-unsubstantial number of empirical investigations contain inaccurately-reported Hardy-Weinberg Equilibrium tests (see also Xu, Turner, Little, Bleecker, and Meyers, 2002). Testing for Hardy-Weinberg disequilibrium appears to be a somewhat overlooked aspect of GxE interaction research.
There is some suggestion that deviation from this equilibrium may be associated with false positive findings or non-replication (Trikalinos, Salanti, Khoury, & Ioannidis, 2006; Xu et al., 2002), and thus results from the sub-sample in Study 2 require replication in a sample that is in Hardy-Weinberg Equilibrium to provide stronger evidence for this GxE interaction. However, it is also argued that deviations from Hardy-Weinberg Equilibrium may result from gene-disease associations, and thus discarding samples in disequilibrium may in turn lead to false-rejection of gene-disease associations and bias subsequent meta-analytic investigations (Rodriguez et al., 2009). The Strengthening the Reporting of Genetic Association Studies (STREGA) guidelines (Little et al., 2009) acknowledge that there are mixed arguments regarding the importance and implications of deviations from the Hardy-Weinberg Equilibrium, and thus advise open and transparent reporting, as was done in the present study.

**9.2.6. Use of retrospective measures**

A final limitation to highlight is that sexual abuse and parental physical punishment in the Study 2 were assessed using retrospective measures. This type of measurement is commonly used in clinical or community-based studies that adopt a cross-sectional design, although is less favourable than assessing constructs concurrently (Mann, 2003). For example, retrospective measures are limited by the possibility of recall bias. This can include both difficulty remembering details of past events or circumstances, but may also include memories that are themselves influenced by a disorder or a genetic factor (e.g., reinterpreting events following a particular diagnosis/outcome, associations between personality factors and recollection of childhood events; Kraemer et al., 1997; Krueger, Markon, & Bouchard, 2003). Such recall bias does not affect fixed markers (e.g., genes), which are in themselves unchanging over time. Therefore, this type of
design is adequate when investing risk factors such as particular genes or information gleamed from medical records before the onset of the disorder (Jacobi et al., 2004). However, for other variables (e.g., environmental factors such as childhood abuse), a cross-sectional retrospective approach may only identify correlates of the disorder rather than risk factors, as per Kraemer et al. (1997).

9.3. Implications of Study 2

Study 2 was conducted as a follow-up investigation to explore whether patterns of results evident in the Study 1 meta-analysis were conceptually replicable in an independent sample. A number of findings in Study 2 mirrored those of the meta-analysis. Across both studies, relatively less severe environmental variables did not appear to interact within a GxE framework, however both studies showed evidence of moderation by 5-HTTLPR in the relationship between ‘extreme’ environmental stressors and eating pathology outcomes. Meanwhile, there was consistently no interaction between 5-HTTLPR and psychological variables to predict EDs or disordered eating outcomes.

9.3.1. GxE interactions involving extreme environmental stressors

In Study 1, GxE interactions were noted on two occasions. Firstly, there was a significant 5-HTTLPR x traumatic life events interaction, with individuals carrying the s-allele who had experienced a higher number of stressful life events displaying a greater likelihood of an ED, both compared to those homogenous for the l-allele, and compared to s-allele carriers who had experienced fewer traumatic life events. In the second analysis examining sexual and physical abuse, a GxE interaction was noted.
(under a multiplicative model of interaction) only when comparing participants who had experienced both sexual and physical abuse with those who had experienced no abuse, or one type of abuse but not the other. Under the additive model, interactions were also seen for 5-HTTLPR x sexual abuse and 5-HTTLPR x physical abuse, but the effect sizes remained smaller than those for participants who had experienced both types of abuse. Meanwhile, in Study 2, an interaction was observed with 5-HTTLPR and severe parental physical punishment to predict disordered eating, while no such interaction was present when mild-to-moderate parental physical punishment was considered. This collection of findings across both studies support the idea that GxE interactions involving 5-HTTLPR may primarily be present when ‘extreme’ environmental conditions are encountered, but less so when considering day-to-day or other milder stressors.

These findings are also echoed to some extent in the depression literature, where others have argued that GxE interactions are mostly evident when environmental factors include cumulative stressors as opposed to ‘one-off’ traumatic events (Moffitt, Caspi, & Rutter, 2006). Indeed, the original study by Caspi et al. (2003) demonstrated GxE interaction when comparing ‘severe’ levels of childhood maltreatment to ‘probable’ or ‘no’ childhood maltreatment, and a similar pattern when considering cumulative life events. Similarly, the interaction between 5-HTTLPR and childhood abuse (in particular, sexual abuse) was found to be largely contingent upon the severity of reported abuse in Fisher et al. (2013). Despite a number of replications (e.g., Åslund et al., 2009; Kaufman et al., 2004), these results must be interpreted in the context of subsequent meta-analyses that argue against a 5-HTTLPR x traumatic life events interaction in predicting depressed mood (Culverhouse et al., 2017; Munafò et al., 2009; Risch et al., 2009).
9.3.2. Gene x psychological factor interactions

Across both the first and second study of the present thesis, genes consistently did not interact with psychological factors to predict EDs or disordered eating risk. Depressed mood was examined within a GxE interaction model in both studies. When no significant moderation by 5-HTTLPR in the relationship between depression and bulimia-spectrum EDs was reported in the secondary data meta-analyses, it was proposed that this null finding was possibly a consequence of the relatively heterogeneous nature of the combined sample, particularly in light of the significant findings in two of the original studies, both included in the secondary data analysis (Mata & Gotlib, 2011; van Strien et al., 2010b; although the former investigated emotional eating, a slightly different construct). However, when the analysis was conducted in a large homogenous sample in Study 2, no significant findings were again reported. Limitations to van Strien et al. (2010b) and Mata and Gotlib (2011), and other factors that may account for this discrepancy, are discussed in Chapter 5. To briefly recap, both van Strien et al. (2010b) and Mata and Gotlib (2011) used sample sizes far too small to reliably detect GxE interactions, particularly in Mata and Gotlib (2011; N = 50), whose sample was also comprised of participants with a mixture of ethnic backgrounds. Further, results in Mata and Gotlib (2011) revealed a significant effect of the s/s but not s/l genotype, which is contrary to how this polymorphism is believed to function (Heils et al., 1996). In sum, results consistently suggest that depressed mood does not interact with 5-HTTLPR to predict EDs or disordered eating.

Impulsiveness, the second psychological variable investigated within a GxE interaction model with 5-HTTLPR, also showed no interactions in the Study 1, and there was no GxE interaction for emotional control and 5-HTTLPR in Study 2. One
possibility discussed in Chapter 6 was that measuring a specific facet of impulsiveness most closely related to eating pathology (i.e. negative urgency; Fischer et al., 2008; in particular related to bulimic behaviours) may prove to be a more logical psychological factor to investigate within a GxE framework. While this variable was not measured in the ATP data set, emotional control, which closely approximates negative urgency (Cyders & Smith, 2008), and is also strongly associated with risk for bulimic behaviours (Pearson et al., 2015b), was selected. Once more, no GxE interactions were identified. These results match the only previous similar GxE study conducted in the ED field (Racine et al., 2009), while to the authors knowledge this specific GxE interaction has not been investigated in other pathologies. Overall, there is little current evidence to suggest that psychological factors, including depression and impulsiveness, interact with genes to predict disordered eating or ED symptomatology.

9.4. Chapter summary

Study 2 involved a thorough data collection process and drew data from a large, high-quality longitudinal study, the ATP. Findings contributed to growing evidence suggesting that 5-HTTLPR may interact with environmental factors within a GxE framework to predict eating pathology, but only in the context of ‘extreme’ environmental stressors. In contrast, the effects of ‘less severe’ stressors and psychological factors appeared to act independently of the 5-HTTLPR polymorphism. The numerous strengths of Study 2 discussed throughout the chapter lend greater credibility to the current findings compared to previous GxE investigations in the ED field, and future studies should aim for replication in samples where the conditions of Hardy-Weinberg Equilibrium are met.
10. CHAPTER 10

Introduction to Study 3

Genetic variations that are argued to play a role within a GxE interaction framework have typically been conceptualised as ‘risk’ factors that increase susceptibility to environmental stressors. Throughout the first two studies of the present thesis, evidence has been presented supporting the role of the 5-HTTLPR s-allele as a factor that increases the likelihood of developing an ED or disordered eating for individuals exposed to environmental stressors of an extreme nature. Another possibility is that the 5-HTTLPR polymorphism may in fact moderate ED outcomes not only in response to environmental adversity, but also in the context of very positive environmental conditions. Under this ‘plasticity’ conceptualisation of GxE interactions, 5-HTTLPR genotype may increase individual response to both positive and negative environments, with individuals with the s-allele who are exposed to adaptive environmental conditions showing a reduced likelihood of developing an ED compared to their l/l counterparts. As discussed in Chapter 3, to date there is some early (albeit mixed) evidence supporting this hypothesis in studies of depression (Belsky & Pluess, 2009; Van Assche et al., 2016; Van Ijzendoorn, Belsky, & Bakermans-Kranenburg, 2012), however no study in the ED field has examined the plasticity hypothesis of GxE interaction.

Exploring the role of parenting behaviours and family interactions provides an ideal opportunity to test the plasticity hypothesis of 5-HTTLPR. Parenting behaviours range on a spectrum from more positive to less positive, and have support for a role in EDs and disordered eating (Tetley et al., 2014). Evidence thus far accrued in this thesis has provided support for the role of ‘extreme’ environmental conditions in GxE interactions. Although parenting behaviours do not generally constitute extreme
environments, they do represent an ongoing risk or protective factor throughout childhood and adolescence (Allen, Gibson, McLean, Davis, & Byrne, 2014) that may be conceptualised as ‘cumulative’ in nature. This type of environmental factor may provide a reliable indication of an individual’s long-term, systematic environmental exposures, with such factors argued by some to be strong candidates for analysis within a GxE framework (Moffitt et al., 2005).

As discussed in Chapter 2, a number of parenting factors have been associated with increased disordered eating or risk of developing an ED. Amongst these, there is relatively consistent support for a role of parental warmth (Tetley et al., 2014). On the theoretically opposite end of the spectrum (Schaefer, 1959) lies parental hostility, which has received little attention thus far in studies examining parenting factors and eating pathology. However, it has been associated with numerous negative outcomes in childhood and adolescence (e.g., depression, personality disorders; Hallquist et al., 2015; Wang & Kenny, 2014), and is closely associated with parental control, a factor implicated in eating pathology (Caglar-Nazali et al., 2014; Tseng et al., 2014). Furthermore, exposure to parental hostility may act as a ‘traumatic event’ in itself for children and adolescents (Dutton, 2000). Therefore, parental warmth and hostility present two parental behaviours that are very well-suited to facilitate investigation of the plasticity hypothesis in eating pathology, and may explain why some individuals are more susceptible to or influenced by parental factors compared to others.

Measuring the role of parenting in eating pathology within a GxE interaction framework can be further enhanced by including high-quality measurement of parenting conducted using a multi-method multi-source approach. Most investigations of parenting in the ED field to date have been conducted using retrospective self-report measurement. Only a small number have investigated this construct with observational
tools (Blair et al., 1995; Humphrey, 1989; Kog & Vandereycken, 1989; Lattimore et al., 2000; Ratti et al., 1996; Stasch & Reich, 2000; Thomas et al., 2012), which provide an objective measure of parenting behaviour, with all families assessed on the same metric. However, past observational studies are limited by an array of factors, including use of unestablished measurement tools, measures with poor psychometric properties, or measures inappropriate for the samples in question. Past observational studies have also contained relatively low sample sizes and tested associations in exclusively female clinical ED samples. As a result, the manner in which parental warmth and hostility are related to disordered eating has not been established using reliable observational measures in a large community sample. Furthermore, there is no research examining GxE interactions using observational family interaction tools in eating pathology, and no studies examining the interaction between 5-HTTLPR and parenting (either observationally measured or self-reported) to predict disordered eating in a community sample.

The aims of the third study of the present thesis are twofold. Study 3a (referred to as Study 1 in the published manuscript) aimed to examine the extent to which certain self-reported parenting behaviours (warmth and harsh discipline) were associated with disordered eating, and whether these effects were moderated by genetic variation in the 5-HTTLPR polymorphism, using a large sample ($N = 650$) of adolescents (age 15 – 16 years). This is an appropriate age bracket in which to test GxE interactions, given findings that the role of genes in EDs is drastically increased post puberty onset (Klump, Culbert, O'Connor, Fowler, & Burt, 2017). Study 3b (referred to as Study 2 in the published manuscript) aimed to replicate any GxE interaction effects in a sub-sample of participants ($N = 304$) by investigating the interaction between observed parenting behaviours (warmth and hostility) and 5-HTTLPR on disordered eating. The
multi-method multi-source and replicative nature of Studies 3a and 3b provides the opportunity for robust conclusions regarding any effects that may replicate across the two studies. Furthermore, as in Study 2, inclusion of both male and female participants and a population-representative community sample allows for results to inform early intervention and prevention of the development of ‘full blown’ EDs. Any evidence of a role for genetic plasticity could provide a further avenue for informing such initiatives.
11. CHAPTER 11

Study 3

This chapter features the following published manuscript for Studies 3a & 3b of the present thesis:


The only changes to the manuscript presented in the current chapter include formatting for consistency with the present thesis. To view the published version of Study 3, please see Appendix D.

Author contributions

VR was responsible for conducting all analyses and preparing all sections of the manuscript. IK, JR, EW, RK, PL, and CO were involved in collecting data and revising the manuscript for important intellectual content. All authors contributed to and approved the final manuscript.
Relationships between self-reported and observed parenting behaviour, adolescent disordered eating attitudes and behaviours, and the 5-HTTLPR polymorphism: Data from the Australian Temperament Project.

Vanja Rozenblat\textsuperscript{a*}, Joanne Ryan\textsuperscript{b}, Eleanor Wertheim\textsuperscript{c}, Ross King\textsuperscript{e}, Craig A. Olsson\textsuperscript{e,d,b}, Primrose Letcher\textsuperscript{d} and Isabel Krug\textsuperscript{a}.

\textsuperscript{a} The University of Melbourne, Psychological Sciences, Faculty of Medicine, Dentistry and Health Sciences, Parkville, 3010 Victoria, Australia
\textsuperscript{b} Murdoch Children’s Research Institute, Royal Children’s Hospital Melbourne, Parkville 3010, Victoria, Australia
\textsuperscript{c} La Trobe University, School of Psychology and Public Health, Faculty of Health, Bundoora 3086, Victoria, Australia
\textsuperscript{d} The University of Melbourne, Department of Paediatrics, The Royal Children’s Hospital Melbourne, Faculty of Medicine, Dentistry and Health Sciences, Parkville 3010, Victoria, Australia
\textsuperscript{e} Deakin University Geelong, Centre for Social and Early Emotional Development, School of Psychology, Faculty of Health, 3220, Victoria, Australia
\textsuperscript{*}Corresponding author. School of Psychological Sciences, The University of Melbourne, Level 12 Redmond Barry Building, Parkville 3010, Australia. \textit{E-mail address}: vanja@rozenblat.net (V.Rozenblat)

This work was supported by an Early Career Researcher Grant (1350035), an Australian Research Council Senior Research Fellowship (DP 130101459), and the Australian Postgraduate Award.
ABSTRACT

This study examined whether self-reported and observationally-measured parental behaviours were associated with disordered eating, and investigated possible moderation by a serotonin transporter polymorphism (5-HTTLPR). Study 1 included 650 adolescents from the Australian Temperament Project (ATP) who completed the EDI-2 Drive for Thinness and Bulimia scales at 15/16 years and were genotyped for 5-HTTLPR. Parents completed an ATP-devised measure of parental warmth and harsh punishment. Study 2 included a sub-group of 304 participants who also engaged in a video-recorded family interaction, with observed parental warmth and hostility coded by the Iowa Family Interaction Rating Scale. Greater self-reported parental warmth was associated with lower bulimia scores. Conversely, observationally-measured parental warmth was associated with lower drive for thinness, but not bulimia. Self-reported parental harsh punishment was associated with bulimia only, with observed parental hostility associated with neither outcome. 5-HTTLPR genotype did not moderate the relationship between parent behaviours and adolescent disordered eating.

Keywords: disordered eating; gene environment interactions; 5-HTTLPR; parenting behaviours; observational measurement.
The biopsychosocial approach to eating disorder (ED) aetiology proposes that risk factors for EDs range from those relating to the individual, such as genes and psychological traits, to those that form part of the environment, including relationships with parents and peers, significant life events, exposure to the ‘thin ideal’, and numerous other factors (Culbert, Racine, & Klump, 2015; Stice, 2002; Trace, Baker, Penas-Lledo, & Bulik, 2013). Risk of developing an ED is believed to be associated with an interplay between these factors. For example, individuals exposed to ‘risky’ environments may develop ED symptoms only if they also carry certain ‘risky’ psychological or genetic factors (Stice, Marti, & Durant, 2011). In particular, following a lack of findings of direct genetic effects (Root et al., 2011), a growing area of ED research involves examining how individual genetic differences may moderate the impact of certain environmental variables on ED symptoms (Rozenblat et al., 2017).

Most research to date has approached investigation of gene x environment (GxE) interactions through the diathesis-stress lens, investigating how genetic ‘vulnerability’ may increase susceptibility to stressful environments (e.g., Stoltenberg, Anderson, Nag, & Anagnopoulos, 2012). However, GxE investigations across other fields increasingly support an alternative model, under which certain genetic factors conceptualised as conferring ‘risk’ may be better conceptualised as ‘plasticity’ factors, that are associated with better outcomes under positive or neutral environmental conditions (Belsky et al., 2009). The current study will examine possible genetic plasticity in disordered eating attitudes and behaviours by examining adolescent exposure to both ‘positive’ and ‘negative’ parenting behaviours, using a multi-method multi-source approach.

The concept of genetic plasticity has received some early support in the depression field (Belsky & Pluess, 2009; Uher & McGuffin, 2008). Belsky and Pluess (2009)
identified a number of studies in which participants with the short (s) allele of the serotonin transporter 5-HTTLPR polymorphism, typically considered a ‘risk’ allele, exhibited lower depression under conditions of few or no stressful life events, compared to those homogenous for the long (l) allele (Brummett et al., 2008; Eley et al., 2004). However, recent meta-analyses cast some doubt over these earlier findings of plasticity (e.g., soon to be published results of a large meta-analysis described by Culverhouse et al., 2013).

5-HTTLPR has been the most heavily studied polymorphism in investigations of GxE interactions, with one or two copies of the s-allele resulting in reduced serotonin transcription (Heils et al., 1996). The s-allele has been implicated in changes in appetite, mood, and the stress-response system (Gotlib, Joormann, Minor, & Hallmayer, 2008; Leibowitz & Alexander, 1998; Ruhé, Mason, & Schene, 2007), and thus is of direct relevance to ED aetiology. As such, most GxE investigations in eating pathology have focussed on 5-HTTLPR, with a recent meta-analysis finding significant interactions between 5-HTTLPR and traumatic life events, as well as sexual and physical abuse, in predicting EDs and bulimia nervosa (BN), respectively (Rozenblat et al., 2017). However, no study in the ED field has examined GxE ‘risk’ from a plasticity perspective.

Parenting factors, such as parental behaviour and child-parent relationship quality, have been implicated in EDs (Quiles, Quiles, Pamies, & Treasure, 2013; Tetzlaff, Schmidt, Brauhardt, & Hilbert, 2016). As parenting practices range from more positive to more negative, they provide an excellent opportunity for investigating the plasticity hypothesis of GxE interactions in relation to eating pathology. This may involve analysing two dimensionally opposite parenting behaviours, such as warmth and
hostility (Schaefer, 1959), which have some theoretical and empirical support for a role in disordered eating. For example, compared to controls, questionnaire-based studies investigating families of ED patients have found families tend to be characterised by lower warmth and fewer positive family bonds (Calam, Waller, Slade, & Newton, 1990; Cunha, Relvas, & Soares, 2009; Vidovic, Juresa, Begovac, Mahnik, & Tocijl, 2004), but greater family conflict and parental control (Calam et al., 1990; Canetti, Kanyas, Lerer, Latzer, & Bachar, 2008). Furthermore, parenting styles (Baumrind, 1971) conceptualised as low on warmth and high on control and hostility (i.e., authoritarian parenting) have been associated with greater disordered eating compared to parenting styles not low on warmth (Lobera, Ríos, & Casals, 2011; Zubatsky, Berge, & Neumark-Sztainer, 2015). Parental warmth is, therefore, a possible protective factor for disordered eating while hostility or harsh punishment may constitute risk factors, although limited research specifically investigates the latter.

Given the importance of accurate measurement of environmental stimuli in GxE research (Moffitt, Caspi, & Rutter, 2005), a second approach may involve investigating parenting behaviours using observational techniques. Unlike idiographic self-report measurement (Margolin et al., 1998), observational measures assess families using the same metric, and may overcome issues such as limitations in self-awareness (Aspland & Gardner, 2003). Other than studies specifically examining parental expressed emotion (Vaughn & Leff, 1976), very few studies have thus far investigated the relationship between (non-mealtime) parental behaviours and eating pathology using observational techniques, and most have not analysed parental warmth or hostility (these include: Blair, Freeman, & Cull, 1995; Humphrey, 1989; Kog & Vandereycken, 1989; Lattimore, Wagner, & Gowers, 2000; Ratti, Humphrey, & Lyons, 1996; Stasch & Reich, 2000; Thomas, Hoste, & Le Grange, 2012). Across these studies, findings
regarding parental warmth and hostility have been mixed (e.g., Humphrey, 1989, cf. Lattimore et al., 2000). This inconsistency is unsurprising given that most existing observational studies are limited by use of poor, unestablished, or inappropriate instruments to code the observational data (e.g., Kog & Vandereycken, 1989; Lattimore et al., 2000), have used small to medium sample sizes (largest $N = 74$, in Humphrey, 1989), and have analysed female-only samples. Furthermore, no studies have examined the relationship between observed parent behaviours and sub-clinical eating pathology. There remains a need to establish solid conclusions regarding how parental warmth and hostility are related to disordered eating using reliable and consistent observational approaches, alongside self-report measures and use of large sample sizes. One further possibility is that genetics may, to an extent, account for some discrepancies in the literature.

To our knowledge, two studies (Karwautz et al., 2011; van Strien, Snoek, van der Zwaluw, & Engels, 2010) have analysed parenting within a GxE framework in the eating disorder field. One study ($N = 265$) found an interaction between problematic parenting styles, in particular parental control, and the s-allele of 5-HTTLPR in predicting AN diagnosis in a female-only discordant AN sister-pair sample (Karwautz et al., 2011). The second study ($N = 279$) found an interaction between parental psychological control and the A1 allele of the Taq1A polymorphism on DRD2 in predicting greater emotional eating in a mixed-gender sample (van Strien et al., 2010). However, neither study explicitly tested for genetic plasticity. Furthermore, both studies focussed largely on parental control and did not shed light on the role of other parenting styles, including positive parent behaviours.
The present investigation aimed to assess the relationship between self-reported and observed parental behaviours and adolescent disordered eating, and to investigate whether 5-HTTLPR moderated this relationship, using the largest sample to date. This is an important improvement on previous ED research, given the critical nature of sample size in genetic research (Duncan & Keller, 2011). The first study included self-reported measures of parenting behaviour to examine the direct effects of parental warmth and use of harsh punishment on disordered eating attitudes and behaviours, as well as possible moderation by 5-HTTLPR. The second study aimed to replicate these analyses with the use of observationally-measured parenting behaviours, to provide insight into how observed parenting behaviours are related to adolescent disordered eating using a sample size multiple times larger than previously assessed, as well as aiming to provide further support for any GxEs identified in Study 1. These studies represent an advancement both in research relating to the role of parenting factors in EDs, which has included predominantly self-report measures, as well as GxE interactions in the ED field, which has focused on presence or absence of negative outcomes only. This is the first investigation of observed parenting within a GxE framework in the ED field, and inclusion of a mixed-gender community sample allows for results to inform ED prevention initiatives.

**STUDY 1**

**METHOD**

**Participants**

Australian Temperament Project participants were initially recruited in infancy (4-8 months) in Victoria in 1983 via maternal and child health centres in urban and rural locations. The first survey included 2,443 infants (48.0% female), with 16 surveys
completed to date. The present study involved a sub-set of 650 participants (50.2% female), who had completed the 11th survey at age 15-16 years and self-identified as Caucasian. Participants provided information on disordered eating attitudes and behaviours, as well as a saliva sample for genotyping. One parent of each participant completed a parent-reported measure of parenting behaviours. To increase sample size for the present study, in 2015, 196 participants who had completed the survey but had not provided saliva for DNA extraction were invited to provide a saliva sample. Of 196 participants, 107 participants were successfully contacted and 83 individuals agreed to provide a sample. Parents and adolescents provided written informed consent for each survey wave and for the collection of saliva samples. The data collection was approved by the Australian Institute of Family Studies Ethics Review and carried out in accordance with the latest version of the Declaration of Helsinki. The final sample included a greater proportion of participants from the highest socio-economic (SES) quartile compared to the original sample in 1983 (see Table 1 for participant characteristics).
Table 1

*Sociodemographic Details of Participants Included in Study 1 and Study 2.*

<table>
<thead>
<tr>
<th></th>
<th>Full Sample</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td><strong>Study 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>650</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>312</td>
<td>49.8</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>338</td>
<td>50.2</td>
<td></td>
</tr>
<tr>
<td>SES Quartile</td>
<td>632</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest</td>
<td>231</td>
<td>36.6</td>
<td>116</td>
</tr>
<tr>
<td>Medium-High</td>
<td>197</td>
<td>31.2</td>
<td>103</td>
</tr>
<tr>
<td>Medium-Low</td>
<td>129</td>
<td>20.4</td>
<td>59</td>
</tr>
<tr>
<td>Lowest</td>
<td>75</td>
<td>11.9</td>
<td>39</td>
</tr>
<tr>
<td>Parent Marital Status</td>
<td>645</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married/Defacto</td>
<td>538</td>
<td>83.4</td>
<td>264</td>
</tr>
<tr>
<td>Separated/Divorced</td>
<td>73</td>
<td>11.3</td>
<td>42</td>
</tr>
<tr>
<td>Single/Widowed</td>
<td>22</td>
<td>3.4</td>
<td>10</td>
</tr>
<tr>
<td>Remarried</td>
<td>12</td>
<td>1.8</td>
<td>8</td>
</tr>
<tr>
<td><strong>Study 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>304</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>143</td>
<td>47.0</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>161</td>
<td>53.9</td>
<td></td>
</tr>
<tr>
<td>SES Quartile</td>
<td>296</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest</td>
<td>103</td>
<td>34.8</td>
<td>53</td>
</tr>
<tr>
<td>Medium-High</td>
<td>100</td>
<td>33.8</td>
<td>55</td>
</tr>
<tr>
<td>Medium-Low</td>
<td>61</td>
<td>20.6</td>
<td>31</td>
</tr>
<tr>
<td>Lowest</td>
<td>32</td>
<td>10.8</td>
<td>20</td>
</tr>
<tr>
<td>Parent Marital Status</td>
<td>299</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married/Defacto</td>
<td>242</td>
<td>81.0</td>
<td>126</td>
</tr>
<tr>
<td>Separated/Divorced</td>
<td>37</td>
<td>12.4</td>
<td>17</td>
</tr>
<tr>
<td>Single/Widowed</td>
<td>12</td>
<td>4.0</td>
<td>8</td>
</tr>
<tr>
<td>Remarried</td>
<td>8</td>
<td>2.7</td>
<td>2</td>
</tr>
</tbody>
</table>
Measures

**Disordered eating attitudes and behaviours.** The Eating Disorder Inventory-2 (EDI-2; Garner, 1991) Drive for Thinness and Bulimia subscales assessed adolescent disordered eating. The Drive for Thinness subscale consists of seven items measuring participants’ desire to lose weight or fear of weight gain (e.g., “I am preoccupied with the desire to be thinner”) with Cronbach’s α = .92 in the current sample. The Bulimia subscale consists of 8 items measuring bulimic behaviours, including binging and purging (e.g., “I stuff myself with food”), with a somewhat lower internal consistency of α = .74 in the present sample. Responses ranged from *Never* to *Always*, and were scored on the original 1 – 6 scale, as recommended for non-clinical samples (Schoemaker, van Strien, & van der Staak, 1994), and then averaged with higher mean scores reflecting greater disordered eating attitudes and behaviours. Wording of the EDI-2 was modified somewhat for an Australian audience, as detailed in Krug et al. (2016).

**Parent behaviours:** The ATP-devised Parenting Practices Scale (Prior, Sanson, Smart, & Oberklaid, 2000) was used to assess parental behaviours, with parents responding to five questions investigating parental warmth (e.g., “In general, how easy is it to spend time with your teenager?”), with α = .77, and 10 questions regarding parental punishment (e.g., “I use threats of punishment to control him/her”), also α = .77. Responses were recorded on a 5-point scale from *Always/Almost Always* to *Never*, and re-coded so higher scores reflected greater warmth or greater punishment.

**5-HTTLPR genotype:** DNA was isolated using Qiagen QIAamp kits from buccal epithelial cells via cotton swabs, and genotyping performed as described previously Jorm et al. (2000). The additional 83 saliva samples obtained for Study 2 were collected
via Oragene saliva tubes, with DNA extracted and genotyped at the Australian Genomics Research Facility (AGRF), Adelaide, Australia. In both cases, 5-HTTLPR genotype was coded into s-present (s/s or s/l genotype) or s-absent (l/l genotype) groups.

**Sociodemographics:** Adolescent age, height in centimetres, and weight in kilograms were self-reported, allowing for calculation of participant body mass index (BMI; kg/m²). Socioeconomic status (SES) was calculated on the basis of parent-reported maternal and paternal education and employment status.

**Data analyses**

Using IBM SPSS Version 21, four multiple linear regression models separately assessed the direct effects of parental warmth and parental harsh discipline on participant EDI-2 Drive for Thinness and Bulimia scores, controlling for BMI and gender. Additional models tested for moderation by the 5-HTTLPR polymorphism by including the gene x environment interaction terms. These models also controlled for the potential confounding effects of gender and BMI as per Keller (2014), by including all the covariate x gene and covariate x parenting style interaction terms in the regression models. Prior to analyses, missing data (23.5%) for the BMI variable were imputed using multiple imputation, with no systematic patterns of missingness observed.

**Power analysis**

Power analysis was conducted using Quanto (http://biostats.usc.edu/Quanto.html), with the following specifications: Continuous outcome, independent individuals design, and a dominant model with s-allele frequency of 43% (based on allele frequencies below). To detect a large to medium sized GxE interaction (R-sq = .02) at an alpha level
of .05, a sample of 389 was required, compared to the present sample of 650. However, this sample was not powered to detect small effects (i.e. \( R^2 < .01 \)).

**RESULTS**

Descriptive statistics for participants in Study 1 are featured in Table 2. Participant 5-HTTLPR genotype distribution (l/l = 197, s/l = 341, and s/s = 112) met the Hardy-Weinberg Equilibrium, \( \chi^2 = 2.96, \ df = 1, \ p > .05 \). Tables featuring results of all regression models are in Supplementary Materials.

Table 2.

*Descriptive Statistics for Key Variables Investigated in Study 1.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Full Sample</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.72 (0.16)</td>
<td>15.74 (0.14)</td>
<td>15.72 (0.17)</td>
</tr>
<tr>
<td>Parental warmth</td>
<td>4.22 (0.63)</td>
<td>4.25 (0.65)</td>
<td>4.19 (0.61)</td>
</tr>
<tr>
<td>Parental punishment</td>
<td>2.00 (0.56)</td>
<td>1.94 (0.55)</td>
<td>2.06 (0.57)</td>
</tr>
<tr>
<td>EDI-2 Bulimia</td>
<td>1.78 (0.65)</td>
<td>1.94 (0.73)</td>
<td>1.62 (0.53)</td>
</tr>
<tr>
<td>EDI-2 Drive for Thinness</td>
<td>2.23 (1.15)</td>
<td>2.81 (1.22)</td>
<td>1.64 (0.70)</td>
</tr>
<tr>
<td>BMI</td>
<td>21.27 (3.28)</td>
<td>21.20 (3.10)</td>
<td>21.34 (3.44)</td>
</tr>
</tbody>
</table>

Across all regression models examining main effects, there was a direct effect of female gender and BMI on Drive for Thinness scores, with the former also predicting Bulimia scores (all \( p < .001 \)). There was a significant relationship between Bulimia, but not Drive for Thinness, and self-reported parental warmth (\( \beta = .11, \ p = .005 \)) and harsh punishment (\( \beta = .11, \ p = .003 \)). There was also an interaction between gender and harsh
punishment in predicting Drive for Thinness ($\beta = .30, p = .038$), with scores for males lower under conditions of higher harsh punishment, while they remained largely unchanged for females. There were no main effects of $5-HTTLPR$, or any significant interaction effects between $5-HTTLPR$ and parent behaviours.

**STUDY 2**

**METHOD**

**Participants**

Study 2 consisted of a sub-sample of 304 participants (53.0% female) from Study 1 who were selected to participate in an observational family interaction task based on responses to the 11th survey, as part of an earlier investigation into factors contributing to risk and resilience for adolescent adjustment. Approximately three-quarters of those invited consented to participate, and were characterised into a problem (16.7%), high risk (21.7%), or low risk (55.2%) group. Participants in the problem group either exhibited elevated levels of depressed mood, as indicated by endorsing five or more DSM-III (APA, 1980) symptoms of depression, reported frequent substance use, or exhibited anti-social behaviour, determined by endorsing four or more delinquent acts (e.g., stealing, fighting, driving a car without permission). Based on a number of factors that differentiated the problem group from the main ATP sample (such as earlier behaviour problems, school adjustment difficulties, and peer relationships), a group of problem free yet ‘high risk’ participants was identified. A gender-balanced low risk group was drawn randomly from the remaining sample. Participants completed the EDI-2 and provided saliva samples as described in Study 1. The final sample for Study 2 included a lower proportion of participants from the lowest SES quartile compared to the original sample. Demographic information is featured in Table 1.
Measures

Disordered eating attitudes and behaviours, BMI, and age were measured as in Study 1.

**Observational measure of parent behaviours:** Trained interviewers performed home visits during which participants and one of their parents (usually the mother) engaged in a video-recorded 15-minute discussion based on a set of cards containing 14 questions about family life (e.g., teenager’s accomplishments and disappointments, parental rules and fairness). Parenting behaviours were coded by a team who had undergone extensive training and were blind to adolescent group membership, using the Iowa Family Interaction Rating Scale (IFIRS; Melby et al., 1998), a macro-level observational coding system that was designed to measure behaviours and emotions in family discussions with adolescents. This is considered a valid tool for measuring behaviours in a variety of dyadic interactions and has been validated against self and other reports from family members (Melby & Conger, 2001). Two scales were selected for the present study, parental warmth and parental hostility.

**Warmth** measured parental expressions of care, concern, and support directed at their child, including expressions of approval, affectionate physical contact, and building upon or reciprocating warmth displayed by their child. It was rated on a 9-point scale from *no warmth* to *frequent warmth*. Average intraclass correlation to assess inter-rater reliability for this scale was .80, based on cross examination of nine videos.

**Hostility** measured the extent to which parents engaged in hostile behaviours directed to their child, including rejection, active ignoring, showing contempt, or expressing complaints or critical remarks. It was rated on a 9-point scale from *no hostility* to *frequent hostility*. The average intraclass correlation for this scale was .75,
which compares favourably to previous studies using the IFIRS and is deemed acceptable (Ge, Best, Conger, & Simons, 1996).

Data analyses

Data were analysed using IBM SPSS Version 21, as in Study 1, via four linear regression models examining the direct effects of observed parental warmth and observed parental hostility on EDI-2 Drive for Thinness and Bulimia scales. Additional models examined possible moderation by 5-HTTLPR, again controlling for gender and BMI. In the analyses investigating parental warmth and 5-HTTLPR, covariate x gene and covariate x environment contrast terms were included, as recommended by Keller (2014). However, this was not possible in the investigation of parental hostility and 5-HTTLPR due to issues of excessive collinearity (further discussed in Results section). Finally, to ensure that any differences between Studies 1 and 2 reflected measurement effects as opposed to sample effects, the direct effects of self-reported parental warmth and self-reported use of harsh punishment were also tested in the Study 2 sub-sample. GxE interactions were not included in this analysis to avoid multiple testing issues. Missing data for BMI (28.29%) were imputed via multiple imputation, with no systematic patterns of missingness observed.

Power analysis

Power analysis was conducted using Quanto (http://biostats.usc.edu/Quanto.html), as for Study 1, with s-allele frequency of 43% (based on allele frequencies for Study 2 participants). The current sample was powered to detect a large to medium sized GxE interaction, R-sq = .03, which requires 258 participants at an alpha level of .05, but was not powered to detect effects smaller than this.
RESULTS

Descriptive statistics for participants in Study 2 are featured in Table 3. 5-HTTLPR genotype distribution (l/l = 90, s/l = 154, and s/s = 60) met the Hardy-Weinberg Equilibrium, $\chi^2 = .16$, $df = 1$, $p > .05$. Results from all regression models are located in the Supplementary Materials.

Table 3.

Descriptive Statistics for Key Variables Investigated in Study 2.

<table>
<thead>
<tr>
<th>Mean (SD)</th>
<th>Mean (SD)</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>15.73 (0.15)</td>
<td>15.73 (0.13)</td>
<td>15.72 (0.10)</td>
</tr>
<tr>
<td>Parental warmth</td>
<td>4.38 (1.93)</td>
<td>4.57 (1.92)</td>
<td>4.16 (1.92)</td>
</tr>
<tr>
<td>Parental hostility</td>
<td>1.92 (1.53)</td>
<td>2.06 (1.65)</td>
<td>1.76 (1.37)</td>
</tr>
<tr>
<td>EDI-2 Bulimia</td>
<td>1.89 (0.73)</td>
<td>2.08 (0.80)</td>
<td>1.68 (0.58)</td>
</tr>
<tr>
<td>EDI-2 Drive for Thinness</td>
<td>2.34 (1.25)</td>
<td>2.99 (1.28)</td>
<td>1.61 (0.67)</td>
</tr>
<tr>
<td>BMI</td>
<td>21.55 (3.59)</td>
<td>21.65 (3.40)</td>
<td>21.45 (3.77)</td>
</tr>
</tbody>
</table>

Female gender predicted greater Drive for Thinness and Bulimia scores (all $p < .001$), while BMI was associated with the former only. There were also significant direct effects of greater observed parental warmth on reduced Drive for Thinness ($\beta = - .10$, $p = .029$), but not Bulimia, and no direct effects of observed parental hostility. No significant main effects of 5-HTTLPR or interaction between 5-HTTLPR and observed parenting behaviours were observed.
The models investigating moderation by 5-HTTLPR in the relationship between observed parental hostility and disordered eating do not include gene x covariate and environment x covariate contrast terms due to issues with collinearity. Although Keller (2014) argues collinearity functions to control for alternate explanations of any GxE interaction, the gene x environment interaction showed minimal change regardless of whether the additional contrast terms were included in the models. Therefore, the simpler model less affected by collinearity is presented.

The follow-up analysis investigating self-reported parental warmth and use of harsh punishment in the present sub-sample revealed main effects of the warmth and punishment on Bulimia scores ($\beta = -0.14, p = .013$ and $\beta = 0.14, p = .011$, respectively) but not Drive for Thinness scores. These results mirrored those of the larger sample in Study 1.

**DISCUSSION**

We adopted a multi-method assessment approach in two studies to investigate the relationship between parental behaviours (warmth, harsh punishment, and hostility) and disordered eating attitudes and behaviours. We also investigated whether the relationship between parenting factors and eating pathology was moderated by the serotonin transporter 5-HTTLPR polymorphism. Results indicated that greater parent-reported warmth was associated with lower bulimia symptoms, and in concordance, parent-reported use of harsh punishment techniques was associated with greater bulimia symptoms. Conversely, greater observed parental warmth was associated with lower adolescent drive for thinness, while greater observed parental hostility did not predict either disordered eating outcome. No moderation by 5-HTTLPR was identified in any of these relationships.
Parental warmth and harsh punishment

Findings of a relationship between parental warmth and use of harsh punishment techniques with disordered eating attitudes and behaviours supports the results of past studies that have assessed these parental behaviours via questionnaires. For example, parental warmth and care have been identified as lower in families of patients with AN and BN compared to controls (Tetley, Moghaddam, Dawson, and Rennoldson, 2014), while parental harsh punishment has been associated with BN in a number of studies (Rorty, Yager, & Rossotto, 1995; Stuart et al., 1990), with these patterns of findings also reflected in studies of child development more broadly (e.g., Bender et al., 2007; Yap, Pilkington, Ryan, & Jorm, 2014).

However, few studies have investigated these associations using observed parenting behaviours, as we have here, and a novel pattern of results was identified compared with research based on questionnaires. Across both samples, the two self-reported measures of parenting behaviours were clearly associated with adolescent bulimia symptoms, but neither was related to drive for thinness. Conversely, the observed parenting behaviours tended to be associated with drive for thinness but not bulimia. Other studies have also found limited correspondence between parent responses on questionnaires and their actual observed behaviours (e.g., Kallstrom-Fuqua, 2004). Such discrepancies are not unexpected for a number of reasons, including socially desirable or idiosyncratic responding to a questionnaire, or issues of ecological validity during observational tasks. It is also possible that the two measures of parental warmth used in the present study tapped into slightly different constructs. The observational measurement may have had a stronger focus on caring and warm behaviour displayed by the parent, irrespective of the child’s behaviour, while the questionnaire measure perhaps reflected more greatly the level of general positive interaction between parent and child, albeit
from the parent’s perspective. This may suggest that the precise relationship between parental behaviours and adolescent disordered eating is highly nuanced and varies according to the specific element of parenting or disordered eating measured.

**Gene x Environment Interactions**

The lack of significant GxE interactions identified in the present study contrasts with Karwautz et al. (2011) \((N = 256)\), who found that problematic parenting styles interacted with the s-allele of 5-HTTLPR to predict AN diagnosis in a discordant sister-pair sample. One possibility is that the influence of 5-HTTLPR may only be evident when clinical-level disordered eating pathology, such as AN, is considered. Another consideration is that Karwautz et al. (2011) examined parental control, a different parental behaviour to those examined in the present study. The other study identifying a possible interaction between parenting and genes \((p = 0.06)\) in the ED field also investigated parental control (van Strien et al., 2010) \((N = 276)\). One possibility therefore is that parental control but not other parenting behaviours may interact with genetic factors to predict eating pathology. However, it remains possible that discrepant findings between the current study and previous investigations are due to low power in both Karwautz et al. (2011) and van Strien et al. (2010), which increases the chances of false positive findings (Duncan, Pollastri, & Smoller, 2014). Sample sizes required for genetic association studies are very large due to the small effect sizes purportedly involved, and the present sample size \((N = 650)\) was more than double that of the previous two studies identifying GxE interactions involving parenting in EDs. Similarly, numerous past studies that have identified plasticity related to the 5-HTTLPR s-allele in other fields, and indeed have been cited as early evidence of this effect, have been vastly underpowered. A large portion of these studies included fewer than one
hundred and fifty participants (e.g., Kim, 2010; Pluess, Belsky, Way, & Taylor, 2010; Taylor et al., 2006; Wilhelm et al., 2006).

The lack of moderation by 5-HTTLPR in the relationship between parental warmth and bulimic behaviours and drive for thinness supports the view that this polymorphism does not act as a plasticity allele in the presence of family environments typically considered ‘protective’. This is also reflected in the fact that, to the authors’ knowledge, no prior study investigating GxEs in the ED field has reported differential susceptibility in response to positive environments (distinct from absence of negative stressors). In other fields, parental warmth and 5-HTTLPR have not been heavily studied within a GxE framework, with the few existing studies presenting a mix of results (e.g., Hankin et al. 2011, Kochanska, Kim, Barry, & Philibert, 2011). Given the large-scale publication bias that purportedly affects studies of GxE interactions (Duncan & Keller, 2011), it cannot be ascertained how many other unpublished investigations have failed to find GxE effects involving parental warmth.

**Strengths and Limitations**

The present paper contributes to research examining parenting factors in disordered eating by analysing a large-scale community sample, with a focus on replication across two samples using multi-method multi-source assessment. In contrast, past studies have largely adopted questionnaire-based measures, often retrospective, with limited investigation using observational measurement of parenting behaviours. A few limitations of the present study must also be noted. The present study did not examine comorbid psychopathology, so was unable to assess whether this factor may possibly mediate the identified relationships between particular parenting behaviours and disordered eating outcomes. The present study also examined the biallelic model of 5-
There is some evidence to suggest that examination of a triallelic model may better reflect \textit{5-HTTLPR} activity (Wendland et al., 2006), with support from some studies in the ED field (Steiger et al., 2009; Stoltenberg, Anderson, Nag, & Anagnopoulous, 2012 but not Richardson et al., 2008), although more broadly findings have been tentative (e.g., Uher & McGuffin, 2008). In the present study data were not available to examine the triallelic model, which may yet have resulted in identification of a GxE interaction in the present sample. Furthermore, the sample size, particularly in Study 2, was relatively underpowered for genetic association studies (although it was above the mean \( N = 288 \) of GxE studies in the ED field; Rozenblat et al., 2017). Conversely, the sample in Study 1 constitutes the third largest investigation of its kind in the field (following Akkermann et al. 2012, \( N = 765 \), and the combined-samples analyses in Rozenblat et al., 2017, \( N > 1,000 \)). This is an important strength. Furthermore, assessing disordered eating attitudes and behaviours dimensionally increases power and sensitivity compared to case-control designs, in which the ‘clinical’ group may represent heterogeneous underlying patterns of eating pathology or genetic influences (Abbott, 2008). Assessing disordered eating symptoms in a population-based sample also increases generalisability of results and utility for informing prevention and early intervention initiatives, although may limit the applicability of results to clinical populations. Finally, as with all studies of candidate genes, \textit{5-HTTLPR} represents only one polymorphism in a system of genetic factors likely to be involved in ED pathology, and thus such investigations constitute stepping stones towards increasing our understanding of the genetic underpinnings of EDs.
Clinical Implications and Future Directions

Results support a clear relationship between parent behaviours and adolescent disordered eating, across both genders, independent of individual genetic predisposition. Findings can inform parenting programs targeting ED prevention or psychoeducation for ‘at risk’ families. For example, one systematic review of parent-focussed ED prevention initiatives stated early positive findings required support from additional research regarding the family setting (Hart, Cornell, Damiano, & Paxton, 2015). Adoption of longitudinal designs would also help establish whether parental behaviours constitute protective/risk factors, or are better conceptualised as correlates of eating pathology. Furthermore, investigations into genetic plasticity could also expand beyond 5-HTTLPR to examine cumulative plasticity across numerous polymorphisms testing multiple positive environmental factors. Selection of positive eating-related outcomes, such as positive body image (Webb, Wood-Barcalow, & Tylka, 2015), as opposed to absence of eating-related pathology, may also be more appropriate for reflecting the relationship between genetic plasticity and environmental factors. However, it is also possible that regardless of precise measurement techniques, the role of 5-HTTLPR in EDs may prove to be very minor at best, such that continued investigation into parenting and other environmental factors may constitute a more fruitful approach to untangling the complex aetiology of eating pathology.

Acknowledgements: The ATP study is located at The Royal Children’s Hospital Melbourne and is a collaboration between Deakin University, The University of Melbourne, the Australian Institute of Family Studies, The University of New South Wales, The University of Otago (NZ), and the Royal Children's Hospital; further information available at www.aifs.gov.au/atp. The views expressed in this paper are
those of the authors and may not reflect those of their organisational affiliations, nor of other collaborating individuals or organisations. We acknowledge all collaborators who have contributed to the Australian Temperament Project, especially Professors Ann Sanson, Margot Prior, Frank Oberklaid, John Toumbourou and Ms Diana Smart. We would also like to sincerely thank the participating families for their time and invaluable contribution to the study. This paper forms part of Vanja Rozenblat’s PhD with publication undertaken at The University of Melbourne.

**Conflicts of interest:** The authors declare no conflicts of interest.
References


12. CHAPTER 12

Further Methodological Details and Discussion of Study 3

The present chapter expands upon the findings in Studies 3a and 3b (originally Study 1 and Study 2 in the published manuscript) by presenting material that was beyond the scope of the original publication. This includes providing details relating to the methodology of the observational component of Study 3b, discussing the advantages and limitations of observational and self-report measures of parenting behaviour, and performing additional statistical analyses that were beyond the scope of the published manuscript. Results are then discussed in the context of existing GxE interaction research findings and current theoretical debates.

12.1. Methodological details pertaining to observational measurement

12.1.1. Home Visit Study

There is substantial overlap in the methodology of Studies 3a and 3b and the previous paper of this thesis, Study 2. For details regarding the ATP, including background information, recruitment, data collection, etc., please refer to Chapter 9. This chapter also features further information regarding collection of saliva samples and the observed parenting variables. The present chapter will elaborate on the Home Visit Study, which involved participants engaging in an observational task that was recorded and coded using the Iowa Family Interaction Rating Scales (IFIRS; Melby et al., 1998; Melby & Conger, 2001), and will further explore the observational and self-reported parenting measures used to examine the relationship between parenting behaviours, 5-HTTLPR, and disordered eating.
The Home Visit study was conducted following the 11th ATP survey (age 15/16 years) to more closely explore the relationship between parent-child interaction and a number of high-risk or problem outcomes in adolescence, as well as to examine links between parenting behaviours and adolescent resilience. Participants were invited to the study based on their responses to the 11th survey. For details regarding sampling, please refer to the methods section of the Studies 3a and 3b published manuscript. In short, participants in the Home Visit Study sub-sample (examined in Study 3b) were classified in one of five groups: Those who reported substance abuse, those who reported conduct issues, those who indicated depressed mood, a group that was considered at ‘high risk’ of these issues based upon underlying patterns of responses to earlier ATP surveys but who did not report any of the problem outcomes at the time of assessment, and a ‘risk free’ comparison group. The composition of this sub-sample does not bear direct relevance to the current investigation, however it is noteworthy to highlight that the sub-sample engaging in the family interaction task largely represented a selective ‘high risk’ group of participants drawn from the larger sample of participants who had completed the 11th survey. Parents of adolescents in both Studies 3a and 3b also responded to the ATP Family Practices Questionnaire (Sanson & Prior, 1999) to provide measures of self-reported parental warmth and use of harsh punishment.

From the 650 participants invited to take part in the sub-study (38% of the total ATP cohort still participating in 1998), 612 families consented, with 445 families taking part in the video-recorded family interaction task resulting in 435 codeable video tapes. Families were invited via mail invitations that included a letter of invitation and provided some information about the study. Follow-up telephone calls were made to gauge participant interest in the study and appointment times were scheduled for a home visit for families who consented to participate. Prior to the scheduled visit, participants
were mailed out a questionnaire and a reply-paid envelope to return the completed booklet.

During the home visit adolescents and one of their parents engaged in two tasks, a discussion of family life (Task 1) and a problem solving task (Task 2). Only data from Task 1 was included in the present investigation, as only a selected number of video tapes of Task 2 were coded due to financial and time constraints during the data collection process. The Family Discussion task involved the parent and adolescent discussing their responses to a number of questions about family life recorded on a set of cards (e.g., parental rules and fairness, the teenagers’ accomplishments and disappointments) while under video-observation. The interviewer directly observed two ‘practice’ discussions and provided feedback if necessary, before leaving the room to provide privacy for the dyads to discuss the topics raised by the remaining 14 cards. Task 1 lasted approximately 15 minutes.

The interviews and coding of the video tapes were conducted by a group of seven extensively trained observers. As part of this training, the observers completed written and observational tests during which they were required to score 80% or greater. Coders were blind to participant group membership (e.g., high risk, substance use, etc.) and did not code the tapes that they were involved in collecting, in order to maintain participant confidentiality. Video recordings were identified by participant ID number only. A total of 20% of the recordings were double coded to measure inter-rater reliability, with coders unaware of which tapes were selected for reliability checks. Any discrepancies (where coders differed by two or more points - see below) were discussed among the coders and resolved.
12.1.2. Iowa Family Interaction Rating Scale

Video tapes were coded using the IFIRS (Melby et al., 1998). The IFIRS is an observational coding system originally developed at Iowa State University’s Institute for Social and Behavioural Research (Melby & Conger, 2001). It is a macro-analytic coding tool designed to measure the global behavioural and emotional aspects of individuals and exchanges between family members, as well as qualities of the overall family processes. In its initial conception, the IFIRS was designed to code observed behaviours in family discussions or problem solving tasks with adolescent aged children (Lorenz & Melby, 1994), such as those specifically used in the present study. The coded variables are believed to represent relatively stable and ongoing behavioural characteristics relating to the interaction styles between participants (Melby & Conger, 2001). Behaviours are rated on the basis of observed behavioural indicators, with behavioural frequency and intensity, affect, and context used to help categorise observed behaviours. The IFIRS provides variables concerning four major types/levels of behaviour: 1) individual characteristics, which assess the general characteristics/traits of the individual (independent of the recipient; e.g., humour, sadness, positive mood); 2) dyadic interactions, which assess the behaviour of the individual towards a specific other individual (this includes parenting scales; e.g., hostility, warmth, communication); 3) dyadic relationships, which measure some characteristics of the relationships between two individuals (e.g., relationship quality); and 4) group scales, which assess the characteristics of the family as a whole (e.g., family enjoyment, agreement). All scales are based upon measuring verbal and non-verbal behaviours, as well as affective and contextual dimensions of family interactions (Melby et al., 1998).
The two variables used in the present study, parental warmth and parental hostility, were drawn from the dyadic interaction scales and measure the behaviour of the parent when directed to their adolescent child. The parental hostility scale reflected participants engaging in behaviour that was hostile, angry, critical, disapproving, rejecting, or contemptuous. It also included engagement in the following behaviours: Verbal attack, physical attack, contempt, angry coercion, escalating hostile behaviour, and reciprocating hostile behaviour. The parental warmth scale measured behaviour that expressed care, concern, support, or encouragement, and also included the following behaviours: Endearment, physical affection, escalating warm/supportive behaviour, and reciprocating warm/supportive behaviour. Scales were rated on a 9-point Likert scale, ranging from (1) not at all characteristic to (9) mainly characteristic.

The IFIRS is an ideal tool for measuring family interaction styles and allows analysis of how these interactions are related to disordered eating. A number of factors support this claim. The IFIRS has established validity (compared to self, sibling, and parent reports of behaviours using correlational and factor-analytic procedures; Melby, Conger, & Puspitawati, 1999) and reliability (intra-class correlations generally ranging from .55 to .85; Melby & Conger, 2001; Williamson, Bradbury, Trail, & Karney, 2011). Results derived from the IFIRS scales have been associated with a range of adolescent adjustment outcomes and adolescent problem behaviour (Ge, Best, Conger, & Simons, 1996; Melby, Conger, Conger, & Lorenz, 1993; Simons & Conger, 2007; Simons, Johnson, Conger, & Elder, 1998). Finally, the IFIRS is administered by trained observers. As such, it is a substantial improvement upon the coding systems used in the seven previously published observational family interaction studies (Blair et al., 1995; Humphrey, 1989; Kog & Vandereycken, 1989; Lattimore et al., 2000; Ratti et al., 1996; Stasch & Reich, 2000; Thomas et al., 2012). Study 3b therefore constituted the first
investigation to date conducting a large scale analysis of the relationship between observationally-measured family interaction styles and disordered eating using a well-established observational coding system such as the IFIRS.

12.1.3. Comparison of self-reported and observational measurement of parenting behaviour

Measurement of family interactions and parenting behaviours is typically conducted using self-report measurement (McLeod et al., 2007). This carries a number of advantages, primarily convenience and ease of measurement, with questionnaires often simple to complete by the participant and quick to score by the researcher. Self-report measures are also able to assess constructs that may not be visible to an observer, such as subjective feelings of low mood, opinions, beliefs, etc. They can often reflect global and persistent qualities of parenting behaviours or parent-child relationships. Questionnaire responses regarding parenting behaviour can be completed by parents or children, although parent response are argued to better align with actual parent behaviours (Taber, 2010), particularly regarding less concrete parenting concepts and behaviours. Meanwhile, measuring child-response may be appropriate in studies seeking to assess the relationship between perceived parenting and other constructs. However, care must be taken regarding shared method or source variance when both a predictor and outcome variable are reported by the same individual (La Greca & Lemanek, 1996). Given the above considerations, the arguably ‘more objective’ parent-response questionnaires were selected for the present study.

Nonetheless, there remain a couple of factors that may introduce bias to self-reported measures of parenting behaviour. Firstly, parents responding to questionnaires may be inclined to do so in a socially-desirable manner (Podsakoff, MacKenzie, Lee, &
Podsakoff, 2003), which may somewhat reduce estimates of less desirable behaviours (e.g., use of harsh punishment) and possibly skew results in favour of positive behaviours. Furthermore, parent responses may be somewhat limited by self-awareness, by individual differences in beliefs regarding what is considered ‘typical’, or by varied understandings regarding the meaning of a given construct (e.g., different conceptualisations of ‘warmth’ or what constitutes supportive behaviour; Bailey, DeOliveira, Wolfe, Evans, & Hartwick, 2012; Gardner, 2000; Podsakoff et al., 2003). Finally, as self-report measures rely on the parent’s own evaluation of their parenting behaviours, they reflect parental perceptions of parenting rather than providing a strictly objective measure of given parenting behaviours.

In contrast, observational measurement of parenting behaviour allows for a more objective and reliable assessment of the behaviours of interest that is consistent across participants (Aspland & Gardner, 2003; Gardner, 2000). As opposed to claiming that the researcher is ‘better at assessing’ parenting behaviours than participants themselves, these techniques are considered ‘objective’ because they assess all participants according to the same metric, using a standardised, established coding tool, that is applied to recordings of the behaviour in a systematic manner by trained observers (Kerig & Lindahl, 2000; Margolin et al., 1998). They also allow for coding particular behaviours of which parents may be unaware, such as certain automatic behaviours present during a parent-child interaction (Gardner, 2000). These factors increase the construct validity of observed parenting behaviours compared to those measured via self-report. Indeed, many longitudinal studies and meta-analyses have revealed excellent predictive power and construct validity using observational measurement of parenting behaviours, including when compared to self-reported measures (Gardner,
Sonuga-Barke, & Sayal, 1999; McLeod et al., 2007; Shlafer, Raby, Lawler, Hesemeyer, & Roisman, 2015; Webster-Stratton, 1998; Zaslow et al., 2006).

Like most psychological assessment, observational techniques for measuring parenting behaviour also carry some limitations. Primarily, there is concern regarding the ecological validity of behaviour displayed during an observational task (i.e., parents and children may behave in an altered or more socially desirable manner due to the knowledge that they are being observed; Gardner, 2000). It is also the case that observational measurement assesses behaviour during a single setting, while self-reported measurement may reflect broader parenting behaviour across time and context (Gardner, 2000). Finally, in terms of practicality, observational techniques are resource intensive to deliver due to the time required to carry out and subsequently code interactions, including the need to ensure sufficient inter-rater reliability (Margolin et al., 1998). However, many issues encountered by observational techniques are also present with the use of self-report questionnaires (e.g., participant affect at time of measurement and socially-desirable responding; Bornstein et al., 2015; Gardner, 2000).

Given these potential issues, the use of observational techniques in Study 3b was aligned with a number of recommendations by Gardner (2000) regarding factors that can help increase the ecological validity of observational measurement. For example, Gardner (2000) found that observational measurement was less influenced by issues of ecological validity when performed in the home (as in the ATP Home Visit Study) compared to a laboratory setting. This is likely due to the fact that the home constitutes a ‘natural’ environment in which parent-child interaction typically takes place, as opposed to a more contrived laboratory setting in which the fact participants are under observation is likely far more salient. Participants may also feel less ‘at ease’ in foreign
settings and adjust their behaviour accordingly. However, the review by Gardner (2000) suggested that presence of an observer (e.g., video recording, as in the present study, or the physical presence of the researcher) does not have a large impact on the types of behaviours displayed by participants or topics discussed (known as ‘observer reactivity’). This suggests that observational tasks conducted in the home setting are most well-suited to measuring parenting behaviour in parent-child interactions.

The type of task used in an observational study may also somewhat influence the types of behaviours elicited. For example, one study found higher levels of criticism, over-control, and anxious behaviour in structured compared to unstructured tasks (Ginsburg, Grover, Cord, & Ialongo, 2006). It is of course important to select a task in which the behaviour under observation (e.g., parental criticism) is likely to be elicited within the duration of the observation (Aspland & Gardner, 2003; Margolin et al., 1998). In the present study, the observed task involved a discussion of several issues pertaining to family life, a number of which were somewhat contentious in nature (e.g., disappointments, parental rules) and likely to provoke some level of parental hostility, or conversely, parental warmth, the variables under investigation in Study 3b.

Finally, the most superior way of reducing error variance due to measurement techniques is to assess parenting via a multi-source multi-method approach (Podsakoff et al., 2003), by including information gleaned from self-report questionnaires in combination with observational measures of parenting behaviours elicited during parent-child interaction (Dishion, Li, Spracklen, Brown, & Haas, 1998). Adopting both methods of measurement capitalises on the potentially unique contribution provided by each measurement type, with self-report and observationally-measured data possibly providing complimentary information regarding parenting behaviour, and reduces issues
relating to shared method variance (La Greca & Lemanek, 1996). Therefore Studies 3a and 3b constituted a unique opportunity to explore the relationship between parenting behaviours and disordered eating using the favoured multi-method multi-source method.

12.2. Additional analyses of data from Studies 3a & 3b

A number of statistical analyses that were beyond the scope of the Study 3a and 3b published manuscript, such as correlational analyses and additional regression models, are explored in the following segment. Firstly, a correlational analysis of the self-reported and observed parenting variables is reported, in order to examine the degree of similarity of scales assessed using the two methods. This is followed by additional regression analyses whereby the parenting variables measured in each study that were originally assessed in separate regression models (warmth and harsh punishment in Study 3a and observed warmth and hostility in Study 3b) are re-analysed within the same regression equation, to assess whether these variables have a unique or overlapping association with the disordered eating outcome variables. These additional analyses help facilitate more accurate interpretation of results, their implications, and avenues for further investigation.

12.2.1. Correlational analysis of self-reported and observed parenting behaviours

The two measurement techniques used to assess parenting behaviour, a self-report questionnaire and an observed parent-child interaction, contain some degree of overlap in what they purport to assess. Both methods provided an assessment of parental warmth, while they measured slightly different variables on the theoretically opposite side of the parenting behaviours spectrum – use of harsh punishment (self-reported) and
hostility (observed). Although the two methods ostensibly assess similar constructs, it is worthwhile to examine the actual degree of association between the self-reported and observed parenting behaviours. A correlational analysis including both parenting measures, EDI-2 (Garner, 1991) scores, and covariates (age, BMI) was therefore conducted (see Table 15).

Inspection of the relationship between the disordered eating variables (Drive for Thinness and Bulimia) and the measures of parenting behaviours reveals that EDI-2 (Garner, 1991) Bulimia was negatively associated with self-reported parental warmth ($r = -.15, p = .009$) and positively related to self-reported use of harsh punishment ($r = .14, p = .018$), while it was not correlated with either observational measure of parenting behaviour (warmth or hostility). Meanwhile, EDI-2 Drive for Thinness was positively associated with observed hostility ($r = .13, p = .020$). These correlations reflect the overall pattern of results across Studies 3a and 3b, with self-reported parenting behaviours associated with EDI-2 Bulimia outcomes, while observed parenting behaviours tended to be related to participant Drive for Thinness scores.

Inspection of correlations amongst the parenting measures themselves revealed that self-reported parental warmth was negatively associated with self-reported parental use of harsh punishment ($r = -.26, p < .001$) and observed hostility ($r = -.13, p = .024$), while the correlation between self-reported and observed parental warmth only approached significance ($r = .11, p = .066$). Meanwhile, observed warmth was negatively correlated with observed hostility ($r = -.120, p = .036$), with its association with self-reported use of harsh punishment only approaching significance ($r = -.106, p = .066$). Self-reported use of harsh punishment and observed hostility showed a similar association ($r = .106, p = .067$).
Table 15

*Correlations Between Self-Reported Parenting Behaviours, Observed Parenting Behaviours, EDI-2 Drive for Thinness and Bulimia scales, Participant BMI, and Participant Gender.*

<table>
<thead>
<tr>
<th></th>
<th>DFT</th>
<th>Bulimia</th>
<th>Self-reported Warmth</th>
<th>Self-reported Punishment</th>
<th>Observed Warmth</th>
<th>Observed Hostility</th>
<th>Gender</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFT</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sg</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Bulimia</td>
<td>R</td>
<td>.503**</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sg</td>
<td>&lt;.001</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Self-report Warmth</td>
<td>R</td>
<td>-.113</td>
<td>-.151**</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sg</td>
<td>.051</td>
<td>.009</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Self-report Punishment</td>
<td>R</td>
<td>.021</td>
<td>.136*</td>
<td>-.257**</td>
<td>-</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Sg</td>
<td>.772</td>
<td>.018</td>
<td>&lt;.001</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Observed Warmth</td>
<td>R</td>
<td>-.038</td>
<td>-.023</td>
<td>.106</td>
<td>-.106</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sg</td>
<td>.505</td>
<td>.688</td>
<td>.066</td>
<td>.066</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Observed Hostility</td>
<td>R</td>
<td>.134*</td>
<td>.027</td>
<td>-.120*</td>
<td>-</td>
<td>.106</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sg</td>
<td>.020</td>
<td>.634</td>
<td>.024</td>
<td>.067</td>
<td>.036</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Gender</td>
<td>R</td>
<td>.555**</td>
<td>.272**</td>
<td>-.045</td>
<td>-.010</td>
<td>.106</td>
<td>.096</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Sg</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.433</td>
<td>.860</td>
<td>.064</td>
<td>.094</td>
<td>*</td>
</tr>
<tr>
<td>BMI</td>
<td>R</td>
<td>.229**</td>
<td>.008</td>
<td>-.013</td>
<td>-.105</td>
<td>.032</td>
<td>.005</td>
<td>.029 *</td>
</tr>
<tr>
<td></td>
<td>Sg</td>
<td>.001</td>
<td>.901</td>
<td>.853</td>
<td>.124</td>
<td>.635</td>
<td>.939</td>
<td>.672 *</td>
</tr>
</tbody>
</table>

Notes. Values in bold indicate significant associations; * = significant at the .05 level; ** = significance at the .001 level; BMI = Body Mass Index; EDI-2 = Eating Disorder Inventory-2 (Garner, 1991); DFT = EDI-2 Drive For Thinness scale; r = Pearson’s r correlation.

The finding that self-reported and observed parental warmth were not more strongly associated is somewhat surprising, and indicates that the two measures of parental warmth may have tapped into slightly different aspects of parental behaviour. Likewise, a somewhat stronger association between (self-reported) use of harsh punishment and (observed) parental hostility may have seemed more intuitive, although
the two measures do not assess directly equivalent parental behaviours. The relatively weak associations between the self-reported and observed parenting behaviours likely explains the rather distinct pattern of findings reported in Study 3, whereby self-reported measures tended to be associated with bulimic symptoms (Study 3a) and observed parental behaviours with participants’ desire for thinness (Study 3b).

12.2.2. Additional regression models of parenting behaviours and disordered eating

12.2.2.1. Study 3a: Self-reported parenting behaviours

In Study 3a, the relationship between parenting variables, 5-HTTLPR, and disordered eating was assessed individually for each parenting variable in a separate linear regression model. Under these analyses, there were direct effects of self-reported warmth and harsh punishment on EDI-2 (Garner, 1991) Bulimia scores, and no direct effects of either self-reported parenting variable on EDI-2 Drive for Thinness scores. Another approach could include to examine both parenting variables in the same regression model, to explore whether parental warmth and use of harsh punishment techniques are separately related to disordered eating, or on the contrary, display ‘overlap’ (i.e., shared statistical variance) in their prediction of the disordered eating outcomes.

Table 16 displays the results of this supplementary analysis examining the role of both self-reported warmth and use of harsh punishment on EDI-2 (Garner, 1991) scales. In the model examining Drive for Thinness, neither of the self-reported parenting variables were found to be significant predictors, as expected based on findings in the separate models reported in Study 3a. In the model examining the outcome variable Bulimia, self-reported parental use of harsh punishment remained a significant predictor.
when examined in the same model as parental warmth, although the latter variable no longer significantly predicted Bulimia scores ($p = .054$). Effect sizes in the combined model were only slightly smaller than when parenting variables were analysed individually: (warmth, $B = -.08, 95\% \text{ CI: } -.16, -.001$, compared to $B = -.11, 95\% \text{ CI: } -.18, -.03$ in Study 3a; and punishment, $B = .10, 95\% \text{ CI: } .01, .19$, compared to $B = .13, 95\% \text{ CI: } .04, .22$ in Study 3a). The pattern of results suggests that the statistical effects of the two parental factors on adolescent bulimic tendencies overlap to a certain extent. It may be the case that the parental warmth or use of harsh punishment variables may reflect an overarching ‘parenting behaviours’ variable that is associated with increased adolescent bulimic tendencies.
Table 16

Main Effects of Self-Reported Parental Warmth and use of Harsh Punishment in Predicting EDI-2 Bulimia and Drive for Thinness Outcomes in a Combined Model, Controlling for Gender and BMI (N = 650).

<table>
<thead>
<tr>
<th>Variable</th>
<th>B (SE)</th>
<th>Lower CI</th>
<th>Upper CI</th>
<th>t-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcome: Drive for Thinness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-1.46 (.47)</td>
<td>-2.38</td>
<td>-.55</td>
<td>-3.15</td>
<td>.002</td>
</tr>
<tr>
<td>Warmth</td>
<td>-.04 (.06)</td>
<td>-.16</td>
<td>.09</td>
<td>-.60</td>
<td>.552</td>
</tr>
<tr>
<td>Harsh Punishment</td>
<td>-.02 (.07)</td>
<td>-.16</td>
<td>.12</td>
<td>-.29</td>
<td>.769</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>-.11 (.08)</td>
<td>-.27</td>
<td>.05</td>
<td>-1.35</td>
<td>.176</td>
</tr>
<tr>
<td>Gender</td>
<td>1.17 (.08)</td>
<td>1.02</td>
<td>1.32</td>
<td>15.54</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>BMI</td>
<td>.10 (.01)</td>
<td>.08</td>
<td>.13</td>
<td>8.09</td>
<td>&lt; .001</td>
</tr>
<tr>
<td><strong>Outcome: Bulimia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1.24 (0.31)</td>
<td>.62</td>
<td>1.85</td>
<td>3.97</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Warmth</td>
<td>-.08 (0.04)</td>
<td>-.16</td>
<td>.001</td>
<td>-1.93</td>
<td>.054</td>
</tr>
<tr>
<td>Harsh Punishment</td>
<td>.10 (0.05)</td>
<td>.01</td>
<td>.19</td>
<td>2.14</td>
<td>.032</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>-.05 (0.05)</td>
<td>-.15</td>
<td>.06</td>
<td>-.88</td>
<td>.380</td>
</tr>
<tr>
<td>Gender</td>
<td>.33 (0.05)</td>
<td>.24</td>
<td>.43</td>
<td>6.70</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI</td>
<td>.01 (0.01)</td>
<td>-.01</td>
<td>.03</td>
<td>1.15</td>
<td>.257</td>
</tr>
</tbody>
</table>

12.2.2.2. Study 3b: Observed parenting behaviours

The aforementioned process of examining disordered eating outcomes when both parenting variables are included in the same model (parental warmth, considered a positive parenting behaviour, and parental use of harsh punishment, considered on the
opposite side of the spectrum) is also worth inspecting for the observationally-measured parenting variables, observed parental warmth and observed parental hostility. In the original analyses in Study 3b, observed parental warmth was related to lower adolescent EDI-2 (Garner, 1991) Drive for Thinness scores, while there was no relationship between observed parental warmth and EDI-2 Bulimia scores, or observed parental hostility and either disordered eating outcome. In the combined analysis (see Table 17), the same pattern of results was noted, albeit the effect size and p-value for parental warmth in the model predicting EDI-2 Drive for Thinness ($B = -.06, 95\% \text{ CI: } -.12, p < .01$) revealed a marginally weaker effect than when parental warmth was investigated in a model alone, as in Study 3b ($B = -.07, 95\% \text{ CI: } -.13, -.01$). This suggests some overlap in ‘shared variance’ between the observed parental warmth and hostility variables when predicting Drive for Thinness, although parental warmth appears to maintain a unique contribution to this outcome variable.
Table 17.

*Main Effects of Observed Parental Warmth and Hostility in Predicting EDI-2 Bulimia and Drive for Thinness Outcomes in a Combined Model, Controlling for Gender and BMI (N = 304).*

<table>
<thead>
<tr>
<th>Variable</th>
<th>B (SE)</th>
<th>Lower CI</th>
<th>Upper CI</th>
<th>t-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcome: Drive for Thinness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-.83 (.48)</td>
<td>-1.18</td>
<td>.13</td>
<td>-1.72</td>
<td>.090</td>
</tr>
<tr>
<td>Warmth</td>
<td>-.06 (.03)</td>
<td>-.12</td>
<td>&lt;.01</td>
<td>-1.96</td>
<td>.050</td>
</tr>
<tr>
<td>Hostility</td>
<td>.06 (.04)</td>
<td>-.02</td>
<td>.14</td>
<td>1.50</td>
<td>.134</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>-.18 (.13)</td>
<td>-.43</td>
<td>.08</td>
<td>-1.36</td>
<td>.175</td>
</tr>
<tr>
<td>Gender</td>
<td>1.35 (.12)</td>
<td>1.12</td>
<td>1.58</td>
<td>11.30</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>BMI</td>
<td>.06 (.02)</td>
<td>.02</td>
<td>.11</td>
<td>3.19</td>
<td>.003</td>
</tr>
<tr>
<td><strong>Outcome: Bulimia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1.44 (0.41)</td>
<td>.59</td>
<td>2.29</td>
<td>3.56</td>
<td>.002</td>
</tr>
<tr>
<td>Warmth</td>
<td>-.02 (0.02)</td>
<td>-.06</td>
<td>.02</td>
<td>-.95</td>
<td>.344</td>
</tr>
<tr>
<td>Hostility</td>
<td>-.003 (0.05)</td>
<td>-.06</td>
<td>.05</td>
<td>-.10</td>
<td>.921</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>-.06 (0.09)</td>
<td>-.23</td>
<td>.12</td>
<td>-.66</td>
<td>.508</td>
</tr>
<tr>
<td>Gender</td>
<td>.40 (0.08)</td>
<td>.24</td>
<td>.56</td>
<td>4.84</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI</td>
<td>-.001 (0.02)</td>
<td>-.04</td>
<td>.04</td>
<td>-.07</td>
<td>.946</td>
</tr>
</tbody>
</table>
12.3. Discussion of main effects of parental warmth and use of punishment/hostility

The findings in Study 3a, Study 3b, and in the present chapter, show an inverse relationship between parental warmth and disordered eating (specifically, bulimic tendencies and drive for thinness). This reflects a substantive body of cross-sectional research that has also found that greater parental warmth is associated with lower disordered eating symptoms (Hosseinzade et al., 2013; Tseng et al., 2014) and reduced likelihood of a clinical ED diagnosis (Caglar-Nazali et al., 2014; Tetley et al., 2014; Ward et al., 2000). This relationship may exist for a number of reasons. For example, as discussed in Chapter 2, exposure to parental warmth is associated with greater adolescent self-esteem (Orth, 2017) and may better equip at-risk adolescents to cope with challenging situations and other specific environmental risks (McVey et al., 2002). Parental warmth may also function as a general protective factor against psychopathology, with an inverse relationship between parental warmth and adolescent psychopathology identified across a number of other pathologies (e.g., depression, anxiety; Yap et al., 2014).

While most of the literature examining the relationship between parental warmth and disordered eating has involved self-reported measurement (Tetley et al., 2014), the present study corroborated these findings using observational measurement techniques. The use of a multi-source multi-method approach to assessing parental warmth resulted in an interesting pattern of findings, with self-reported warmth in Study 3a related to lower bulimic tendencies, while observed warmth in Study 3b was found to be inversely related to the tendency to aspire towards thinness. As discussed in Studies 3a and 3b, it is possible this reflects a slight difference between the precise elements of the parent-child relationship measured by the two ‘parental warmth’ variables in the two studies.
For example, while the observed measure in Study 3b was based on coding parental behaviours that expressed care, concern, support, or encouragement, the self-reported measure of parental warmth in Study 3a tended to focus more so on parent perceptions of the closeness and level of positive interaction in the parent-child relationship (e.g., “Most of the time, how well do you get along with your teenager?”). Indeed, the correlational analysis in Table 15 of the present chapter does not show a significant correlation between the two measures of warmth. This may suggest that bulimic tendencies and adolescent drive for thinness are related to positive parental behaviours in very specific and somewhat idiosyncratic ways.

Meanwhile, relatively few studies have examined the specific relationship between eating pathology and use of harsh punishment and parental hostility. Existing studies have found some evidence for a relationship between parental harsh punishment and eating pathology (Rorty et al., 1995), although one recent systematic review of 3 studies indicated that the relationship between harsh punishment and binge eating remained unclear (Saltzman, 2016). Thus, results of Study 3a provide timely further evidence supporting the presence of a relationship between greater parental use of harsh punishment and bulimia-spectrum pathology, using the largest sample tested to date, to the author’s knowledge.

Likewise, the role of parental hostility (as opposed to more general ‘negative’ parental measures, such as adverse parenting; Fairburn & Harrison, 2003) has not been specifically investigated as a factor in eating pathology, except in studies assessing broader concepts such as expressed emotion (Vaughn & Leff, 1976) and parenting styles (Baumrind, 1971). This body of research would suggest a likely role for parental hostility in EDs. For example, hostility is a feature of authoritative parenting style,
which is associated with EDs (Zubatsky et al., 2015) and is a domain of expressed emotion, with evidence that ED families are characterised by greater levels of expressed emotion than families of controls (Anastasiadou et al., 2014; Schmidt et al., 2015). However, results of Study 3b do not support a role for observed parental hostility in adolescent drive for thinness or bulimic tendencies. This may suggest that while expressed emotion, authoritarian parenting, and general ‘adverse parenting’ might be related to eating pathology, hostility specifically is not a key factor in this relationship. It is also possible that parents in Study 3b were less likely to display hostile behaviours in the family interaction task due to the knowledge they were being filmed, and thus this weakened any possible relationship between hostility and disordered eating. However, similar issues of desirable responding would also be present if using questionnaire data, as in Study 3a. The most prudent approach may be to supplement the findings based on observed measurement with findings based on other methodology that include specific investigation of parental hostility, before firm conclusions regarding the relationship between this parenting behaviour and adolescent disordered eating are established.

12.4. Discussion of gene x environment plasticity, parenting, and 5-HTTLPR

Aside from assessing the direct relationships between self-reported (Study 3a) and observed (Study 3b) parenting behaviours and adolescent disordered eating, Study 3 also aimed to test the plasticity hypothesis of GxE interactions (5-HTTLPR x parenting) in predicting the disordered eating outcomes. No GxE interaction or evidence for plasticity was found. To better contextualise this finding, it is prudent to briefly reconsider previous studies examining parenting within a GxE model in the ED field, and to also examine such studies in other pathologies.
As discussed in previous chapters, only three studies to date in the ED field have examined whether the relationship between parenting behaviours and ED or disordered eating outcomes is moderated by a genetic factor (Karwautz et al., 2011; Micali et al., 2017a; van Strien et al., 2010a). In these studies, Karwautz et al. (2011) reported a significant interaction between 5-HTTLPR and maladaptive parenting styles in predicting AN in a discordant sister-pair sample, while van Strien et al. (2010a) reported an interaction between the Taq1A polymorphism on DRD2 and parental control to predict greater emotional eating. While neither study explicitly tested for plasticity, visual inspection of figures included in both manuscripts suggests that participants with the allele typically considered ‘risky’ displayed lower disordered eating compared to those without the allele, under conditions of fewer problematic parenting styles in Karwautz et al. (2011), and lower parental control in van Strien et al. (2010a). It is worth mentioning that this was not the case in Study 1 or Study 2 of the present thesis for any of the significant GxE interactions identified (traumatic life events, sexual and physical abuse, and severe parental physical punishment). In the third study, Micali et al. (2017a), ‘mixed’ support was found for a role of maternal care and OXT-R in predicting ED behaviours (one out of twelve tested interactions was significant at the \( p = .03 \) level). No mention of plasticity or a visual diagram of the GxE interaction identified was included in this publication.

However, a number of cautions must be raised regarding these studies. Firstly, two of the studies used sample sizes considered low for studies of genetic association (\( N = 265 \) in Karwautz et al., 2011; \( N = 279 \) in van Strien et al., 2010a), increasing the likelihood that results represent false-positive findings (Duncan & Keller, 2011). Furthermore, in van Strien et al. (2010a), \( p \)-values were only of borderline significance (\( p = .06 \)) and authors did not perform \( p \)-value adjustment to correct for multiple testing,
which is noted to be a limitation of existing GxE interaction research (Duncan et al., 2014). While the sample size in Micali et al. (2017a) was the largest of any published candidate GxE interaction study in the ED field ($N = 3135$), the measurement of environmental factors was far less thorough than in other studies. For example, the outcome variable, ‘ED behaviours’, rather than being measured according to an established tool such as the EDI-2 (Garner, 1991) or EAT-26 (Garner et al., 1982), was coded as a binary yes/no based on whether women had self-reported any one of the following behaviours throughout their lives: Dietary restriction (skipping a day’s worth of food or three meals per week for three months), purging (once per week for three months), or engaging in any binge eating (once per week for three months). Furthermore, a host of statistical tests for GxE interactions were carried out in this study, between two OXT-R polymorphisms and three outcome variables (binge eating, purging, both binge eating and purging), indicating that multiple testing was a limitation of the present study. The alpha-threshold of $p < .05$ was not adjusted in any way to account for the multiple testing. Indeed, the significant GxE was only identified in a post-hoc test (examining a composite outcome variable, ‘binging and purging behaviours’) but not in the main analysis examining binging or purging behaviours alone (it is unclear why the third ED behaviour, dietary restraint, was not analysed). Overall, the level of multiple testing and lack of $p$-value adjustment in Micali et al. (2017a) is a major limitation, and suggests that results should be viewed with a high degree of caution.

In other fields, studies have also investigated the possible interactions between genetic factors and parenting behaviours in predicting psychological outcomes for children and adolescents. A recent study most closely mirroring Study 3 is an investigation by Van Assche et al. (2016) in the depression field ($N = 797$), who
examined five child and parent-reported parenting behaviours, including parental support, proactive control (defined as ‘strict, but fair guidance’), psychological control (hostility and emotional control), non-physical punishment, and physical punishment. No significant GxE interactions were reported. This study has several variables in common with those in Study 3, and across both studies no significant GxE were reported between 5-HTTLPR and parental support, parental hostility/psychological control, or parental use of (harsh) punishment (albeit the outcome measured in Van Assche et al., 2016, was depression, not disordered eating as in the present thesis). Encouragingly, Van Assche et al. (2016) adopted stringent multiple-testing controls to account for the 35 GxE interaction analyses reported (with five parenting styles – measured separately for child and parent report - and three different genetic factors investigated: 5-HTTLPR, DAT1, and MAO-A), using Bonferroni correction to produce a corrected alpha level of .0014 for each GxE interaction. Similar results were reported in van Roekel et al. (2011; N = 306), who conducted a longitudinal investigation of the interaction between parental support and 5-HTTLPR in predicting adolescent depressive feelings, and reported no interaction between 5-HTTLPR and either maternal or paternal support in predicting subsequent adolescent depressive symptoms.

As discussed in Chapter 3, an assortment of studies in other fields have identified gene x parenting interactions under a plasticity framework, contrary to results of Studies 3a and 3b. However, closer inspection of such studies tends to reveal methodological limitations. For example, Belsky and Beaver (2011; N = 1586) investigated the interaction between ‘parent quality’ and a cumulative participant plasticity score (based on polymorphisms previously identified by authors as contributing to plasticity: DAT1, DRD2, DRD4, 5-HTTLPR, and MAO-A; Belsky et al., 2009) to predict adolescent self-regulation ability. They identified a plasticity effect for males but not females. Belsky
and Beaver (2011) contained a large sample and a strong suite of purportedly relevant genetic polymorphisms, although the quality of their environmental variable was questionable. ‘Parent quality’ was a variable created via combining three measured scales, maternal involvement, maternal disengagement, and maternal attachment. However, these scales themselves were based upon ten, five, and two self-reported questions respectively, with Cronbach’s alpha as low as $\alpha = .50$ for the first scale and $\alpha = .64$ for the third scale. In light of debate regarding the importance of well-measured environmental factors in studies of GxE interactions (see further discussion below), this poses a serious threat to the validity of results.

Similarly, Hankin et al. (2011) found evidence of genetic plasticity involving 5-\textit{HTTLPR} when investigating the relationship between supportive and unsupportive parenting styles and adolescent positive affect. Strengths of this study include the fact that authors replicated this effect across three sub-studies, with investigations of parenting using parent-report, observation, and child-report. However, sample sizes in the first two sub-studies were low ($N = 307$ and $N = 197$, with $N = 1370$ in the third sub-study), and effects were found for only s/s and not s/l genotypes, which is at odds with theoretical understandings of 5-\textit{HTTLPR}-related serotonin production and past empirical evidence (Karg et al., 2011; Lesch et al., 1996; Munafò et al., 2009). Furthermore, it is unclear whether observed parent-child interactions were coded using an established family interaction coding system, or a scale devised by authors on an ad-hoc basis. Authors further asserted that due to the low sample size in the observational investigation, comparison was made only between participants with the s/s genotype with those with one or more l-alleles, which is contrary to how 5-\textit{HTTLPR} production is believed to operate (Heils et al., 1996; Lesch et al., 1996) and a further limitation of the analysis. Finally, GxE interactions reported across the three studies in Hankin et al.
(2011) were only significant at the .05 level, with no exact $p$-values provided by authors, and no correction for multiple testing performed. Although this study offers the strongest support of plasticity involving $5-HTTLPR$, due to its focus on replication of findings across several analyses (as was attempted in the present thesis), the aforementioned limitations must be noted and results interpreted with an according degree of caution.

A host of early studies of $5-HTTLPR$ that sparked initial interest in this avenue of investigation and are often cited as evidence for the plasticity hypothesis should now be recognised as vastly underpowered. For example, an investigation by Pluess et al. (2010) suggesting that $5-HTTLPR$ interacted with traumatic life events to predict neuroticism included only $N = 118$, while a study by Kochanska, Kim, Barry, and Philibert (2011) identifying an interaction between $5-HTTLPR$ and maternal care to predict academic skills, social functioning, and moral internalisation included a sample of $N = 100$. Similar sample sizes were included in other studies cited as ‘early evidence’ of a plasticity effect, (e.g., Taylor et al., 2006, $N = 118$; Wilhelm et al., 2006, $N = 127$). In fact, likely due to issues of low power, some studies found contrary results, whereby the s-allele reduced environmental sensitivity. For example, a study by Steiger et al. (2008a) found that individuals with the s-allele of $5-HTTLPR$ were less likely to respond to treatment than their I/I counterparts ($N = 111$). Overall, the picture from existing studies investigating GxE interactions involving parenting behaviours is one of mixed findings, with studies reporting significant interactions peppered with limitations.
12.5. Implications for measurement of candidate gene x environment interactions

The results of Studies 3a and 3b contain some implications for current debate in the field of genetic association studies regarding importance of large sample size versus value of high-quality measurement of phenotypes and environmental factors. As discussed throughout the present thesis, a number of research groups strongly advocate for the use of very large sample sizes in studies exploring GxE interactions, and argue that existing studies with ‘small’ samples (e.g., \( N < 1,000 \)) are underpowered to detect the small effect sizes likely involved in GxE interactions. As such, they claim that published significant findings in these studies most likely represent false-positive results and the preponderance of smaller studies with significant findings reflects the force of publication bias in the genetic association field (de Vries et al., 2016; Dick et al., 2015; Duncan & Keller, 2011; Ioannidis et al., 2014; Sham & Purcell, 2014). For more extensive discussion of these issues, please refer to Chapter 5.

In contrast, other research groups argue that studies with smaller sample sizes generally include more extensive assessment of environmental and outcome variables (e.g., assessment of depression using a semi-structured interview as opposed to a self-report measure), and thus these factors (i.e., less error variance due to better measurement techniques; Podsakoff et al., 2003) increase the statistical power of samples typically considered small for studies of genetic association (Caspi et al., 2010; Moffitt et al., 2006; Uher & McGuffin, 2008). Further, authors argue that in order to perform hypothesis-testing investigations driven by theory (i.e., using a construct validity approach as opposed to a purely statistical exploratory approach) the primary emphasis should be placed on high-quality measures and study design, with sample size
a secondary concern (Caspi et al., 2010). For example, Moffitt and Caspi (2014) point out that the original study by Caspi et al. (2003), which showed a 5-HTTLPR x stressful life events interaction in predicting depressive symptoms, used face-to-face interviews to assess environmental and outcome measures. Therefore, larger studies attempting replications of this result that do not use such methods may report null findings due to use of poorer measurement tools. Indeed, Caspi et al. (2010) argue that non-replication of GxE interaction effects tends to be associated with studies that use lower-quality measures. Some authors even claim that under conditions where the environmental and outcome variables are continuous in nature, there is statistical support for studies with high-quality measurement (e.g., repeated and precise measurements) having equivalent power to samples 20 times larger using poorer measurement techniques (Wong et al., 2003).

In Study 3b, the observational measurement of parenting applied to a sub-group of the overall sample may be viewed as one example of a ‘more reliable’ measure of the environmental factor, compared to the self-report measure used in Study 3a. Use of an observational method for assessing parenting behaviours, if indeed more ‘objective’ and with better construct validity than self-report measures, can decrease the error variance in the statistical analyses and provide a more nuanced account of the GxE interaction (Podsakoff et al., 2003). However, despite the use of an observational tool for measuring parenting in Study 3b, no GxE interactions were identified. This partly contradicts the view of authors who have argued that use of techniques that more thoroughly assess environmental and psychological factors in smaller studies is related to the significant GxE interaction findings identified in these studies, compared to large-scale investigations that utilise less intensive measurement techniques (Caspi et al., 2010; Moffitt et al., 2006; Uher & McGuffin, 2008). In Study 3b, ‘thorough’
(observational) measurement of parenting behaviour did not result in a significant GxE interaction. However, there are several other reasons that may account for this lack of GxE interaction, such as issues relating to the observational measure selected (i.e., it remains possible that the IFIRS was not superior to the self-report measure) or the use of a self-reported disordered eating outcome variable (i.e., instead of using a semi-structured interview such as the EDE; Fairburn & Cooper, 1993). Finally, perhaps the null findings across Studies 3a and 3b simply reflect the fact that there is truly no GxE interaction between 5-HTTLPR and the presently assessed parenting behaviours to predict disordered eating outcomes. The results therefore do not necessarily discredit the notion that ‘small’ samples sizes ($n < 1,000$), even when using presumably excellent measurement tools, are inadequate for identifying GxE interactions. Nonetheless, at face value the results from Studies 3a and 3b more evidently support the notion that large sample sizes are needed to identify the small effect sizes believed to be involved in GxE interactions, and that using higher-quality measurement tools may not compensate for a low sample size.

12.6. Future directions for investigation of gene x environment plasticity

As Studies 3a and 3b did not show evidence of a GxE interaction, they also did not support presence of a plasticity effect. One possibility is that the outcome variables used in Studies 3a and 3b (i.e., degree of disordered eating pathology) may not be ideally suited for reflecting genetic plasticity. Plasticity occurs when individuals with a specific genetic variant that is usually conceptualised as increasing susceptibility to environmental stressors respond better under positive or neutral environmental conditions (Belsky et al., 2009). It may therefore be the case that to best capture this effect, it is necessary to assess ‘positive’ outcomes, such as healthy body image or
positive attitudes towards one’s appearance (Levine & Smolak, 2016). Another possibility could be that plasticity may be evident only in conditions of ‘extreme’ positive environments, reflecting the pattern established across the studies of the present thesis. It may be that in order to identify such an effect, a number of other positive parenting factors in addition to parental warmth would need to be assessed and included into the regression model (e.g., family cohesiveness; Lampis et al., 2014). These are all factors that may be worth considering in future studies of GxE interactions, using large sample sizes and high quality measures of environmental and psychological variables that may act as both risk and protective factors.

Overall, the findings in Studies 3a and 3b that the relationship between parenting factors and disordered eating is not moderated by 5-HTTLPR provide further support to the theory that 5-HTTLPR may only be predictive of ED outcomes in the presence of particularly ‘severe’ environmental stressors. For example, Study 1 found that the s-allele increased susceptibility to BN when participants reported history of both sexual and physical abuse, but not ‘only’ one. Similarly, Study 2 found that bulimic tendencies were predicted by an interaction between 5-HTTLPR and severe parental physical punishment, but that this interaction was not present when ‘only’ mild-to-moderate parental physical punishment was considered. Meanwhile, Studies 3a and 3b, which did not include any ‘extreme’ environmental stressors did not show evidence of a GxE interaction. This is further discussed in the subsequent chapter, which presents an integrated summary of the various findings and implications of the current thesis, including an inspection of the overall limitations and future directions for genetic investigations in the ED field.
13. CHAPTER 13

Integrative Conclusion and Future Directions

The present thesis has focused on uncovering the role of GxE interactions in the development of EDs and disordered eating. This avenue of investigation was conducted in order to increase knowledge regarding the source of individual differences in susceptibility to EDs, particularly within environments that are generally considered ‘risk factors’ for these disorders. The present thesis first conducted a thorough review of the literature to provide a clear picture of the current knowledge regarding the role of numerous risk factors and correlates of EDs, with a particular focus on genes, individual psychological differences, and exposure to challenging environments (Chapters 1, 2, and 3). Based on the reviewed findings, it was hypothesised that GxE interactions may play a role in ED etiology. The first study of the present thesis (Study 1, Chapter 4) thus involved a systematic review and meta-analysis of existing GxE interaction studies in the ED field, finding significant interactions between 5-HTTLPR and traumatic life events, as well as 5-HTTLPR and sexual and physical abuse, but no interactions between 5-HTTLPR and psychological factors (depression and impulsivity). Main effects of 5-HTTLPR were absent in this study and throughout all investigations (published and unpublished) in the present thesis. Chapters 5 and 6 expanded on the findings reported in the publication and critically evaluated existing GxE interaction research in the ED field, noting several key limitations and areas for improvement in future research. The second study (Study 2, Chapter 8) then attempted to conceptually replicate the results of Study 1 with methodological improvements based on discussion throughout Chapters 5 and 6, in a large, independent sample from the ATP. Tentative support was found for an interaction between 5-HTTLPR and severe parental physical
punishment in predicting bulimic behaviours, although results were based on a sample not in Hardy-Weinberg Equilibrium. As in the meta-analysis (Study 1), no support was found for an interaction between psychological factors (depressed mood and emotional control) and disordered eating attitudes and behaviours. Chapter 9 featured an extended discussion of the results and implications of Study 2. Finally, the third study (Chapter 11) investigated whether possible GxE interactions may extend beyond traumatic experiences to ongoing and systematic environmental exposures, specifically, parental behaviours. However, it found no evidence of an interaction between 5-HTTLPR and parental warmth, parental use of harsh punishment, or parental hostility in predicting disordered eating related outcomes across two separate studies (Studies 3a and 3b). Methodological details and implications of results were further discussed in Chapter 12. The current Chapter features an overview and integrative summary of the findings of the present thesis within the context of existing ED and GxE interaction research, discusses theoretical and treatment implications of the present thesis, and recommends avenues for future investigations based on the current findings.

13.1. Situating current empirical findings in the broader ED and genetic association literature

13.1.1. Gene x environment interactions

On the basis of the three studies in the current thesis (Studies 1, 2, 3a, and 3b), a number of conclusions were drawn. Firstly, across all three studies the environmental factors considered most ‘extreme’ (i.e., greater number of traumatic life events, the experience of both sexual and physical abuse, severe parental physical punishment) appeared to interact with 5-HTTLPR to predict increased disordered eating symptoms or ED risk. More ‘mild’, systematic environments, such the family interaction environment
(e.g., parental hostility), appeared not to interact with 5-HTTLPR to predict eating pathology. Secondly, there were consistently no GxE interactions with 5-HTTLPR involving psychological variables (depression, impulsivity, emotional control). Meanwhile, direct effects of these psychological variables were demonstrated, suggesting that perhaps such factors exert strong main effects on disordered eating and ED risk, such that they constitute likely risk factors irrespective of individual 5-HTTLPR genotype or other specific genetic factors. Conversely, individual response to stressful environments may be more dependent on these individual genetic differences.

The finding that GxE interactions involving 5-HTTLPR tended to be associated with extreme environmental factors, as opposed to ongoing systematic environments (i.e., parental behaviours) may theoretically align with the notion that 5-HTTLPR is involved in regulating human stress response (van Eekelen et al., 2012). Under this model, an individual with a low functioning s-allele may not experience altered stress response functioning under mild to moderate conditions, however, may experience compromised stress management when severe strain is encountered. In contrast, those with the high-functioning l/l genotype appear to be comparatively resilient to such stressors. Accordingly, a number of theories linking stress and disordered eating behaviours were discussed in Chapters 2 and 3, and suggest, for example, that individuals with compromised stress-response functioning may engage in binge eating to distract or alleviate negative affect associated with stress, or adopt restrictive eating habits in order to regain control in at least one element of their lives (Heatherton & Baumeister, 1991).

It is also possible that the reason GxE interactions were only identified when ‘extreme’ environmental stressors were examined was due to the size of their effect. Perhaps only interactions involving extreme stressors produced an effect size large
enough to be detected by the present samples. Had the samples used in the current thesis been even larger, so that the studies were powered to detect small GxE effect sizes, it is possible that significant interactions could have been observed for some of the other environmental variables investigated (e.g., parental behaviours).

Another possible explanation for the identified relationship between 5-HTTLPR and extreme environments in predicting eating pathology is that this GxE interaction may also increase comorbid symptoms (e.g., low mood, impulsive behaviour), in addition to direct ED symptomatology (Richardson et al., 2008). An increase in comorbid pathology may in turn magnify the intensity of ED symptoms. For example, there is some evidence of a 5-HTTLPR x environmental stress interaction in predicting greater depressed mood (Karg et al., 2011; Sharpley et al., 2014, cf. Culverhouse et al., 2017; Munafò et al., 2009; Risch et al., 2009). An increase in depressed mood may consequently result in lower self-esteem regarding appearance, thus increasing the tendency for individuals to engage in restrictive eating and binge-purge cycles (Stice, Presnell, & Spangler, 2002). These comorbid symptoms of EDs or disordered eating may be even more intensified under conditions of particularly extreme environmental stress, and thus further contribute to the increase in eating pathology seen for individuals with the s-allele of 5-HTTLPR who have experienced extreme stressors.

13.1.2. Genetic plasticity

No evidence for plasticity was found in Studies 3a and 3b to suggest those with the s-allele may fare better under positive or neutral environmental conditions, compared to those homogenous for the l-allele. Indeed, it was noted in Chapter 12 that many studies upon which initial assertions of genetic plasticity were based were vastly underpowered for analysis of genetic association (e.g., Caspi et al., 2003). As is often the case in
empirical endeavours, the importance of these ‘early’ studies may have been over-
exaggerated because plasticity is an appealing avenue of investigation that provides the
potential for researchers to identify a ‘silver lining’ in fields of research where the focus
is primarily on factors that increase risk to pathology and poor outcomes. However, the
evidence for plasticity thus far is unclear. Importantly, in order to consistently identify
plasticity effects, it is also necessary to consistently identify GxE interactions. As
discussed throughout this thesis, support for candidate GxE interactions in psychiatry
continues to be mixed. In the present study, it is possible that limitations relating to
sample size may have obscured any GxE interactions, and thus evidence of plasticity. In
the ED field overall, we have identified some early positive GxE interaction findings
(Study 1), although they should be considered only preliminary until independent and
consistent replication is achieved. Meanwhile, no evidence of plasticity has thus far
been reported in studies of eating pathology (see Chapter 3), notwithstanding limitations
of existing GxE research. Despite the appeal of exciting ideas such as genetic plasticity
and positive outcomes for those typically considered to be at greater genetic risk of
pathology, it is important to continue to critically assess such research and draw
conservative conclusions in the face of the continued uncertainty regarding findings of
GxE interactions involving candidate genes.

13.1.3. The role of psychological variables

The finding that GxE interactions were not present when psychological variables
were analysed in Studies 1 and 2 supports the view that such interactions, as their name
would suggest, are restricted to an interplay between genetic factors and how
individuals respond to given environmental stressors. Meanwhile, psychological factors
tended to be rather strong direct predictors of eating pathology across the present
studies. This may be because of the strong relationship between certain psychological factors and disordered eating or ED pathology (Martinussen et al., 2016; Puccio et al., 2016; Waxman, 2009), which is far larger than any effects thus far identified for an individual genetic factor. It is therefore not surprising that the effect of certain psychological factors appears to be uninfluenced by a single genetic factor such as 5-HTTLPR. A further point for consideration is that personality and other psychological factors themselves are under a moderate to high level of genetic influence (Jang, Livesley, and Vemon, 1996), which thus may dilute any interactions with ‘genetic susceptibility’ polymorphisms, compared to factors more typically considered ‘environmental’ (e.g., experiencing sexual abuse).

The finding that psychological factors appear to be moderately strong predictors of eating pathology independent of interaction with specific genetic factors (although personality traits are considered highly heritable; Bratko, Butković, & Vukasović Hlupić, 2017) has some positive implications for ED treatment initiatives. For example, personality traits such as impulsivity (assessed in Study 1), represent measureable predictors for eating pathology that can be directly targeted in prevention and treatment initiatives. Indeed, greater impulsivity has been associated with greater risk of treatment drop-out for patients with an ED (Fassino, Pierò, Tomba, & Abbate-Daga, 2009) and poorer weight loss and binge-eating reduction during treatment for children with obesity (Nederkoorn, Braet, Van Eijs, Tanghe, & Jansen, 2006). This further supports the idea that directly targeting impulsivity (for example) in ED treatment could be a fruitful future treatment option. Furthermore, while it is possible that a patient with an ED may have some success modifying or learning to cope with certain maladaptive psychological factors (e.g., emotional control, as assessed in Study 2), with current scientific knowledge it is not possible to alter genetic factors that increase susceptibility
to diseases. There are, however, different ways in which findings of GxE interactions involving personality factors or comorbid psychopathological factors could also deliver numerous benefits. For example, knowledge regarding what extent of a patient’s ED symptomatology may be attributed to genetic factors (including an understanding of the specific genetic mechanisms) and how much is likely an independent result of psychological or environmental conditions is directly relevant to current treatment initiatives. This provides a clearer picture of what is realistically preventable and how much is beyond our current clinical capabilities, which is potentially very valuable information to both clinicians and patients alike. Further discussion of treatment implications of GxE interaction research is discussed more extensively later in this chapter.

13.2. Summary of limitations

13.2.1. Cross-sectional design

A number of limitations of the investigations reported in the present thesis are important to highlight. Firstly, analysis of environmental and psychological variables occurred concurrently with assessment of disordered eating and ED symptoms. Ideally, such variables would be assessed at earlier time points in longitudinal studies, with their effects on later disordered eating pathology evaluated (Jacobi et al., 2004). As this was not possible due to resource restraints, the present findings must be interpreted as providing evidence for ‘correlates’ of disordered eating. One exception is that 5-HTTLPR is a genetic factor present from conception, and thus may be more clearly referred to as a potential ‘risk factor’ for disordered eating (although no evidence supporting direct 5-HTTLPR risk was found throughout the present thesis). However, this thesis still comprises a valuable contribution to the literature despite adopting a
cross-sectional design. Indeed, preliminary risk studies such as the present investigations of GxE interactions tend to be cross-sectional in nature and aim to first establish correlates of given diseases or outcomes (Kazdin et al., 1997). These studies constitute an important first step in identifying candidate variables, which can then be used to justify and support resource allocation to further examination of these variables in longitudinal studies, in order to determine whether they are possible ‘risk factors’ for a disease. Following this rationale, the most recent wave of ATP data collection (undertaken in 2015) features further assessment of disordered eating, in order to best assess any potential longitudinal links.

13.2.2. Publication bias

Publication bias is a major issue discussed throughout this thesis, which likely affects a substantial portion of published GxE interaction research, including possibly the meta-analysis in Study 1 (see Chapter 5). Discussion of publication bias features prominently in the present thesis for two main reasons. Firstly, there is substantial evidence of publication bias in studies of genetic association (de Vries et al., 2016) and psychology more broadly (Ferguson & Heene, 2012). This has been discussed at length and the reader may view Chapter 5 for more information. The second main issue is that publication bias carries potentially severe consequences. If publication bias is not kept in check, research findings in the field will no longer represent an accurate and balanced picture of the current state of knowledge (Ferguson & Heene, 2012). Suppression of negative findings in favour of more ‘interesting’ findings can lead to a large-scale misrepresentation of GxE interaction effects in the field.

Indeed, in the present instance, publication of Study 2 was a challenge. Despite the relatively large sample sizes and the expensive, genetic data examined, a number of
leading ED journals opted not to publish the manuscript, despite no major methodological issues identified by reviewers. Reasons provided for rejection of the manuscripts tended to relate to findings not having sufficient ‘impact’ or ‘priority’. While it is not possible to ascertain how these responses would have differed had the manuscript contained more ‘interesting’ (i.e., significant) findings, it is quite possible that these roadblocks to publication would not have been met, as was the case for Studies 1 and 3. Indeed, Study 2 was for this reason submitted to an open-access journal with a publication fee. Persisting with publication was considered an essential step by authors, in order to not contribute to the ‘file-drawer’ phenomenon and continue to positively skew published findings of GxE interactions. However, this step required both monetary resources and a very persistent approach from the researchers involved. It is likely that this approach is often unfeasible for researchers and that similar studies, along with their non-significant findings, are regularly relegated to the file-drawer.

Publication bias is highly problematic both from an epistemological perspective, as it results in the proliferation of biased information that is presented as the most up-to-date evidence-based knowledge in the field, and also from a practical viewpoint, with the potential for misallocation of funding and other resources that may be better directed to different lines of inquiry. For example, if a more accurate picture of findings in certain fields were available, funding bodies and researchers themselves may opt to allocate time and funds to investigating different or more advanced avenues of research that may provide greater benefit to society. This is particularly relevant given the global trend for a tightening of research grants and other funding, suggesting it is more important than ever to ensure that decisions regarding resource allocation be made on the basis of the most up-to-date and scientifically vigorous existing research findings, to maximise progress in human understanding of complex diseases and their causes.
13.2.3. Sample size and other limitations

Other limitations are discussed in greater depth throughout the methodological and discussion chapters of the present thesis, and include sample size restrictions, issues relating to multiple testing, improper control of confounding variables, and publication bias (see Chapters 5, 9, and 12). Overall the most substantive limitations of the present studies, and of existing GxE research in the ED field, relate to sample size. Although the studies in this thesis constitute the largest GxE investigations in the ED field to date (with the exception of Akkermann et al., 2011, and the recently published Micali et al., 2017a), the sample sizes are still considered modest for examination of genetic association. As a result, findings in this thesis must be viewed as preliminary, and require replication in larger, independent samples. For extensive discuss of this issues, please refer to Chapters 5 and 9.

13.3. Effect sizes in gene x environment interactions

Throughout the present thesis and in published studies of GxE interactions in the ED field and beyond, focus has largely rested on identification of statistically significant moderation by genetic factors in general linear models or similar types of analyses. In interpreting such results from statistical analyses of GxE interaction, effect size has been an important yet often overlooked consideration. In the present thesis, despite identification of some very small $p$-values in a number of the analyses, indicating a low likelihood that the null hypothesis of no GxE interaction was falsely rejected, effect sizes were modest. In the meta-analytic investigation (Study 1), the odds ratio for the interaction between 5-HTTLPR and traumatic life events in predicting a likely eating disorder was $\text{OR} = 1.23$, 95% CI [1.06, 1.44]. This was somewhat larger for sample estimate of the 5-HTTLPR x sexual and physical abuse interactions in predicting
bulimia-spectrum pathology, \( \text{OR} = 3.15, 95\% \ CI [1.09, 9.09] \). The fact that the effect sizes in the meta-analysis in Study 1 were based on published studies, which may have been subject to the file-drawer phenomenon (Rosenthal, 1979), suggests that the size of these effects are likely top-range estimates. Meanwhile, the standardised beta regression coefficient for the interaction found between severe parental physical abuse and 5-\textit{HTTLPR} to predict bulimic behaviours in Study 2 was \( \beta = .22 \), also showing only a modest effect.

These findings reflect some of the larger ‘small’ effect sizes identified in the ED literature. A large portion of previous GxE investigations also report odds ratios marginally above 1 (Risch et al., 2009) or fail to report effect sizes altogether (Karg et al., 2011). Indeed, Dick et al. (2015) argues that expected effect sizes for genetic main effects and GxE interaction effects for a single gene are around \( \text{OR} = 1.1 \). Despite these consistently low effect sizes, emphasis in the field has largely been placed on \( p \)-values (e.g., discussion of Bonferonni correction, issues related to multiple testing, etc.; Duncan & Keller, 2011). Greater focus on effect sizes would enhance understanding of implications associated with significant GxE interaction findings. Indeed, the consistently low effect sizes of GxE interactions identified in the present research (with the exception of the sexual and physical abuse analyses in Study 1) and in the broader literature may suggest that even these ‘significant’ GxE interactions may have limited relevance or applicability in a broader context (e.g., in terms of implications for prevention or treatment). Such limitations should be highlighted in situations where effect sizes are very small.

Although the effect sizes of existing GxE interactions involving 5-\textit{HTTLPR} may be considered vastly underwhelming in light of the ‘hype’ surrounding such avenues of investigation, it must be remembered that 5-\textit{HTTLPR} represents only one genetic
polymorphism in a large system of genes. It is therefore unsurprising, if not expected, that effect sizes are likely to be low. However, while in themselves the findings involving single genes and their associated effects may not constitute ‘ground-breaking’ results, they are important stepping stones towards building a larger picture of the role of genes and genetic factors in ED and disordered eating aetiology. Indeed, given the high heritability of EDs identified in twin studies (approximately 50%; Yilmaz et al., 2015), the question is no longer whether genetic factors play a role in EDs, but rather how genetic factors play a role in EDs. Given the current lack of knowledge regarding which genetic mechanisms are involved in EDs, investigations at the level of individual polymorphisms should by no means be disregarded.

13.4. Implications of the present findings for treatment

13.4.1. Findings relating to environmental and psychological factors

While the present thesis identified significant GxE interactions in a number of instances, these generally were not without limitations, and there were many null findings reported. Meanwhile, there was continuously strong support for the role of psychological and environmental factors. Depressed mood and emotional regulation difficulties consistently predicted greater levels of disordered eating (Studies 1 and 2), and there was evidence of clear associations between certain parenting behaviours or the experience of childhood abuse and later disordered eating and ED symptomatology (Studies 2, 3a, and 3b).

At face value, it may be considered disappointing that candidate gene research in general has not produced findings that point to a clearer gene-disease association, particularly given the large volume of resources devoted to these investigations and the potential implications of well-established effects. However, as previously alluded to, the
stronger support of a role for environmental and psychological factors can be considered a positive conclusion. There is far greater scope for individuals and clinicians to help modify environments (e.g., via promoting safer settings for children by raising awareness regarding family violence or other patient-specific environmental factors), or to help individuals to better cope with certain psychological traits, than to modify genetic factors. For example, focusing on treatment for depression or reducing negative affect in an individual with disordered eating may also help reduce disordered eating symptoms and prevent the development of a ‘full-blown’ ED (e.g., as per the dual pathway model; Stice, 2001). Psychoeducation regarding links between negative affect and EDs may help ED patients become more aware of sliding into maladaptive eating patterns (Stice, Shaw, & Marti, 2007). Likewise, although emotional control is not a modifiable ‘external’ factor per se, helping those at-risk of experiencing an ED address their emotional regulation difficulties may in turn help reduce binge eating or purging practices (Clyne & Blampied, 2004; Corstorphine, 2006).

13.4.2. Clinical implications of genetic findings

Research into candidate genes however is not without implications for prevention and therapeutic intervention of EDs. At a broader level, research into the genetic correlates of EDs has already made a substantial impact on the way EDs are perceived and treated. Results of twin and family studies have indicated that a large portion of ED risk appears to be genetic (Yilmaz et al., 2015). Genetic findings have also clarified that EDs aggregate in families largely due to genetic factors, while shared environments have been shown to have a relatively low contribution to ED pathology (Culbert et al., 2015). This greater understanding regarding the genetic underpinnings of EDs allows for patients to be better informed regarding why they may have developed this disorder,
which can help reduce guilt associated with ED diagnosis (Pinto-Gouveia et al., 2016). Relatedly, sharing information relating to the genetic factors involved in ED aetiology reduces the guilt and blame placed on families (Crisafulli, Von Holle, & Bulik, 2008), who may have in the past felt responsible for their child’s ED. Such knowledge can also be used as a basis for targeted prevention for individuals with a family history of EDs.

Specific research into candidate genes also has numerous current and potential future clinical implications. With our current level of understanding regarding the role of candidate genes in EDs and disordered eating, clinicians are able to incorporate nuanced information regarding genetic risk factors when they provide psychoeducation to at-risk and newly diagnosed individuals. Explaining to individuals that certain (common) genetic variants may increase their susceptibility to an ED is likely to alleviate guilt associated with diagnosis (Easter, 2012). Indeed, there is evidence to suggest that blame and stigma related to EDs is reduced when aetiology is framed in terms of biology and genetics compared to socio-cultural factors (Bannatyne & Abel, 2015; Doley et al., 2017). Further, explaining to individuals that certain genes may increase risk for EDs only under particular environmental conditions may additionally decrease any feelings of helplessness or loss of hope associated with concerns regarding genetic determinism. Understanding the interplay between candidate genes and the environment can also help patients better understand why they have developed an ED based on their genetic sensitivity to particular environments.

There are also numerous ways that this line of research can lead to future treatment advancement. Firstly, enhanced knowledge regarding the candidate genes and genetic systems involved in ED aetiology may shed light into the biological basis of eating pathology (e.g., involvement of 5-HTTLPR in GxE interactions may broadly imply a role for serotonin in the aetiology of the disorder). Such information that implicates
specific genetic and more broadly, biological mechanisms, could pave the way for further pharmacological advances in treatment of EDs. Understanding which genes may be involved in increasing ED risk could also be used to enhance prevention and early intervention initiatives. For example, individuals exposed to risky environments may be able to provide saliva samples for genotyping, with those identified at risk subsequently offered psychoeducation or the opportunity to engage in other preventative initiatives (Eley, 2014). This could be incorporated into existing services that offer various treatment or educational programs and may have frequent contact with ‘at risk’ populations (e.g., hospitals, psychology clinics, schools, sporting clubs). Reducing risk of EDs through these types of prevention initiatives can potentially have very beneficial effects in curbing ED incidence and reducing long term ED prevalence rates.

Although genotyping saliva from a large number of individuals may be costly, with costs of such processes becoming increasingly accessible, it would overall constitute a far more cost-effective method for addressing ED prevalence than if such a measure were not in place. At present, the economic burden associated with EDs is very large. One Australian estimate by the Butterfly Foundation in 2012 stated that the annual socio-economic cost of EDs in Australia was AUD$69.7 billion (The Butterfly Foundation for Eating Disorders, 2012). This was attributed to numerous reasons, in large due to productivity impacts (e.g., young people are unable to reach their employment potentials or unable to participate in the workforce), and in smaller part due to the cost of formal and informal treatment and care for those with an ED. Treatment of individuals with EDs can involve ongoing outpatient therapy and lengthy inpatient hospital visits, both of which come at a considerable financial cost to the healthcare system and/or families (Striegel-Moore, Leslie, Petrill, Garvin, & Rosenheck, 2000; Toulany et al., 2015). In addition, the long-term success rates of
current treatment approaches are only moderate, with around one third to half of
individuals who receive evidence-based psychological treatment not showing evidence
of remittance (Berkman, Lohr, & Bulik, 2007; Steinhausen & Weber, 2009). Indeed, the
duration of illness for those with an ED often exceeds a decade (Milos, Spindler,
Schnyder, & Fairburn, 2005; Reas, Williamson, Martin, & Zucker, 2000) and ongoing
ED symptomatology is associated with greater subsequent comorbidities (e.g., anxiety,
depression, substance use; Micali et al., 2017b). Meanwhile, there is strong evidence
that prevention programs tend to reduce risk for EDs (Stice, Becker, & Yokum, 2013;
Stice et al., 2007). Increasing focus on prevention initiatives would reduce disease
burden and economic burden by curbing the development of early disordered eating
symptoms before they develop into full-blown EDs. Candidate gene research could
potentially make a sizable contribution in such prevention programs.

The future use of individualised genotyping of candidate genes in a clinical
prevention and treatment capacity is advocated by Eley et al. (2012) in a somewhat
futuristic-seeming set of ideas labelled ‘therapy genetics’. Therapy genetics suggests a
number of direct ways in which understanding the role of candidate genes in
psychopathology may lead to enhanced treatment options. Eley (2014) posits that
individual treatment choice may be informed by patient genotype, if it is discovered that
particular candidate genes are associated with greater (or conversely, poorer) outcomes
under particular treatment regimes. Eley (2014) also suggests that genotyping via saliva
samples may become part of the standard diagnostic assessment process undertaken by
clinicians upon patient admission. For example, cumulative genetic risk scores that
aggregate risk over a number of polymorphisms (De Jager et al., 2009) could be used to
enhance clinician understanding of client risk, alongside established assessment of
patient history and other factors, in order to aid patient formulation and inform an
appropriate treatment plan. The idea of therapy genetics evolved from an initial finding by Eley et al. (2012), in which children (N = 359) who received a cognitive behaviour therapy intervention for anxiety showed better outcomes if they carried two copies of the 5-HTTLPR s-allele, compared to those with the l-allele. However, this investigation is subject to all the usual limitations of GxE interaction research discussed throughout the present thesis. While evidence for ‘therapy genetics’ is currently scarce and the theory may appear somewhat far-fetched in light of all the issues plaguing GxE interaction research, it is nonetheless possible that in the future research on differential susceptibility markers will provide a more personalised basis from which to design and implement more effective forms of intervention for EDs.

13.5. Future directions of genetic research in the eating disorder field

13.5.1. Suggestions for addressing sample size issues

Given that sample size remains a key limiting factor in genetic investigations of disordered eating and EDs, a number of options can be considered for future studies in order to expand the sample sizes used in genetic research or to overcome this issue in other ways. At present, even the largest candidate GxE interaction studies in the ED field (with all but two forming part of the present thesis) are sufficiently powered to detect large effects only. Within the context of the present research, we may conclude that there is some mixed evidence for large GxE interaction effect sizes in the ED field. However, it is more likely that effect sizes under question are small to very small, particularly when a single genetic polymorphism is in question.

As discussed throughout this thesis and demonstrated in Study 1, cross-institute collaboration should be prioritised in order to combine resources and maximise sample
sizes of genetic association studies in the field. To facilitate the combining of data sets across various research groups, researchers should collaboratively decide upon a set list of preferred measurement tools. Measures with the highest available psychometric properties should be selected, and could be stored in a searchable online repository (e.g., similar to the RAND Online Measure Repository [ROMR] for traumatic brain injury research; www.rand.org). Indeed, decreasing the size of error variance present when measuring environmental and outcome variables can lead to substantial increases in power for a given sample size (Lenth, 2001). Such collaborations are slowly gaining momentum (e.g., the Eating Disorders Working Group of the Psychiatric Genomics Consortium; PGC-ED), and would benefit from an ongoing focus on measuring high-quality environmental variables.

In cases where large-scale collaboration is not possible, other techniques that do not necessarily lead to an increase in sample size, but nonetheless potentially address issues of validity related to low sample sizes, could be considered. One option discussed by Sham and Purcell (2014) is to move from a frequentist to a Bayesian model for investigating GxE interactions. At present, the frequentist significance testing approach is used to measure GxE interactions, whereby genetic and environmental factors are typically entered into regression-based models to produce significance values that determine the probability that a given effect should occur, over the long run, if the null hypothesis of no interaction were true (Fisher, 1925). However, this method encounters numerous limitations, including a great dependency on sample size and issues relating to multiple testing. In contrast, Sham and Purcell (2014) propose that GxE analyses could be conducted using a Bayesian approach (Gelman, Carlin, Stern, & Rubin, 2014), which would help reduce false-positive findings and allow for results to be interpreted independently of sample size. The difficulty of this approach is that it requires a greater
complexity of statistical analyses and depends on a level of prior knowledge regarding likely effect sizes and agreement on prior distributions for model parameters.

Another technique to maximise validity of results in future meta-analytic studies (which can provide the highest level of evidence for GxE interaction effects, although this is contingent upon the quality and sample size of the included individual studies) could be to use simulation techniques. John, Lencz, Malhotra, Correll, and Zhang (2016) argue that performing a meta-analysis based on summary statistics leads to biased results when additive genetic effects are involved. They propose and provide evidence for statistical simulation of participant-level data from summary statistics to increase validity of findings. It is possible that similar computation techniques may be used in the future to expand available sample sizes for studies of genetic association. In sum, it appears that a fruitful future direction in the genetic association field would involve a greater focus on statistical and computational opportunities, to help overcome some of the current methodological limitations of GxE investigations.

13.5.2. Polygenetic effects, genome wide association studies, and epigenetics

It might be the case that despite cross-institute collaboration, or other techniques used to increase the power of samples, the effect sizes associated with single candidate genes remain too small to be identified by available samples. An alternate approach could be to investigate systems of genes, such as those involved in stress-response or appetite regulation. Testing plasticity or genetic effects across numerous polymorphisms may implicate larger effect sizes than those likely associated with single genes (Dudbridge, 2013), and thus be better suited to the sample sizes that are presently available for studies of genetic association in the ED field.
In the ED field, only one study (Groleau et al., 2012) examined multiple polymorphisms in a candidate GxE interaction study. However, authors did not test for polygenetic or cumulative effects, but rather examined the alleles in separate regression models. The polygenic approach is thus untested within a candidate GxE study in the ED field. In other fields, studies have examined numerous polymorphisms in the same analysis to create a ‘plasticity score’ for each participant, with a number of findings indicating significant polygenetic x environmental interactions to predict mental health outcomes (Mullins et al., 2016; Purcell et al., 2009). Polygenetic investigations are a possible future avenue for the ED field, particularly where existing un-analysed data sets are available. Nonetheless, these efforts would undoubtedly also benefit from cross-institutional collaboration, and would likely require an even higher level of combined effort and coordination.

Studies of polygenetic risk may be enhanced through initial investigations that identified appropriate candidate genes by examining the entire genome. Genome-wide association studies (GWAS) aim to explore large sections of the genotype to identify possible associations between psychological outcomes and particular single-nucleotide polymorphisms (SNPs). These investigations often involve hundreds of thousands, or more, SNPs (Manuck & McCaffery, 2014), and are exploratory in nature rather than driven by hypotheses regarding the function of particular genetic variants. They offer the opportunity to uncover previously unknown biological pathways related to eating pathology, and provide potential leads for future candidate gene studies. Naturally, great caution must be particularly taken in these studies to guard against multiple testing and other sources of spurious findings.

Thus far only a handful of GWAS have been conducted in the ED field (Boraska et al., 2012; Boraska et al., 2014; Duncan et al., 2017; Nakabayashi et al., 2009; Wade et
Although most of these studies were underpowered for GWAS analyses. No consistent results have thus far eventuated from this line of investigation. The largest of these studies, by Boraska et al. (2014), examined 5,551 individuals with AN and 21,080 controls, and found that no SNPs reached genome-wide significance. Authors concluded that their study was underpowered for such analysis.

More recently, in a large collaboration by the PGC-ED, Duncan et al. (2017) examined 3,496 individuals with AN and 10,982 controls, reporting one locus that reached genome-wide significance (rs4622308 on chromosome 12). This initial finding awaits independent replication in even larger samples. Ongoing success using the GWAS approach requires continued emphasis on achieving the vast number of participants necessary for effective analyses, which much like candidate gene research, requires ongoing global collaboration such as that undertaken by the PGC-ED group.

Another growing line of genetic investigation in psychiatry involves research into epigenetic mechanisms. Epigenetics refers to the process by which genetic expression, but not DNA coding, is modified based on (often early) environmental influences (Klengel & Binder, 2015). The epigenetic process has been proposed to possibly underlie the observed relationship between early life stressors and mental health issues later in life (Cruceanu, Matosin, & Binder, 2017; Kessler, Davis, & Kendler, 1997). Under this model, molecular-level changes in response to early adversity can modify gene expression and may increase sensitivity to future stressful life events. Indeed, early adversity is associated with changes in hormonal functioning of the stress response system (e.g., hypothalamus, anterior pituitary gland, and adrenal gland; Lupien, McEwen, Gunnar, & Heim, 2009). These epigenetic processes may therefore account for the lack of direct gene-disease association currently identified in eating pathology and identify potential new genetic variants that play a role in EDs (Strober, Peris, &
Epigenetic findings in the ED field could also be useful in a treatment capacity, as they imply a lower extent of genetic determinism compared to current genetic approaches. The epigenetic approach places greater importance on environmental factors in GxE interaction models of disease development, thereby enhancing the prospects of successful prevention initiatives despite ‘risky’ genotypes or family history of EDs. Furthermore, many epigenetic changes at the molecular level are considered reversible (Sweatt, 2013).

An increasing number of epigenetic investigations populate the ED field, mostly investigating alterations in DNA methylation (Steiger & Thaler, 2016; Yilmaz et al., 2015). However, studies to date have been heterogeneous in nature and results remain preliminary (Campbell, Mill, Uher, & Schmidt, 2011; Yilmaz et al., 2015). Limitations to these investigations include the increase in level of resources and methodological complexity compared to studies of genetic association or candidate GxE interactions. Epigenetic studies would still require large sample sizes and longitudinal investigation, limiting the feasibility of this approach. However, if these limitations are successfully navigated, epigenetics could potentially reveal an important new perspective on ED aetiology.

Despite the promising sound of novel techniques for investigating the genetic underpinnings of EDs, caution must be taken when moving forward to such approaches. Such studies are also constrained by most of the identified limitations of candidate GxE research. Furthermore, in light of the vast quantity of purportedly false-positive findings published in the genetic association field (Sullivan, 2007), the genes and systems selected for investigation must be based on a strong theoretical rationale (Dick et al., 2015). Indeed, the ability of researchers to predict a priori which genetic polymorphisms are likely to be relevant for a given outcome or in combination with a
specified environmental factor are poor (Sullivan, Daly, & O’Donovan, 2012). In studies of candidate GxE interactions, this issue can be more readily addressed by examining polymorphisms with the strongest theoretical and empirical support for a role in the stress-response system (and EDs, as was done in the present thesis). This rigorous approach reduces the chances of false-positive results compared to investigations that examine a mix of polymorphisms, or several polymorphisms, using an ‘exploratory’ approach. This is an issue that can potentially affect the aforementioned GWAS studies and investigations of genetic ‘systems’ or polygenic effects, and thus future investigations must remain vigilant to these threats to validity.

**13.5.3. Future directions for candidate gene studies**

Despite the scepticism regarding candidate gene research and the potential promising avenues available via GWAS and epigenetic research, there is still much that can be uncovered from candidate gene studies in the ED field. Given the limitations of sample size and small effects, as well as issues regarding the heterogeneous nature of the ED phenotype, future candidate-gene research could benefit from investigating specific symptoms or physiological traits that underlie ED pathology (e.g., as has been done in studies examining autism and schizophrenia; Goodbourn et al., 2014). By ‘zoning in’ on these ‘lower level’ phenotypes (e.g., metabolic processes or physiological responses to food cues), there can be much greater accuracy of measurement for each participant, thus greatly increasing the power of a given analysis at a particular sample size. This approach has the advantage of utilising currently available technology and accessible candidate-gene ED samples, which can be examined for a fraction of the cost of GWAS and other methods. Furthermore, this would hopefully enable researchers to establish an understanding of how GxE
interactions may operate in EDs at the lowest level, which could allow subsequent research to build on this and explore higher-order psychological ED traits in a more informed and guided manner.

‘Small’ studies of candidate genes (e.g., \( N = 600 \)) can still also contribute over the long run to knowledge regarding the aetiology of EDs. While caution should be taken in drawing conclusions based on any single study, the accumulation of evidence across numerous small studies in meta-analyses has the potential to provide reliable evidence regarding GxE interactions. For example, in a paper offering a new perspective on what authors term the ‘so called’ replication crisis, Schmidt and Oh (2016) argue that replication (attempts) of studies are very prevalent in psychology, as is evidenced by the large number of meta-analytic reviews in the field. They argue that meta-analyses are ideal for the reliable accumulation of knowledge, and state that the greatest limitations to this form of accumulated knowledge relate to publication bias and questionable research practices (e.g., dropping measures that are non-significant, conducting multiple significance tests and only reporting those that reach significance, hypothesising after results are known, etc.). Thus, the continued publication of both significant and non-significant genetic findings, as well as strict adherence by authors to impeccable research practices (or clear communication regarding the limitations of their studies), is essential in order for the success of future genetic explorations in the ED field and beyond.

13.6. Final summary

The present thesis presents a thorough and complete picture of current candidate GxE interaction research in the ED field. Findings of the current thesis revealed some evidence of an interaction between \( 5-HTTLPR \) and ‘extreme’ environmental conditions.
in predicting ED symptoms, shedding some light onto the likely genetic mechanisms underlying the strong heritability in ED aetiology. However, in order to continue to expand knowledge regarding the genetic underpinnings of EDs in a scientifically rigorous and valid manner, future studies must take into consideration the numerous limitations of existing GxE studies as discussed throughout the present thesis, including sample size limitations, and selecting appropriate and high-quality measures of environmental and psychological traits. Finally, researchers and journal editors alike must make concerted efforts to address the issue of publication bias and reward good research practices. Continuing investigation into the genetic underpinnings of EDs following these principles will best facilitate findings and recommendations that are important to furthering our understanding of why some individuals suffer and, not uncommonly, die from eating disorders.
14. References


de Vries, Y., Roest, A., Franzen, M., Munafò, M., & Bastiaansen, J. (2016). Citation bias and selective focus on positive findings in the literature on the serotonin transporter gene (5-HTTLPR), life stress and depression. *Psychological Medicine, 46*(14), 2971.


---

389


Jeronimus, B., Kotov, R., Riese, H., & Ormel, J. (2016). Neuroticism's prospective association with mental disorders halves after adjustment for baseline symptoms and psychiatric history, but the adjusted association hardly decays with time: A meta-analysis on 59
longitudinal/prospective studies with 443,313 participants. *Psychological Medicine*, 46(14), 2883-2906.


obesity in women with seasonal affective disorder: A seasonal thrifty phenotype hypothesis. *Neuropsychopharmacology, 31*(11), 2498-2503.


on treatment outcome in bipolar patients. *Journal of Affective Disorders, 119*(1), 205-209.


e51.


depressive traits that may be partially responsible for the phenotypical variability of bulimia nervosa. *Journal of Psychiatric Research, 42*(1), 50-57.


A systematic review and secondary data analysis of the interactions between the serotonin transporter 5-HTTLPR polymorphism and environmental and psychological factors in eating disorders


Objectives: To summarize and synthesize the growing gene x environment (GxE) research investigating the promoter region of the serotonin transporter gene (5-HTTLPR) in the eating disorders (ED) field, and overcome the common limitation of low sample size, by undertaking a systematic review followed by a secondary data meta-analysis of studies identified by the review.

Method: A systematic review of articles using PsycINFO, PubMed, and EMBASE was undertaken to identify studies investigating the interaction between 5-HTTLPR and an environmental or psychological factor, with an ED-related outcome variable. Seven studies were identified by the systematic review, with complete data sets of five community (n = 1750, 64.5% female) and two clinical (n = 426, 100% female) samples combined to perform four secondary-data analyses: 5-HTTLPR x Traumatic Life Events to predict ED status (n = 909), 5-HTTLPR x Sexual and Physical Abuse to predict bulimic symptoms (n = 1097), 5-HTTLPR x Depression to predict bulimic symptoms (n = 1256), and 5-HTTLPR x Impulsiveness to predict disordered eating (n = 1149).

Results: Under a multiplicative model, the low function (s) allele of 5-HTTLPR interacted with traumatic life events and experiencing both sexual and physical abuse (but not only one) to predict increased likelihood of an ED and bulimic symptoms, respectively. However, under an additive model there was also an interaction between sexual and physical abuse considered independently and 5-HTTLPR, and no interaction with traumatic life events. No other GxE interactions were significant.

Conclusion: Early promising results should be followed-up with continued cross-institutional collaboration in order to achieve the large sample sizes necessary for genetic research.

© 2016 Elsevier Ltd. All rights reserved.
1. Introduction

Over the past decade, etiological models of eating disorders (EDs) have increasingly acknowledged the role of genetics, with twin studies estimating a notable heritable component (approximately 40–60%: Bulik et al., 2006, 2010; Fairweather-Schmidt and Wade, 2015; Trace et al., 2013). Investigations so far have not consistently identified specific candidate genes associated with increased ED risk, suggesting that hereditary factors in EDs may not operate via simple genetic association (Trace et al., 2013). Hence, studies are now increasingly examining whether environmental factors moderate the influence of candidate genes on risk for pathological eating behavior. Gene x environment (GxE) interaction research in the ED field is still relatively novel, with early studies identifying potential candidate genes associated with ED risk under specific environmental conditions (e.g., history of abuse; Steiger et al., 2012). In anticipation of the increased popularity of this research focus, it is timely to evaluate the current state of evidence and to highlight existing limitations, in order to guide the direction and methods of future GxE studies in eating pathology.

Previous research examining genetic influences on eating pathology has primarily focused on genes in the serotonin and dopamine systems linked to functions relevant to EDs, including appetite, mood, and reward sensitivity (e.g., SLC6A4, HTR2A, DRD2, DRD4, DAT1, and COMT; see Culbert et al., 2015, and Trace et al., 2013, for a review). Direct genetic association studies have not provided a clear picture of the links between specific genes and EDs or disordered eating symptoms, with many initial significant findings failing to achieve consistent replication (see Calati et al., 2011; Culbert et al., 2015; Lee and Lin, 2010; Scherag et al., 2010; Trace et al., 2013).

One reason for a lack of direct association between allele frequency and ED risk may be that this relationship is moderated by environmental factors. Under the diathesis-stress model of GxE interactions, individuals carrying a ‘risk’ allele may be more susceptible to EDs when exposed to environmental stressors, but show no differences in outcome in the absence of challenging environmental circumstances, compared to those without the risky genotype (Caspi et al., 2003; Monroe and Simons, 1991). The role of GxE interactions in psychology has gained increasing attention since Caspi et al. (2003) found that stressful life events increased susceptibility to depression for those with one or two copies of the short (s) allele of the serotonin transporter gene (5-HTTLPR polymorphism).

Studies have since largely focussed on 5-HTTLPR due to its biological relevance to psychiatric disorders (with the s-allele reducing serotonin transporter transcription efficiency; Heils et al., 1996), and early significant findings in the depression literature (Karg et al., 2011). Despite substantial research investigating GxE interactions with 5-HTTLPR and other polymorphisms, many studies are limited by small sample size, and replicability remains a major issue (see Duncan et al., 2014 for a review; Risch et al., 2009). Furthermore, most studies to date failed to control for confounding influences on the GxE interaction by not including all required covariate x gene and covariate x environment contrast terms in the regression model (Keller, 2014). Studies examining case-control samples have also tended to evaluate the GxE effect using logistic regression and have thus tested departures from a multiplicative model of interaction, which is believed to be less biologically plausible than an additive model (Rothman, 1976; Rothman and Greenland, 1998).

GxE studies of candidate genes in eating pathology have been scarce. A recent review by Culbert et al. (2015) highlighted the heterogeneity of candidate GxE research in eating pathology. Their investigation identified five studies examining candidate GxE interactions with eating pathology outcome variables. Two studies reported a significant GxE interaction for 5-HTTLPR (Karwautz et al., 2011; parenting styles; Akkermann et al., 2012; traumatic life events), while one study investigating a psychological factor did not (Racine et al., 2009; impulsivity). The two remaining studies examined other genes (NR3C1 x childhood abuse, Steiger et al., 2011; BDNF x restricted food intake; Akkermann et al., 2011).
finding significant interactions to predict bulimia nervosa (BN) spectrum pathology. This paper presents a good start in summarising candidate GxE literature in eating disorders (although it was not a systematic review and thus omitted several studies, e.g., Stoltenberg et al., 2012; van Strien et al., 2010), and reflects the growing focus on gene x environment interactions in the eating disorders field.

While candidate GxE research in eating pathology is still in its infancy, it is not premature to consider how to best utilise academic resources to avoid the pitfalls GxE research has faced in other fields, such as lack of consistent replication and small sample sizes (Dick et al., 2015). This will aid greater accuracy in GxE findings, which is a vital step in increasing understanding of how individual differences at the genetic level can influence susceptibility to eating pathology. In the depression field, a protocol for a collaborative meta-analysis to achieve these aims has been published (N = 33,761), with authors aiming to re-analyse their data using a standardised analysis script to increase consistency of analytic methods and phenotypic definitions (Culverhouse et al., 2013). Future collaborations could integrate complete datasets for combined re-analysis rather than relying on summary statistics. No such study has been undertaken in the ED field so far.

The objective of this study is to provide a systematic, detailed overview and re-analysis of current GxE studies investigating 5-HTTLPR in eating pathology, to clarify the current state of knowledge and to encourage future research to build upon this via HTTLPR in eating pathology, to clarify the current state of knowledge how a genetic variant modifies risk for EDs (e.g., impulsivity in BN, Steiger et al., 2005). Indeed, many studies have investigated psychological factors within a GxE framework both in the ED literature (Akkermann et al., 2011; Racine et al., 2009; Mata and Gotlib, 2011; van Strien et al., 2010) and in other psychopathologies (Lu et al., 2011; Mandelli et al., 2009; Wang et al., 2013). Results were limited to published studies. A total of 1353 papers were initially identified (701 duplicates), with 35 selected for closer reading. Of these, 7 papers met criteria for the systematic review, with a summary provided in Fig. 1.

2.2. Quality appraisal

Quality of each study in the systematic review was evaluated using a framework by Downs and Black (1998). As this tool was created to assess clinical trials, criteria were adapted to evaluate GxE research in eating disorders, with 14 non-applicable criteria excluded. A brief description of the items is presented, with notes in parentheses detailing changes in their current application:

1) Clear description of the hypothesis/aim/objectives; 2) Clear description of main outcomes in introduction/method; 3) Participant characteristics clearly described (as appropriate for GxE and ED research); 4) Clear description of main findings; 5) Characteristics of participants lost to follow-up described; 6) Exact probability values reported (or confidence intervals included); 7) Participants representative of population (including clinical, but not convenience samples); 8) Any “data-dredging” explicitly noted; 9) Appropriate statistical tests used; 10) Main outcome measures valid and reliable; 11) Participants in different groups (if case-control study) recruited contemporaneously; 12) Adequate adjustment for (potential) confounding variables (e.g., BMI; according to Keller (2014), this requires inclusion of all covariate x gene and covariate x environment interaction terms in the model); and 13) Sufficient power (to detect a GxE interaction, as guided by Duncan and Keller, 2011).

Studies were evaluated independently by two coders, V.R. and D.O., and cross-checked for consistency. Discrepancies were discussed amongst the raters with a third author (I.K.) consulted where necessary. Another author with particular expertise in statistical methods in psychology (M.F.T.) additionally evaluated criterion 9. To avoid biases or conflicts of interest, no other co-authors provided input to the evaluation.

Table 1 presents results of the quality evaluation. Discrepancy between coders was lower than 5%. The evaluation found that studies largely adopted valid and reliable methods with good reporting of results. The main issues pertained to insufficient power to detect the small-to-medium effect sizes likely involved in GxE interactions (Duncan and Keller, 2011), and that no study properly controlled for potential confounds on the interaction effect by including covariate x gene and covariate x environment interaction terms (Keller, 2014). Some studies tested three-category polymorphic groupings using cross-product terms in regression models, which was recently suggested to be statistically flawed due to the possibility of both false positive and negative results (Aliev et al., 2014). Nonetheless, the studies present promising initial findings and constitute good building blocks for continued GxE analyses in the field.

2.3. Summary of findings

The systematic review identified 7 studies (see Table 2). Samples were from North American or European countries and n varied from 50 to 384. Participants were mainly adolescents and young adults, with mean age spanning from 13.4 years to 25.6 years. Five studies investigated community samples (total N = 2017, 78.0% female), with two of these studies investigating mixed gender samples. Two studies examined clinical ED patients (N = 348, 100%
female), with one of these a discordant sister-pair sample (N = 128 controls, 100% female).

Three studies found a significant 5-HTTLPR x Traumatic Life Events interaction, although each predicted a different ED pathology; two disordered eating (Akkermann et al., 2012 — EDI-2 Bulimia subscale only; Stoltenberg et al., 2012 — EAT-26 total score) and one Anorexia Nervosa (AN) diagnosis (Karwautz et al., 2011). Notably, unlike in Akkermann et al. (2012) and Stoltenberg et al. (2012), Karwautz et al. (2011) found an interaction only when analysing risky parenting styles and not for broader traumatic life events. One study found a significant sexual abuse x 5-HTTLPR interaction (Akkermann et al., 2012, predicting EDI-2 Bulimia and Drive for Thinness scales), while the other did not (Steiger et al., 2007; predicting BN-spectrum clinical diagnosis).

Neither study reported a significant physical abuse x 5-HTTLPR interaction. Mata and Gotlib (2011) and van Strien et al. (2010) both reported a significant depression x 5-HTTLPR interaction in predicting overeating and emotional eating, respectively, although this effect was only for the s/s genotype in the former study. Racine et al. (2009) found no interaction between 5-HTTLPR or HTR2A (T102C polymorphism) and impulsivity or dietary restraint in predicting binge eating or emotional eating symptoms.

3. Secondary data meta-analyses

3.1. Method

3.1.1. Inclusion criteria

From the final 7 studies identified through systematic review, those that tested equivalent environmental or psychological variables were considered for a secondary data meta-analysis. Six suitable studies were identified (see Fig. 1). Data from one additional study (Richardson et al., 2008) were included in the secondary data analysis but not the systematic review, as it contained relevant variables (drawing from the same larger sample as Steiger et al., 2007), but did not explicitly analyse the GxE interaction with an ED-related outcome variable.
Table 2

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Total no. of participants (no. women)</th>
<th>Mean age, yrs (SD)</th>
<th>Clinical sample</th>
<th>5-HTTLPR genotype %</th>
<th>Outcome (measures)</th>
<th>Environmental factor</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steiger et al. (2007)</td>
<td>92 (92)</td>
<td>25.4 (6.4)</td>
<td>BN</td>
<td>LL LS SS</td>
<td>34 47 20</td>
<td>BN (EDE, EAT-26, DSM-IV diagnosis)</td>
<td>Childhood sexual/physical abuse, (CTI) Impulsivity (Barratt Impulsiveness Scale) Dietary restraint (EDE-Q/DEBQ composite)</td>
</tr>
<tr>
<td>Racine et al. (2009)</td>
<td>344 (344)</td>
<td>19 (1.4)</td>
<td>No</td>
<td>27 56 17</td>
<td></td>
<td>Binge eating (MEBS), Emotional eating (DEBQ)</td>
<td></td>
</tr>
<tr>
<td>Mata and Gotlib (2011)</td>
<td>50 (50)</td>
<td>13.9 (1.9)</td>
<td>No</td>
<td>28 44 28</td>
<td>Overeating (EDI-C)</td>
<td>Depression (CDI)</td>
<td>Interaction between s/s (but not s/l) genotype and depression</td>
</tr>
<tr>
<td>van Strien et al. (2010)</td>
<td>584 (295)</td>
<td>13.4 (0.6)</td>
<td>No</td>
<td>32 50 18</td>
<td>Emotional, eating (DEBQ)</td>
<td>Depression (Depressive Mood List)</td>
<td>Interaction between b/w s-allele and depression on DEBQ scores</td>
</tr>
<tr>
<td>Karwautz et al. (2011)</td>
<td>256 (128 discordant sister-pairs)</td>
<td>25.6 (8.4)</td>
<td>Half AN</td>
<td>38 43 18</td>
<td>AN (EATATE-I interview, DSM-IV diagnosis)</td>
<td>Life events (Oxford Risk Factor Inventory)</td>
<td>Interaction between s-allele and life events, specifically problematic parenting styles</td>
</tr>
<tr>
<td>Akkermann et al. (2012)</td>
<td>252 (252)</td>
<td>17.8 (0.5)</td>
<td>No</td>
<td>103 (l/l) 136 (s/-)</td>
<td>Drive for thinness, Bulimia (EDI-2)</td>
<td>Life events (self-devised scale), including sexual, physical, and emotional abuse</td>
<td>Interaction between s-allele and life events on bulimia only (interaction with sexual abuse for both outcomes, none for physical abuse)</td>
</tr>
<tr>
<td>Stoltenberg et al. (2012)</td>
<td>439 (284)</td>
<td>22.5 (6.2)</td>
<td>No</td>
<td>33 46 21</td>
<td>Disordered eating (EAT-26)</td>
<td>Life events (Traumatic Antecedent Questionnaire)</td>
<td>Interaction between s-allele and traumatic events for females only</td>
</tr>
</tbody>
</table>

Notes. GxE = gene x environment interaction; AN = anorexia nervosa; BN = bulimia nervosa; CDI = Children’s Depression Inventory (Kovacs, 1985); CTI = Childhood Trauma Interview (Pink et al., 1995); DEBQ = Dutch Eating Behavior Questionnaire (van Strien, 2002); EDE = Eating Attitudes Test (Garner et al., 1983); EATATE-I = EATATE Lifetime Diagnostic Interview (Anderluth et al., 2009); EDE = Eating Disorders Examination (Fairburn and Cooper, 1993); EDI-2 = Eating Disorders Inventory-2 (Garner, 1991a); EDI-C = Eating Disorders Inventory for Children and Adolescents (Garner, 1991b); MEBS = Minnesota Eating Behavior Survey (von Ranson et al., 2005). Results are significant at p < 0.05 unless otherwise specified.

3.1.2. Design

Data from six studies (Akkermann et al., 2012; Karwautz et al., 2011; Racine et al., 2009; Richardson et al., 2008; Stoltenberg et al., 2012; Steiger et al., 2007; and van Strien et al., 2010) were combined to test four separate secondary data analyses: 5-HTTLPR x Traumatic Life Events to predict ED diagnosis or equivalent, 5-HTTLPR x Sexual and/or Physical Abuse to predict a BN-spectrum ED or equivalent, 5-HTTLPR x Depression to predict BN-spectrum ED or equivalent, and 5-HTTLPR x Impulsiveness to predict ED diagnosis or equivalent.

3.1.3. Data synthesis

Full data sets from each study were provided. Overlapping participant data in Steiger et al. (2007) and Richardson et al. (2008) were removed by contributing authors prior to sending their data. Karwautz et al. (2011) was part of a European multi-centre collaboration (The European Project) and data for the present study were drawn from the larger unpublished sample, including additional data from clinical BN patients. Data from The European Project were only included if they contained item-level responses to the Oxford Risk Factor Inventory (ORFI; Fairburn et al., 1998) to ensure consistent measurement of the environmental factor ‘traumatic life events’ across studies. Item-level data were unavailable from some participating research centres and therefore the present sample size does not match that of Karwautz et al. (2011), but includes additional participants with a BN diagnosis.

Prior to combining datasets according to the below-mentioned procedures, missing data were imputed at the item-level where necessary using the median value (Tabachnick and Fidell, 2013), with missingness lower than 5%. Participants with missing genetic data or summary scales (where item-level data were unavailable), were excluded from the analyses.

3.1.3.1. Analysis 1 – traumatic life events

Traumatic life events were determined according to 17 events (e.g., traumas/accidents, abuse, major health problems) that overlapped between scales used in Akkermann et al. (2012; self-devised scale), and Stoltenberg et al. (2012; Traumatic Antecedent Questionnaire, Herman & van der Kolk, 1987). Fourteen of these events overlapped with The European Project (ORFI; Fairburn et al., 1998) data, which was scaled to match the 18-item (0–17 events) solution.

ED status or equivalent was determined by a total score above 5 and 3 on the Drive For Thinness and Bulimia Scales of the Eating Disorder Inventory-2 (EDI-2; Garner, 1991a), respectively, which are the recommended scale-level cut-offs for clinical-level disordered eating (Nevonen and Broberg, 2001; Norring and Söhlberg, 1988). In Stoltenberg et al. (2012), ED status or equivalent was determined by a total score of 20 or greater on the Eating Attitudes Test-26 (EAT-26; Garner et al., 1982), the established cut-off for likely clinical-level eating pathology. A semi-structured clinical interview, the EATATE (Anderluth et al., 2009), was used to identify ED diagnosis based on DSM-IV criteria (American Psychiatric Association [APA], 2000) in the European Project.
3.1.3.2. Analysis 2 — sexual and physical abuse. Sexual abuse and physical abuse were coded dichotomously in the European Project and Akkermann et al. (2012), and re-coded into yes/no format in Richardson et al. (2008) and Steiger et al. (2007) if participants endorsed anything above ‘low’ sexual or physical abuse, and in Stoltenberg et al. (2012) if abuse was ‘occasional’ or greater. BN status or equivalent was determined based on whether participant responses to items on the EDI-2 (Garner, 1991a) in Akkermann et al. (2012) and on the EAT-26 (Garner et al., 1982) in Stoltenberg et al. (2012) endorsed DSM-IV (APA, 2000) BN criteria, namely, engaging in regular binge eating, with loss of control, and engagement in compensatory behavior. In addition, participants whose scores on the EDI-2 Bulimia scale and EAT-26 Bulimia and Food Preoccupation scale were substantially elevated, suggesting likely clinical-range BN, were classified in the BN group. BN was determined according to the EATATE (Anderluh et al., 2009) and DSM-IV criteria (APA, 2000) in the European Project, and by the Eating Disorders Examination (EDE; Fairburn and Cooper, 1993) in Steiger et al. (2007) and Richardson et al. (2008).

3.1.3.3. Analysis 3 — depression. Depression was coded dichotomously in the European Project using the ORFI (Fairburn et al., 1998) and in Richardson et al. (2008) as determined by the Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-I, First et al., 1996). Dimensional measurements were obtained in Akkermann et al. (2012) via the self-report version of the Montgomery-Åsberg Depression Rating Scale (MADRS-S; Montgomery and Åsberg, 1979), and van Strien et al. (2010) via the Depressive Mood List (Kandel and Davies, 1982). For compatibility with the European Project and Richardson et al. (2008), these were dichotomised. A cut-off score of 15 was selected for the MADRS-S according to research examining criterion validity (Svanborg and Åsberg, 2001; Svanborg and Ekselius, 2003). No cut-off score has been established for the Depressive Mood List. However, as there was complete overlap between these measures, participant scores on the Depressive Mood List were scaled to match MADRS-S responses and the same cut-off value was applied. BN status or equivalent was determined as in Analysis 2 for the European Project, Richardson et al. (2008), and Akkermann et al. (2012). BN status was based on participant responses to categorical questions investigating binge frequency, loss of control, and engagement in compensatory behaviours in van Strien et al. (2010).

3.1.3.4. Analysis 4 — impulsiveness. All studies assessed impulsiveness using the Barratt Impulsiveness Scale (BIS-11; Patton et al., 1995). ED status or equivalent was determined as in Analysis 1 for Akkermann et al. (2012) and Stoltenberg et al. (2012), while for Racine et al. (2009) this was determined by a mean score of 2.3 or greater on a self-report version of the EDE (EDE-Q; adopted from Fairburn and Cooper, 1993), as suggested by Mond et al. (2004).

3.1.4. Statistical analyses

Analyses were conducted using binary logistic regression to test main and interaction effects of 5-HTTLPR and the environmental or psychological factor in predicting ED/BN status. 5-HTTLPR was coded according to presence or absence of the low-function s-allele. In light of past findings suggesting the s-allele operates in a genetically dominant manner (e.g., Lesch et al., 1996), and in order to avoid issues relating to multiple testing, genotype grouping (s/s, s/l, l/l) was not investigated. All analyses controlled for age by including the age, age x environment, and age x 5-HTTLPR terms to the overall model. BMI was also controlled for where data were available. These interaction terms are necessary to adequately control for potential confounders, although have been omitted from most GxE investigations in psychiatry to date (Keller, 2014). It was not possible to control for sex due to frequency distribution issues in the logistic regression. When examining sex differences by comparing a female only sub-sample to the overall sample in each analysis (a male-only sub-sample was not possible due to frequency distribution), results were similar across all analyses, therefore only results for the larger, complete sample are displayed.

Finally, whereas the interaction term between gene and environmental or psychological factor is sufficient for testing a GxE interaction in logistic regression under a multiplicative model (as per past studies; e.g., Caspi et al., 2003; Karwautz et al., 2011; Steiger et al., 2012; also see Munafò et al., 2009), three additional statistics were computed to quantify the interaction from an additive perspective: the relative excess risk due to interaction (RERI), the attributable portion due to the interaction (AP), and the synergy index (S). When an interaction is present in the data, RERI and AP will be greater than 0, whereas S will be greater than 1. These additive models were conducted using Stata version 13. Estimates of these interaction effects were derived from relative risk ratios rather than odds ratios, as: (1) the formulae for RERI, AP, and S were designed to use with RR values, and (2) substituting OR values for RR values in this formula will over-estimate the interaction effects in cases where the baseline prevalence is not rare (e.g., greater than 10% prevalence; VanderWeele and Knol, 2014). To facilitate calculation of RR values, the two continuous predictors – traumatic experiences and impulsivity – were converted into categorical forms. Trauma history was split into no instances reported versus 1 + instances reported. As the appropriate cut-point for the impulsivity measure is unclear, several percentiles were trialled (5th, 10th, 15th, 20th, and 25th). Substantive conclusions did not change depending on the cut-off applied, and as such, results are reported for the lowest cut-off (5th percentile) to conceptually reflect those with lowest reported levels of impulsivity.

3.2. Results

3.2.1. Analysis 1: traumatic life events

The sample comprised 909 individuals (65.7% female), from the following studies: two community samples, Stoltenberg et al. (2012); N = 436, 65.1% female), Akkermann et al. (2012; N = 369, 56.6% female), and a discordant sister-pair sample, the European Project (N = 104, 100% female).

Overall, 169 (18.6%) participants met criteria for an ED or equivalent. 5-HTTLPR frequencies (l/l = 29%, s/l = 41%, s/s = 16%) met the Hardy-Weinberg equilibrium, \( \chi^2 = 2.55, df = 1, p > 0.05 \). Traumatic Life Events were scored 0 to 17, (M = 2.38 events, SD = 2.54), and were positively skewed. Results of the logistic regression are displayed in Table 3.

As evident in Table 3, while there was no effect of traumatic events or genotype alone, presence of the s-allele was related to significantly greater likelihood of an ED for those who had experienced more traumatic life events compared to those with the l/l genotype (OR = 1.23, see Fig. 2). A small but significant main effect of age was also noted. From an additive perspective however, none of the interaction indices reported significant findings to support an interaction effect: RERI = −0.90 (95% CIs: −4.17, 2.36), \( p = 0.587 \); AP = −0.53 (95% CIs: −2.85, 1.79), \( p = 0.654 \); and S = 0.44 (95% CIs: 0.01, 16.53), \( p = 0.657 \).

3.2.2. Analysis 2: sexual and/or physical abuse

The sample comprised 1097 individuals (71.8% female), from the following studies: two community samples, Stoltenberg et al. (2012); N = 436, 65.1% female), Akkermann et al. (2012; N = 369, 56.6% female), one clinical sample from Steiger et al. (2007) and Richardson et al. (2008), (N = 127, 100% female) and a discordant sister-pair sample, the European Project (N = 168, 63% controls, 100% female).
likelihood of BN was also significantly predicted by an interaction between (younger) age and each of the abuse variables. From an additive perspective, a number of the interaction indices displayed significant findings to also support an interaction effect for physical abuse: RERI = 2.26 (95% CIs: 0.05, 4.47), p = 0.045; AP = 2.70 (95% CIs: 0.43, 0.70), p = 0.041; but not S = 0.49 (95% CIs: 0.13, 0.85), p = 0.038.

Results outlined in Table 3 show significant main effects for sexual abuse (OR = 8.56), physical abuse (OR = 4.79), and both sexual and physical abuse combined (OR = 11.53). There was a significant GxE interaction (OR = 3.15), whereby participants with the s-allele who experienced both sexual and physical abuse were more likely to endorse BN status compared to those with the l/l genotype (Fig. 2). There was a main effect of age, and increased
3.2.3. Analysis 3: depression

The sample comprised 1254 individuals (62.5% female), from the following studies: two community samples, Akkermann et al. (2012; N = 369, 56.6% female), and van Strien et al. (2010; N = 623, 51.2% female), one clinical sample, Richardson et al. (2008; N = 89, 100% female) and a discordant sister-pair sample, the European Project (N = 168, 63% controls, 100% female).

Overall, 172 (13.7%) participants met criteria for BN or equivalent, while 184 (14.7%) participants met criteria for depressed mood. 5-HTTLPR frequencies (l/l = 438, l/s = 612, s/s = 205) met the Hardy-Weinberg equilibrium, $\chi^2 = 0.14$, $df = 1$, $p > 0.05$. Results of the logistic regression revealed no main or interaction effects of depression and 5-HTTLPR in predicting BN status (Table 3). Similar to the pattern observed in Analysis 2, younger age interacted with depressive status to predict greater likelihood of BN. There was also no support for an interaction effect under an additive model, $\text{RERI} = 0.15$ (95% CIs: $-0.95, 1.26$), $p = 0.785$ and $\text{AP} = 0.13$ (95% CIs: $-0.77, 1.03$), $p = 0.778$. S could not be reliably computed for this interaction.

3.2.4. Analysis 4: impulsiveness

The sample comprised 1122 individuals (72.2% female) from three community samples, Stoltenberg et al. (2012; N = 436, 65.1% female), Akkermann et al. (2012; N = 369, 56.6% female), and Racine et al. (2009; N = 317, 100% female).

Overall, 224 (20.0%) participants met ED criteria or equivalent. 5-HTTLPR frequencies (l/l = 384, l/s = 545, s/s = 193) met the Hardy-Weinberg equilibrium, $\chi^2 < 0.01$, $df = 1$, $p > 0.05$. Impulsivity was measured using the Barratt Impulsiveness Scale Version 11 (BIS-11; Patton et al., 1995), and was normally distributed. Data did not meet the assumption of linearity between continuous independent variables and the logit ($p = 0.003$), suggesting that results may present an underestimation of the relationship (Hosmer and Lemeshow, 1989). Results of the logistic regression revealed no main or interaction effects of impulsiveness and 5-HTTLPR in predicting ED status (Table 3), which was supported by the indices measuring additive interaction, $\text{RERI} = -1.18$ (95% CIs: $-4.22, 1.86$), $p = 0.448$; $\text{AP} = -0.85$ (95% CIs: $-2.49, 0.78$), $p = 0.307$; and $S = 0.24$ (95% CIs: $0.03, 1.83$), $p = 0.170$.

4. Discussion

To our knowledge, this is the first systematic review and secondary data meta-analysis investigating the role of 5-HTTLPR x environment and psychological factor interactions in risk for eating pathology. The aim was to summarize and re-analyse existing GxE research on eating disorder-related outcomes investigating the 5-HTTLPR polymorphism, in the largest sample tested to date, in order to elucidate the current state of knowledge and provide guidance for future GxE studies in the field. Results of the secondary data meta-analysis revealed that when testing deviations from an additive model of interaction, the experience of sexual abuse, physical abuse, and both sexual and physical abuse each interacted with the s-allele of 5-HTTLPR to predict increased risk of bulimia-spectrum eating pathology. The significant interaction between 5-HTTLPR and both sexual and physical abuse (but not only one) was also supported from a multiplicative perspective, although there was no support for sexual abuse or physical abuse considered alone. In addition, there was a significant interaction between traumatic life events and 5-HTTLPR to predict an increased risk of eating pathology under the multiplicative model only. No effects were noted for the potential risk factors of depression and impulsiveness under either model.

Other noteworthy results include the large direct effects of sexual abuse and physical abuse on BN-spectrum disorders, an association demonstrated in previous meta-analyses (Chen et al., 2010; Norman et al., 2012; Smolak and Murnen, 2002). Conversely, there were no main effects of 5-HTTLPR genotype in any analyses, contrary to some past findings (Calati et al., 2011; Lee and Lin, 2010), although aligned with others (Castellini et al., 2012; Solmi et al., 2016). The current GxE findings suggest that individuals with the ‘risky’ genotype may be relatively resilient to low levels of environmental risk, but disproportionately affected by greater environmental adversity (e.g., experiencing numerous types of abuse). From a biological perspective, it is plausible that this may function via the lowered serotonin transcription associated with the s-allele of 5-HTTLPR, leading to reduced availability of a key neurotransmitter in the stress response system (van Eekelen et al., 2012).

The present results demonstrate some links to findings in the depression field, where greater traumatic life events, including childhood abuse, have been found to interact with 5-HTTLPR to predict depression (Karg et al., 2011; Nugent et al., 2011), although the interaction between life events and 5-HTTLPR is not undisputed (Munafó et al., 2009; Risch et al., 2009). One caveat is that ‘traumatic life events’ is a heterogeneous concept. The types of events measured, scaling process, timing of events, age of participants,
etc., may vary greatly, perhaps accounting for some inconsistency in GxE findings in the depression field (Uher and McGuffin, 2008). Careful consideration of these factors is encouraged for future analyses.

Aside from consistent measurement of environmental variables, another key issue affecting GxE research is low statistical power. Use of small sample sizes with insufficient power to detect GxE interactions has been a major point of criticism in GxE research for increasing risk of both false negative and false positive findings (Button et al., 2013). Sample sizes necessary to detect GxE effects are far bigger than typically involved in psychology (Luan et al., 2001), with one calculation of minimum sample size necessary to detect a large GxE interaction effect at 80% power, assuming no measurement error, \( N = 600 \) (Duncan and Keller, 2011). This increases substantially if only moderate effect sizes are involved. The median sample size of studies identified by the systematic review was 288, which is considered substantial in the ED field but lacking for genetic analyses. This is a particularly challenging limitation in light of the difficulty of obtaining genetic samples and highlights the immense value of the present collaboration, which has allowed us to utilise existing resources to maximize sample size and further knowledge regarding GxE effects in eating pathology.

One factor that may yet affect accuracy of the present findings is the possibility of publication bias among the studies identified by the systematic review. This has been noted in past GxE research, with one review reporting that significant findings were observed in 96% of initial GxE investigations but in only 27% of subsequent replication attempts (Duncan and Keller, 2011; Duncan et al., 2014). However, others argue that many instances of non-replication are related to methodological issues, including inadequate measurement of traumatic life events (Caspi et al., 2010; Monroe and Reid, 2008). In any case, the tendency for positive findings to be more readily published, and null findings perhaps less likely to be initially submitted, can have a large effect on the accuracy of published studies by inflating false positive results (Dick et al., 2015; Ioannidis et al., 2014). It is therefore vital, for the success of future collaborative meta-analyses, for researchers to publish both significant and non-significant findings and for journals to support this initiative, while emphasising the use of reliable environmental measures.

Aside from the benefits of large sample sizes and resource efficiency in the present investigation, it further improved upon existing GxE research in eating pathology by correctly controlling for the potential effect of confounding variables (Keller, 2014). The inclusion of all necessary interaction terms was also facilitated by the large sample sizes investigated, and should be aimed for in future studies. Another strength of the present study was that it examined GxE interactions under both additive and multiplicative models of interaction. Most previous studies using a logistic regression model to assess their data have tested deviations from a multiplicative model. Conversely, studies of community samples with continuous outcome variables typically use linear regression models, which test deviations from an additive model. As the two methods produce somewhat different results, with the latter generally more conservative, caution should be taken in comparing the results of these models, and indeed, this may account for some of the discrepant findings in GxE research.

The present secondary data meta-analysis is not without limitations, primarily due to the need to harmonise heterogeneous datasets, which tested both community and clinical samples and contained varied measures of environmental and psychological factors and eating symptoms. The investigation of the 5-HTTLPR \( \times \) depression interaction was in particular hindered by variability in the measurement of depression between studies. These methodological issues may explain why this interaction was not found to be significant in the present analysis, contrary to findings in two of the initial studies (Mata and Gotlib, 2011; van Strien et al., 2010).

Nonetheless, the present study provides a detailed overview of current GxE findings involving 5-HTTLPR in the ED field, including studies assessing psychological variables. Subsequent research should focus on continued replication with large sample sizes, possibly best achieved through ongoing collaboration between researchers, given the resource-intensive nature of genetic research and scarcity of clinical ED samples. Such investigations would be best facilitated by researchers adopting standardised, or easily comparable, measures of environmental and psychological factors and eating symptoms that have excellent psychometric properties. Selection of measures should be carefully deliberated, both to maximize construct validity and to reduce measurement error, which can substantially increase statistical power (Bakermans-Kranenburg and van Ljzendorn, 2014). Polymorphisms and environmental or psychological factors selected should also be carefully justified, particularly in light of sample size restrictions (Dick et al., 2015).

Future studies may also benefit from adopting a differential susceptibility approach to GxE investigations. This hypothesis posits that certain alleles may be better conceptualised as conferring ‘plasticity’ in response to environmental stimuli, with alleles linked to poorer outcomes under negative environments conversely linked to better outcomes in positive environments (Belsky and Pluess, 2009). Such an analysis was not possible in the current paper due to lack of data assessing positive environments, however this pattern has been demonstrated for various polymorphisms, including 5-HTTLPR, in non-ED literature (see Bakermans-Kranenburg and van Ljzendorn, 2011; for a meta-analysis; Hankin et al., 2011). Accordingly, studies should include environmental measures that range from positive to negative in nature, such as parenting, peer relationships, or positive life events.

In sum, the present collaboration constitutes a large step forward in increasing knowledge of how genetics may moderate the manner in which environmental and psychological influences affect the likelihood of ED development. Given the ongoing uncertainty regarding why thus far identified risk factors appear to contribute towards ED development for some individuals but not others, genetics may be an important missing puzzle piece in identifying the source of individual variation in susceptibility to eating pathology.

Role of funding source

Financial support was received from the European Union (Framework-V Multicenter Research Grant, QK1-1999-916), the University of Melbourne Early Career Researcher Grants Scheme (2014, 1350035), U.S. National Institute of Mental Health (1R15MH077654-01A1), the Estonian Ministry of Education and Science (IUT20-40 and IUT 42-2), the National Institute of Health and Michigan State University (T32-MH070343 and #05-IRGP-883), the Quebec government’s Joint CQRS-FRSQ-MSSS Program in Mental Health (SR-4306), the Canadian Institute of Health Research (MOP-57929), the Dutch Organization for Scientific Research (no. 400-05-051) and the Radboud University Nijmegen. None of these institutions had any role in the study design, collection, analysis and interpretation of data, preparation of the manuscript, or decision to submit the manuscript for publication.

Contributors

Ms. Rozenblat was responsible for conducting all analyses and preparing all sections of the manuscript. Ms. Ong contributed to the systematic review section, including searching, recording results,
evaluating the studies, and contributing to that section of the manuscript. Drs. Krug and Fuller-Tyszczewicz contributed to study design and editing drafts of the manuscript, and Dr. Fuller-Tyszczewicz also contributed to the analyses section. Remaining authors were involved in the collection of data. All authors contributed to and approved the final manuscript.

Conflict of interest
None declared.

Acknowledgements
This paper forms part of Vanja Rozenblat’s PhD with publication undertaken at The University of Melbourne.

References


References


APPENDIX B

Additional Analyses of Data from Study 1

This appendix presents results from a number of additional analyses that were not featured in Study 1 due to space restrictions. This includes examination of sex differences, and additional exploration of the 5-HTTLPR x traumatic life events analysis. The appendix concludes with a discussion of how the meta-analysis undertaken in Study 1 addressed the issues relating to GxE interactions that were identified in the previous chapter.

Additional investigation of Analysis 1 (traumatic life events): Examination of main effects

Although not a primary goal of this thesis, it is also worthwhile to examine any main effects of traumatic life events on the ED outcome variable in Analysis 1 (traumatic life events) in Study 1. Main effects within a regression model may be best examined when there is no interaction term included in the model, as inclusion of the contrast term complicates the interpretation of the main effect (Schielzeth, 2010). Thus, a simplified version of the model featured in Study 1 is presented below, in order to observe possible main effects of traumatic life events. One note of caution, however, is that these additional post-hoc analyses should be recognised as only secondary to the main aims of this thesis and interpreted with caution due to issues relating to ‘data dredging’.
Table 13

Main Effects of Traumatic Life Events and 5-HTTLPR on the Composite ED Outcome Variable, Controlling for Age and Gender

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Odds Ratio</th>
<th>95% CI for Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Constant</td>
<td>-6.00</td>
<td></td>
<td>.79</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>.14</td>
<td>1.15</td>
<td>.79</td>
</tr>
<tr>
<td>Traumatic Events</td>
<td>.08</td>
<td>1.09</td>
<td>1.02</td>
</tr>
<tr>
<td>Gender</td>
<td>1.70</td>
<td>5.46</td>
<td>3.21</td>
</tr>
<tr>
<td>Age</td>
<td>.06</td>
<td>1.06</td>
<td>1.03</td>
</tr>
</tbody>
</table>

Note. This is an additional analyses not included in Study 1.

According to the modified logistic regression model not containing the GxE interaction terms, there was no main effect of 5-HTTLPR genotype on likelihood of having an ED (see Table 13). Meanwhile, there was a relationship between experiencing greater traumatic life events and ED diagnosis. This result, using one of the largest samples to date, corresponds with findings from a multitude of studies testing the relationship between adverse life events and eating pathology (Johnson et al., 2002; Meiser & Esser, 2017; Pike et al., 2006; Speranza et al., 2003). However, the associated effect size (OR: 1.09, 95% CI: 1.02, 1.16) was relatively small, indicating that the strength of the relationship in the present sample is moderate at best.

Additional investigation of Analysis 1 (traumatic life events): Excluding abuse items

In previous research, examination of the 5-HTTLPR x stressful life events interaction has typically involved a wide variety of measures, with some including sexual and physical abuse and others testing non-abuse related stressors (e.g., see Tielbeek et al., 2016, for a review of GxE interaction studies in the anti-social literature). In the depression literature, one meta-analysis by Risch et al. (2009) states that most studies tested traumatic life events as specified
by the Brugha List of Threatening Experiences (Brugha, Bebbington, Tennant, & Hurry, 1985), a list of 12 event categories that does not include a measure of sexual or physical abuse. However, closer inspection reveals that many studies in the Risch et al. (2009) review (e.g., Chorbov et al., 2007; Middeldorp, Cath, Beem, Willemsen, & Boomsma, 2008) utilised study-specific measures that did examine sexual and physical abuse. In a second systematic review and meta-analysis of the 5-HTTLPR x stressful life event interaction in the depression field by Munafò et al. (2009), it is further evident that many studies (e.g., Kaufman et al., 2006; Scheid et al., 2007) classified sexual and physical abuse as a ‘stressful’ life event. Indeed, in a third meta-analysis on this topic, by Karg et al. (2011), this distinction was specifically tested. They found that while studies specifically examining childhood abuse as the environmental variable produced strong evidence of an interaction with 5-HTTLPR to predict depression ($p = .00007$), the interaction with stressful life events excluding childhood abuse showed a far weaker effect ($p = .033$).

In Study 1, both in Analysis 1, investigating traumatic life events, and Analysis in 2, examining sexual and physical abuse, there were significant GxE interactions to predict eating pathology. Conversely, no direct effects of traumatic life events on ED status were found in the main regression model (in Study 1), while there was a moderate to strong main effect of sexual and physical abuse on bulimia spectrum pathology. This indicates the possibility of a stronger link between abuse and eating pathology compared to the association between general life stressors and eating pathology. Given that the traumatic life events scale tested in Study 1 included the sexual and physical abuse items, it is possible that the significant GxE interaction was partly driven by the inclusion of the abuse variables. By removing items examining sexual and physical abuse from Analysis 1, it will be possible to evaluate the impact of general life stressors, such as illness, injury, separation from family, etc., on eating pathology, independently from the experience of abuse.
To achieve this, participant scores on the measure of Traumatic Life Events were re-calculated to exclude the items measuring sexual and physical abuse. Data from Akkermann et al. (2012) and Stoltenberg et al. (2012) were consequently recoded from 16 items to 11 items, and data from The European Project were recoded from 14 items to 7 items, with the total score re-scaled to 11 to attain equivalence with the two community samples assessed in Akkermann et al (2012) and Stoltenberg et al. (2012). As in the original analysis (Study 1), a logistic regression model tested for main and interaction effects of 5-HTTLPR and traumatic life events on the eating-disorder outcome variable, controlling for age as per Keller (2014).

Table 14

*Interaction Between 5-HTTLPR and Traumatic Life Events, Excluding Sexual and Physical Abuse, on Bulimic Status (N = 909)*

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Odds Ratio</th>
<th>Lower</th>
<th>Upper</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-4.21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>.91</td>
<td>2.47</td>
<td>.70</td>
<td>8.70</td>
<td>.158</td>
</tr>
<tr>
<td>Traumatic Events</td>
<td>.18</td>
<td>1.20</td>
<td>.90</td>
<td>1.59</td>
<td>.223</td>
</tr>
<tr>
<td>5-HTTLPR x Traumatic Events</td>
<td>.16</td>
<td>1.18</td>
<td>.97</td>
<td>1.42</td>
<td>.092</td>
</tr>
<tr>
<td>Age</td>
<td>.12</td>
<td>1.13</td>
<td>1.07</td>
<td>1.18</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Age x 5-HTTLPR</td>
<td>-.05</td>
<td>.95</td>
<td>.90</td>
<td>1.01</td>
<td>.090</td>
</tr>
<tr>
<td>Age x Traumatic Events</td>
<td>-.01</td>
<td>.99</td>
<td>.98</td>
<td>1.00</td>
<td>.993</td>
</tr>
</tbody>
</table>

*Notes.* Odds ratio = Exp(B). These are additional analyses not included in Study 1.

The findings in Table 14 show that with the exclusion of sexual and physical abuse from the measure of traumatic life events, the interaction between this variable and the 5-HTTLPR s-allele is no longer significant. There are several possible interpretations of this finding. Firstly, it is possible that sexual and/or physical abuse, or the combination of the two, was the main driving factor of the significant traumatic life events x 5-HTTLPR interaction in the
original analysis. Given the strong main effects of sexual and physical abuse on BN-spectrum pathology identified in Study 1 (and supported by past meta-analyses; Chen et al., 2010; Munafò et al., 2009; Norman et al., 2012; Smolak & Murnen, 2002), it is possible that individuals with the s-allele are more poorly equipped to cope with this established ED risk factor, thus accounting for the GxE interaction independently of other traumatic life events. However, it is also possible that by considering fewer items, the modified measure of traumatic life events represents a diluted picture of the life stressors experienced by participants. As a result, the associated effect size might be smaller and thus require a larger sample than presently available to capture any significant GxE interaction. This is a particularly important consideration, given the very large sample sizes estimated as necessary for the detection of low to moderate effect sizes in GxE research (Duncan & Keller, 2011).

While experiences of sexual and physical abuse are certainly types of traumatic life events (Finkelhor, 1987), it would be worthwhile for future studies and reviews to more explicitly and consistently define what they classify as a ‘life stressor’, to increase the generalisability of results and ease subsequent replication attempts.

**Additional analysis: Investigation of main effects of depressed mood**

As discussed in regards to the traumatic life events analyses, investigation of the main effects of depression on BN was not the intended purpose of the Study 1. However, it may be worthwhile examining whether such effects exist when the model does not include the depression x 5-HTTLPR interaction term. In particular, given that this contrast did not meet the threshold for significance, it may be that a simplified model provides a better fit for the data. Once again, this post-hoc analysis should be regarded as supplementary and interpreted with a degree of reserve.
Table 15

Main Effects of Depression and 5-HTTLPR on the Composite BN Outcome Variable, Controlling for Age, Gender, and BMI

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Odds Ratio</th>
<th>95% CI for Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-5.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>.27</td>
<td>1.31</td>
<td>.86 2.02</td>
</tr>
<tr>
<td>Depression</td>
<td>.22</td>
<td>1.25</td>
<td>.73 2.13</td>
</tr>
<tr>
<td>Gender</td>
<td>1.58</td>
<td>1.21</td>
<td>1.10 1.42</td>
</tr>
<tr>
<td>Age</td>
<td>.21</td>
<td>1.23</td>
<td>1.19 1.27</td>
</tr>
<tr>
<td>BMI</td>
<td>.01</td>
<td>1.01</td>
<td>.98 1.04</td>
</tr>
</tbody>
</table>

Note. These are additional analyses not included in Study 1

Inspection of Table 15 shows that even in the simplified regression model, there were no main effects of depression or 5-HTTLPR. Given the strong past evidence supporting a relationship between depression and eating pathology (Puccio et al., 2016), it is possible that the lack of main effect identified both in Study 1 and the additional analyses in Table 15 may partly result from methodological considerations. In particular, due to the need to harmonise five data sets to form the combined analysis investigating the 5-HTTLPR x depression interaction, the slightly different criteria used to assess depression between the five studies may have resulted in a more ‘blunt’ measure of depression. Meanwhile, age and gender were strong predictors of bulimic status, with female gender and older age independently related to greater likelihood of BN. Given that the clinical samples included in the combined analyses tended to feature older participants, the finding that older age was associated with bulimic status is not unexpected, while the association between gender and BN reflects heavily established research findings regarding gender and EDs (Allen et al., 2016; Murnen & Smolak, 2015).
Impulsivity

Once again, additional analyses were conducted in the present chapter to re-analyse the main effects of 5-HTTLPR and impulsivity as per the simplified model. This resulted in some evidence of a relationship between higher scores on the BIS and disordered eating, however the effect size was very small (OR: 1.02, 95% CI: 1.00, 1.03; see Table 16). Interestingly, in the simplified model there was a significant main effect of 5-HTTLPR, however due to the very marginal significance value ($p = .048$) and effect size (OR: 1.40, 95% CI: 1.00, 1.96), and issues related to multiple testing, this result should be viewed with extreme caution.

Table 16

Main Effects of Impulsivity and 5-HTTLPR on the Composite Disordered Eating Outcome Variable, Controlling for Age and Gender.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Odds Ratio</th>
<th>Lower</th>
<th>Upper</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-6.46</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>.34</td>
<td>1.40</td>
<td>1.00</td>
<td>1.96</td>
<td>.048</td>
</tr>
<tr>
<td>Impulsiveness</td>
<td>.02</td>
<td>1.02</td>
<td>1.00</td>
<td>1.03</td>
<td>.023</td>
</tr>
<tr>
<td>Gender</td>
<td>1.79</td>
<td>6.01</td>
<td>3.59</td>
<td>10.07</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Age</td>
<td>.02</td>
<td>1.02</td>
<td>.99</td>
<td>1.06</td>
<td>.209</td>
</tr>
</tbody>
</table>

Note. These are additional analyses not included in Study 1.

The role of gender in gene x environment interactions

One limitation of Study 1 is that it did not focus on measuring gender differences. Given that a number of the GxE studies identified by the systematic review in Study 1 noted different GxE effects for males and females (Akkermann et al., 2011; Stoltenberg et al., 2012; van Strien et al., 2015; van Strien et al., 2010b), it is worthwhile to also explore any potential gender differences in the four analyses included in Study 1. The primary reason that gender
was not controlled for in the publication of Study 1 is that it was not possible to add the gender term as a covariate into the regression model (along with the required gene x covariate and environment x covariate contrast terms as per Keller, 2014), due to issues with the probability distribution in the logistic regression model. Specifically, Study 1 included too few male participants who met criteria for an ED and had experienced abuse, a given level of traumatic life events, or had high scores on the measure of depression. As a result, logistic regression was unable to produce models when controlling for gender.

Another approach to identifying GxE interaction effects according to gender is to perform the analyses in two distinct samples, one testing females and the other testing males. It was not possible to test a male-only sample due to the aforementioned restrictions. This limitation did not affect female-only samples. The following section will therefore briefly present the results of each analysis re-examined in a female-only sample to explore any possible gender differences.

**Additional analyses: Analysis 1 (traumatic life events) in a female-only sub-sample**

An additional regression model testing the interaction between 5-HTTLPR and number of traumatic life events in predicting ED status was conducted in a female-only sub-sample \((N = 597)\) taken from the original sample \((N = 909)\). This subsample contained \(n = 209\) participants from Akkermann et al. (2012), \(n = 284\) from Stoltenberg et al. (2012), and all original participants from the European Project, \(n = 104\), which was originally a female-only sample.

In this analysis, 25.5% of females met criteria for an ED or equivalent (compared to 18.6% in the overall sample). 5-HTTLPR frequencies \((l/l = 211, l/s = 280, s/s = 106)\) met the Hardy-Weinberg equilibrium, \(\chi^2 = .61, df = 1, p > .05\). Traumatic life events were once again positively skewed, with \(M = 2.49\) events, \(SD = 2.69\) events (compared to \(M = 2.38\) events, \(SD \)
Results of the logistic regression model are displayed in Table 17.

As in the overall sample, a significant interaction effect between 5-HTTLPR and number of traumatic life events in predicting ED status was found, with the same effect size noted (OR = 1.23). As in the main analysis, a small but significant main effect of age was present, with no other significant main or interaction effects. In sum, these results do not represent a deviation from those obtained from the overall (male and female) sample.

Additional analyses: Analysis 2 (sexual and physical abuse) in a female-only sub-sample

An additional regression model examining the interaction between 5-HTTLPR and sexual and/or physical abuse in predicting BN status was conducted in a female-only sub-sample (N = 788) taken from the original sample (N = 1097). This sub-sample contained n = 209 participants from Akkermann et al. (2012), n = 284 from Stoltenberg et al. (2012), and all original participants from Steiger et al. (2007) and Richardson et al. (2008), n = 127, and The European Project, n = 168, which were female-only samples.

In this analysis, 27.2% of females met criteria for BN or equivalent (compared to 20.1% of the overall sample). Two hundred and ten (26.6%) of female participants reported experiencing physical abuse (compared to 28.5% of individuals in the overall sample), 157 (19.9%) reported experiencing sexual abuse (compared to 15% in the overall sample), and 80 (10.2%) reported both physical and sexual abuse (compared to 7.7% in the overall sample). 5-HTTLPR frequencies (l/l = 285, l/s = 357, s/s = 146) met the Hardy-Weinberg equilibrium, $\chi^2 = 3.31$, df = 1, p > .05.

As in the overall sample, there was no interaction effect between 5-HTTLPR and sexual abuse in predicting BN status, although the interaction between 5-HTTLPR and physical abuse approached significance (p = .05; see Table 17). There remained a main effect of physical abuse on BN status (OR = 7.78, 95% CI: 1.93, 31.35), which was of a slightly
greater magnitude than in the overall sample (OR = 4.79, 95% CI: 1.37, 16.70). Conversely, in the female-only sample the main effect of sexual abuse on BN status was somewhat smaller (OR = 4.85, 95% CI: 1.08, 21.67) compared to in the overall sample (OR = 8.56, 95% CI: 2.16, 33.92).

Results in the female-only sample again revealed a significant GxE interaction effect between 5-HTTLPR and sexual and physical abuse, with a comparable effect size (OR = 3.41, 95% CI: 1.13, 10.32) to the overall sample (OR = 3.15, 95% CI: 1.09, 9.09). As in the overall sample, experiencing both sexual and physical abuse was associated with a significantly greater chance of BN status for those who had at least one copy of the s-allele, compared to those with the l/l genotype. Interestingly, the main effect of both sexual and physical abuse was no longer significant (result in overall sample was OR = 11.53, 95% CI: 2.26, 58.68). This may suggest that the original effect may have largely been driven by the male participants.

Given that only a small number of males in the present sample experienced both sexual and physical abuse (1.6%), this increases the likelihood that the finding of a strong effect size of both sexual and physical abuse on BN status may be, in part, due to chance. Another possibility was that this effect was attenuated due to the lower power present in the female-only sample, and if analysed with fewer predictors (i.e., by removing all contrast terms from the model to examine main effects only), this may result in a significant finding. The latter analysis is not undertaken in this chapter to avoid ‘data mining’, as it is not the primary goal of this investigation, although it is worthwhile noting that a recent meta-analysis of childhood abuse (sexual, physical, and emotional) did not report any gender differences in the relationship between abuse and EDs (Caslini et al., 2016). Given this inconsistency, it would remain worthwhile to replicate the present results in an independent sample containing a larger number of male participants.
Table 17

Main and Interaction Effects of 5-HTTLPR s-allele with Traumatic Life Events and Sexual and/or Physical Abuse in Predicting ED Status and BN Status in a Female Sample Only, Controlling for Age and for BMI where Possible.

<table>
<thead>
<tr>
<th>Analysis 1: Traumatic life events (N=597)</th>
<th>95% CI for Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B</strong></td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>Constant</td>
<td>-3.29</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>.78</td>
</tr>
<tr>
<td>Traumatic Events</td>
<td>-.07</td>
</tr>
<tr>
<td>5-HTTLPR x Traumatic Events</td>
<td>.20</td>
</tr>
<tr>
<td>Age</td>
<td>.11</td>
</tr>
<tr>
<td>Age x 5-HTTLPR</td>
<td>-.06</td>
</tr>
<tr>
<td>Age x Traumatic Events</td>
<td>-.001</td>
</tr>
</tbody>
</table>

| Analysis 2: Sexual/Physical abuse (N=788)  |

Sexual abuse

| Constant       | -4.09     |       |       |      |
| 5-HTTLPR       | -.003     | 1.00  | .28   | 3.61 | .997 |
| Sexual Abuse   | 1.58      | 4.85  | 1.08  | 21.67| .039 |
| 5-HTTLPR x Sexual Abuse | .51     | 1.66  | .70   | 3.91 | .247 |
| Age            | .12       | 1.13  | 1.08  | 1.18 | <.001|
| Age x 5-HTTLPR | .004     | 1.00  | .95   | 1.06 | .885 |
| Age x Sexual Abuse | -.05 | .95   | .90   | 1.00 | .065 |

Physical abuse

<p>| Constant       | -4.53     |       |       |      |
| 5-HTTLPR       | .09       | 1.09  | .29   | 4.07 | .89  |
| Physical Abuse | 2.05      | 7.77  | 1.93  | 31.35| .004 |
| 5-HTTLPR x Physical Abuse | .76     | 2.13  | 1.00  | 4.54 | .050 |
| Age            | .14       | 1.15  | 1.10  | 1.20 | &lt;.001|
| Age x 5-HTTLPR | -.003    | 1.00  | .95   | 1.05 | .913 |</p>
<table>
<thead>
<tr>
<th>Model</th>
<th>Coefficient</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sexual and Physical Abuse</strong></td>
<td>-0.06</td>
<td>0.94</td>
<td>0.89</td>
<td>0.99</td>
<td>0.019</td>
</tr>
<tr>
<td>Constant</td>
<td>-4.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5-HTTLPR</strong></td>
<td>0.16</td>
<td>1.17</td>
<td>0.33</td>
<td>4.22</td>
<td>0.808</td>
</tr>
<tr>
<td>Sexual and Physical Abuse</td>
<td>1.56</td>
<td>4.74</td>
<td>0.76</td>
<td>29.52</td>
<td>0.096</td>
</tr>
<tr>
<td><strong>5-HTTLPR x Sexual and Physical Abuse</strong></td>
<td>1.23</td>
<td>3.41</td>
<td>1.13</td>
<td>10.32</td>
<td>0.030</td>
</tr>
<tr>
<td>Age</td>
<td>0.12</td>
<td>1.13</td>
<td>1.08</td>
<td>1.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age x <strong>5-HTTLPR</strong></td>
<td>-0.003</td>
<td>1.00</td>
<td>0.95</td>
<td>1.05</td>
<td>0.904</td>
</tr>
<tr>
<td>Age x Sexual and Physical Abuse</td>
<td>-0.06</td>
<td>0.94</td>
<td>0.89</td>
<td>1.01</td>
<td>0.070</td>
</tr>
</tbody>
</table>

**Analysis 3: Depression**

(N= 785)

<table>
<thead>
<tr>
<th>Model</th>
<th>Coefficient</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-6.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5-HTTLPR</strong></td>
<td>-0.38</td>
<td>0.69</td>
<td>0.06</td>
<td>8.31</td>
<td>0.767</td>
</tr>
<tr>
<td>Depression</td>
<td>-0.96</td>
<td>0.39</td>
<td>0.01</td>
<td>10.56</td>
<td>0.385</td>
</tr>
<tr>
<td><strong>5-HTTLPR x Depression</strong></td>
<td>1.03</td>
<td>2.81</td>
<td>0.83</td>
<td>9.56</td>
<td>0.098</td>
</tr>
<tr>
<td>Age</td>
<td>0.23</td>
<td>1.26</td>
<td>1.06</td>
<td>1.49</td>
<td>0.009</td>
</tr>
<tr>
<td>BMI</td>
<td>0.05</td>
<td>1.05</td>
<td>0.86</td>
<td>1.28</td>
<td>0.647</td>
</tr>
<tr>
<td>Age x BMI</td>
<td>-0.001</td>
<td>1.00</td>
<td>0.99</td>
<td>1.06</td>
<td>0.752</td>
</tr>
<tr>
<td>Age x <strong>5-HTTLPR</strong></td>
<td>0.05</td>
<td>1.05</td>
<td>0.97</td>
<td>1.13</td>
<td>0.712</td>
</tr>
<tr>
<td>Age x Depression</td>
<td>-0.08</td>
<td>0.92</td>
<td>0.86</td>
<td>1.00</td>
<td>0.041</td>
</tr>
<tr>
<td>BMI x <strong>5-HTTLPR</strong></td>
<td>-0.03</td>
<td>0.97</td>
<td>0.88</td>
<td>1.07</td>
<td>0.584</td>
</tr>
<tr>
<td>BMI x Depression</td>
<td>0.10</td>
<td>1.10</td>
<td>0.98</td>
<td>1.25</td>
<td>0.119</td>
</tr>
</tbody>
</table>

**Analysis 4: Impulsivity**

(N= 810)

<table>
<thead>
<tr>
<th>Model</th>
<th>Coefficient</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-3.44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5-HTTLPR</strong></td>
<td>0.57</td>
<td>1.77</td>
<td>0.13</td>
<td>25.10</td>
<td>0.672</td>
</tr>
<tr>
<td>Impulsiveness</td>
<td>0.02</td>
<td>1.02</td>
<td>0.96</td>
<td>1.08</td>
<td>0.542</td>
</tr>
<tr>
<td><strong>5-HTTLPR x Impulsiveness</strong></td>
<td>0.01</td>
<td>1.01</td>
<td>0.97</td>
<td>1.04</td>
<td>0.712</td>
</tr>
<tr>
<td>Age</td>
<td>0.07</td>
<td>1.07</td>
<td>0.91</td>
<td>1.27</td>
<td>0.424</td>
</tr>
<tr>
<td>Age x <strong>5-HTTLPR</strong></td>
<td>-0.04</td>
<td>0.97</td>
<td>0.89</td>
<td>1.04</td>
<td>0.377</td>
</tr>
<tr>
<td>Age x Impulsiveness</td>
<td>&lt;0.001</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.997</td>
</tr>
</tbody>
</table>

Odds ratio = Exp(B).
Additional analyses: Analysis 3 (depression) in a female-only sub-sample

The additional analysis of the female-only sample for Analysis 3, which investigated whether 5-HTTLPR interacted with depression to predict BN status, consisted of $N = 785$ participants (Study 1 $N = 1254$). This comprised $n = 209$ from Akkermann et al. (2012), $n = 319$ from van Strien et al. (2010b), and full samples from The European Project, $n = 168$, and Richardson et al. (2008), $n = 89$, who both tested female-only samples. In this sample, 17.2% of participants met criteria for depression (compared to 14.7% in the overall sample) and 20.8% met criteria for BN (compared to 13.7% in the overall sample). 5-HTTLPR frequencies ($l/l = 278, l/s = 376, s/s = 131$) met the Hardy-Weinberg equilibrium, $\chi^2 = .04, df = 1, p > .05$.

Results from this analysis are displayed in Table 17. As in the overall sample, there were no significant main or interaction effects of genotype or depression identified. Age remained a significant predictor of BN. These results suggest that the lack of significant findings in the overall sample was not a result of gender obscuring any existing GxE interaction effects.

Additional analyses: Analysis 4 (impulsivity) in a female-only sub-sample

The additional analyses investigating the interaction between 5-HTTLPR and impulsiveness in predicting ED status in a female-only sample consisted of $N = 810$ participants (Study 1 $N = 1114$), with $n = 209$ from Akkermann et al. (2012), $n = 284$ from Stoltenberg et al. (2012), and the full sample from Racine et al. (2009), $n = 317$, which consisted only of female participants. 25.6% of females met criteria for an ED (compared to 20.0% in the overall sample). Impulsiveness was normally distributed, with $M = 63.1$ and $SD = 9.7$. 5-HTTLPR frequencies ($l/l = 262, l/s = 410, s/s = 138$) met the Hardy-Weinberg equilibrium, $\chi^2 = 1.09, df = 1, p > .05$. Results of this analysis are displayed in Table 17. As was found for the original analysis of the entire sample in Study 1, the present additional
analyses in a female-only sample revealed no significant main or interaction effects for any of the variables included in the model.

**Summary of gender differences**

In sum, the investigation of GxE interactions in four female-only samples did not reveal substantive differences compared to results in the overall sample. The only differences included that the main effect of both physical and sexual abuse on BN status was no longer significant in the female-only sample, and that the 5-HTTLPR x physical abuse interaction in predicting BN status approached borderline significance. It is possible that physical abuse may be experienced as more traumatic for females than males, and thus constituting a greater risk factor for females with existing genetic vulnerability (i.e., the s-allele) compared to males. However, given the borderline significance value ($p = .05$), no conclusions should be drawn from this finding until it is replicated in an independent sample.

One pertinent consideration when interpreting the results derived from the female-only samples is that they contained a smaller number of participants compared to the original analyses in Study 1, which is a substantial drawback given the large sample sizes required to ensure that genetic studies are adequately powered (Duncan & Keller, 2011). For this reason, along with the fact that the pattern of results in this sample reflected the results of the combined sample, only findings taken from the larger, male and female sample, were included in the publication. A final point to note is that the higher rate of BN and ED identified in the female samples compared to the combined samples was in part due to the fact that the female-only samples contained a higher portion of clinical ED participants, as all three clinical samples included in the meta-analysis consisted of only female participants.
Investigating Direct Links between Depression, Emotional Control, and Physical Punishment with Adolescent Drive for Thinness and Bulimic Behaviors, Including Possible Moderation by the Serotonin Transporter 5-HTTLPR Polymorphism

Vanja Rozenblat1,*, Joanne Ryan2, Eleanor H. Wertheim3, Ross King4, Craig A. Olsson2,4,5 and Isabel Krug1

1 School of Psychological Sciences, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, VIC, Australia, 2 Murdoch Childrens Research Institute, Royal Children's Hospital Melbourne, Parkville, VIC, Australia, 3 School of Psychology and Public Health, Faculty of Health, La Trobe University, Melbourne, VIC, Australia, 4 School of Psychology, Faculty of Health, Centre for Social and Early Emotional Development, Deakin University, Geelong, VIC, Australia, 5 Department of Paediatrics, The Royal Children's Hospital Melbourne, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, VIC, Australia

Objectives: To examine the relationship between psychological and social factors (depression, emotional control, sexual abuse, and parental physical punishment) and adolescent drive for Thinness and Bulimic behaviors in a large community sample, and to investigate possible genetic moderation.

Method: Data were drawn from the Australian Temperament Project (ATP), a population-based cohort study that has followed a representative sample of 2443 participants from infancy to adulthood across 16 waves since 1983. A subsample of 650 participants (50.2% female) of Caucasian descent who provided DNA were genotyped for a serotonin transporter promoter polymorphism (5-HTTLPR). Adolescent disordered eating attitudes and behaviors were assessed using the Bulimia and Drive for Thinness scales of the Eating Disorder Inventory-2 (15–16 years). Depression and emotional control were examined at the same age using the Short Mood and Feelings Questionnaire, and an ATP-devised measure of emotional control. History of sexual abuse and physical punishment were assessed retrospectively (23–24 years) in a subsample of 467 of those providing DNA.

Results: EDI-2 scores were associated with depression, emotional control, and retrospectively reported parental physical punishment. Although there was statistically significant moderation of the relationship between parental physical punishment and bulimic behaviors by 5-HTTLPR \((p = 0.0048)\), genotypes in this subsample were not in Hardy–Weinberg Equilibrium. No other G×E interactions were significant.
INRODUCTION

Eating disorders (EDs) are believed to have a substantial heritable component (Bulik et al., 2016), with estimates from twin studies ranging from 40 to 60% (Yilmaz et al., 2015). Thus far, research examining molecular genetic mechanisms that may increase risk for eating pathology has largely investigated whether certain genetic polymorphisms (e.g., serotonin transporter linked polymorphism, 5-HTTLPR) are found in different frequency in those with a clinical ED compared to controls. These studies have largely produced inconsistent findings (Calati et al., 2011; Solmi et al., 2016), supporting the notion that genetic risk operates in a manner more complex than simple association. One line of research receiving increasing attention is the possibility that certain polymorphisms may induce differential risk, depending upon exposure to certain environmental factors, via gene by environment (G×E) interaction.

Studies examining whether G×E interactions play a role in ED etiology have largely focused on 5-HTTLPR, with the short (s) allele associated with lower serotonin transcription activity compared to the long (l) allele (Heils et al., 1996). Serotonin plays a role in mood regulation, appetite, and weight (Blundell, 1984; Leibowitz and Alexander, 1998; Kalra et al., 1999; Ruhé et al., 2007), all known to be involved in eating pathology. Serotonin is also involved in the stress-response system (Gotlib et al., 2008; van Eekelen et al., 2012), and as 5-HTTLPR is a functional polymorphism it may conceivably play a role in EDs, directly or indirectly through interaction with other environmental stressors. However, in other fields of psychiatry, the role of 5-HTTLPR in moderating the effects of environmental stressors remains controversial; for example, in depression, where independently conducted meta-analysis continue to contradict one another (cf. Munafò et al., 2009; Risch et al., 2009; Karg et al., 2011). Lack of consensus stems from a range of methodological limitations, such as insufficient sample size, inappropriate statistical techniques and multiple testing, as well as substantial publication bias favoring significant G×E findings (Duncan and Keller, 2011; Duncan et al., 2014; Dick et al., 2015; de Vries et al., 2016).

To date, seven publications have investigated the role of G×E interactions in the ED field involving 5-HTTLPR (Rozenblat et al., 2017). Systematic review and meta-analysis of these studies suggested that 5-HTTLPR may moderate the risk relationship between experiencing both sexual and physical abuse and bulimic symptomatology (combined N = 1,096), and traumatic life events and ED symptomatology (N = 909). This was not the case, however, for risk relationships between depressive and bulimic symptomatology (N = 1,254) or impulsivity and disordered eating (N = 1,122). Findings from this review suggest that risk associated with 5-HTTLPR may be intensified under increasingly severe social stress, but not psychological distress.

However, findings from this work were based on a combined sample that was derived by summing across small highly heterogeneous samples [e.g., two community samples, N = 369, Akkermann et al., 2012; N = 623, van Strien et al., 2010; a clinical sample, N = 89, Richardson et al., 2008; and a discordant sister-pair sample, N = 168 from European cross-institutional data set used in Karwautz et al. (2011)]. Testing interactions in one large, homogenous sample would be preferable (Cochran, 1954). This, for example, may explain why 5-HTTLPR was found to moderate the effects of depression on eating outcomes in two of the original studies (van Strien et al., 2010; Mata and Gotlib, 2011) but not in the combined data analysis (Rozenblat et al., 2017). Furthermore, the lack of significant interaction between impulsivity and 5-HTTLPR in the combined-sample may be partly due to the analysis of impulsivity as an overall construct, rather than separately testing the particular facets of impulsivity that have previously been associated with EDs, such as negative urgency (Race et al., 2009, 2013). Negative urgency refers to the tendency to act rashly or feel strong impulses when experiencing negative affect (Whiteside and Lynam, 2001), and, along with the broader ability to regulate one’s emotions, has wide empirical support for a role in EDs, particularly bulimia nervosa symptomatology (Fischer et al., 2003; Claes et al., 2005), with some evidence linking emotional regulation and 5-HTTLPR function (Hariri and Holmes, 2006). From a theoretical perspective, lowered emotional control may lead to greater eating pathology, as individuals may attempt to control their emotional states via altered food intake (e.g., binge eating or restricted intake; Haynos and Fruzzetti, 2011; Pearson et al., 2015). Meanwhile, the other psychological factor that has been analyzed in a G×E framework, depressed mood, is believed to precipitate bulimic behaviors under a number of key ED models (e.g., Dual Pathway Model; Stice, 2001) with support from longitudinal investigations of high-risk samples (Stice et al., 2017), although there is evidence suggesting depressed mood may also arise as a consequence of eating pathology (Puccio et al., 2016).

While many prior studies investigating G×E interactions have focussed on patients with clinical EDs (Rozenblat et al., 2017), analysis of disordered eating in community samples is of equal, if not greater importance. Developing a better understanding of the correlates and risk factors for pre-clinical eating pathology, which may later develop into a ‘full blown’ ED (Herpertz-Dahlmann et al., 2013), can help promote prevention at the earliest possible opportunity to reduce ED incidence (Stice et al., 2007). From
a practical perspective, this also allows for the collection of far larger samples compared to studies using case-control designs, which is a key consideration in genetic association research (Duncan and Keller, 2011).

To further test preliminary findings from Rozenblat et al. (2017) in a homogenous sample, the present study used data from 650 participants who provided DNA in the Australian Temperament Project (ATP), a population based cohort study that has followed a representative sample of around 2000 participants from infancy to adulthood since 1983. The first aim was to assess the direct effects of depressed mood, emotional control, sexual abuse, and parental physical punishment on adolescent drive for thinness and bulimic behaviors. The second aim was to examine the extent to which the relationships between these factors and eating pathology were moderated by 5-HTTLPR. Results of this study constitute an important step toward accumulating evidence regarding whether genetic factors may moderate the influence of psychological and environmental risk factors on EDs.

MATERIALS AND METHODS

Participants
Australian Temperament Project participants were initially recruited in infancy (4–8 months) in Victoria in 1983, using a stratified random sampling framework, via maternal and child health centers in urban and rural locations. The first survey included 2,443 infants (48.0% female), with 16 surveys completed to date. The present study involved a sub-set of 650 participants (50.2% female) who had completed the 11th survey at age 15–16 years, providing information on drive for thinness and bulimic behaviors, depressive symptoms and emotional control, and who also provided a saliva sample for genotyping at this time (N = 567), or in their early 30s (N = 83, in 2015 as part of a separate sub-study). Of the 650 participants, 467 participants also provided retrospective information regarding sexual abuse and parental physical punishment in the 14th survey at age 23–24, and were included in the respective analyses. To avoid issues related to genetic heterogeneity, 23 participants who self-identified as non-Caucasian were excluded prior to forming the sample. The final analysable sample had a higher proportion of participants from the highest SES quartile than the original sample (for full socio-demographic information, see Table 1). Due to missing data, the final sample comprised 643 participants for the depression analyses and 649 for the emotional control analyses. Parents and adolescents provided written informed consent for each survey wave and for the collection of saliva samples. The data collection was approved by the Australian Institute of Family Studies Ethics Review and carried out in accordance with the latest version of the Declaration of Helsinki.

Measures
Disordered Eating
Drive for thinness and bulimic behaviors were assessed at age 15–16 via the Eating Disorder Inventory-2 (EDI-2; Garner, 1991) and the Eating Disorder Inventory-2 (EDI-2; Garner, 1991) Drive for Thinness and Bulimia scales. The Drive for Thinness scale consists of 7-items measuring participants’ desire to lose weight or fear of weight gain (e.g., “I am preoccupied with the desire to be thinner”). Internal consistency in the current sample was α = 0.92. The Bulimia scale consists of 7 items measuring bulimic behaviors, including binging and purging (e.g., “I stuff myself with food”), with Cronbach’s α = 0.74 in the current sample. For details of scoring and some minor modifications made for an Australian context, refer to Krug et al. (2016).

Psychological Stress Exposures
Depression was assessed at the same time-point as disordered eating via the Short Mood and Feelings Questionnaire (SMFQ) (Angold et al., 1995), a 13-item subscale derived from the original 33-item questionnaire. The SMFQ is intended as a screening measure for children and adolescents that queries depressive symptoms according to DSM-III criteria (American Psychiatric Association [APA], 1980; e.g., “I feel miserable or unhappy”), with responses provided on 3-point scale (rarely/never, sometimes, often/always). Participants with missing data on five or more items were excluded from analyses (α = 0.83 in current sample).

Emotional control measured participants’ capacity to control their emotions and was also assessed at age 15–16 using an ATP-devised measure consisting of 10-items (e.g., “I am able to keep my feelings under control” and “I am able to calm down if I am feeling nervous”) rated on a 6-point scale from never to always. This measure has been previously used in studies examining internalizing problems (Toumbourou et al., 2011), with α = 0.70 in the present sample.

Sexual and Physical Stress Exposures (Retrospective)
A number of retrospective indicators were used at age 23–24 to assess sexual abuse and parental physical punishment during childhood and adolescence. Sexual abuse was based on a ‘yes’ response to the questions: “You had a sexual experience with a person who was not a family member prior to 16” and a follow up ‘no’ response to the question “Was this consensual?” or, a ‘yes’ response to the question “A family member did, or tried to do, sexual things to you.”

Mild-to-moderate parental physical punishment was based on a ‘yes’ response to the question “Your parent/s used harsh physical treatment (e.g., smacking, hitting) to discipline you,” and severe parental physical punishment was based on an additional ‘yes’ response to a follow up question, “Did you ever suffer effects that lasted to the next day or longer (e.g., bruising, marking, pain, soreness)?”, creating two distinct severity categories.

5-HTTLPR Genotyping (Moderation Variable)
Following the 11th survey, DNA for 567 participants was isolated using Qia-gen QIAamp kits from buccal epithelial cells via cotton swabs, with further details described in Jorm et al. (2000). Saliva samples for an additional 83 participants were collected following the 16th survey in 2015 using Oragene saliva pots or tubes and analyzed at the Australian Genomics Research Facility (AGRF), Adelaide, SA, Australia. Genotype frequencies were similar in the original and 2015 samples. For all samples, 5-HTTLPR genotype
was coded as per the di-allelic model into s-present (s/s or s/l genotype) or s-absent (l/l genotype) groups, as the s-allele is believed to operate in a genetically dominant manner (Lesch et al., 1996).

**Potential Confounding Factors**

Age, height, and weight were self-reported at age 15–16, with the latter two figures used to calculate participant BMI. SES status was measured according to maternal and paternal education and occupation as reported by parents in the first survey in 1983.

**Data Analysis**

The main and interaction effects of 5-HTTLPR and the two psychological stress exposures (depression and emotional control), as well as the three social stress exposures (sexual abuse, mild-to-moderate parental punishment, and severe parental physical punishment), were assessed using separate linear regression models. Outcome variables were Drive for Thinness and Bulimia scores. G×E models were adjusted for sex and BMI, as per Keller (2014), by including all the covariate × gene and covariate × environment interaction terms in the regression models. Prior to analyses, missing data (23.5%) for the BMI variable were imputed using multiple imputation in IBM SPSS Version 21, with no systematic patterns of missingness observed. A total of 10 tests were conducted with p-values adjusted accordingly (adjusted p-value = 0.05/10, corrected p = 0.005), to correct for multiple-testing, a frequent limitation of genetic association studies (Munafo and Flint, 2009). Standardized effect sizes are reported.

**RESULTS**

5-HTTLPR genotype distribution (l/l = 197, s/l = 341, and s/s = 112) for the overall sample met the Hardy–Weinberg Equilibrium, \( \chi^2 = 2.96, df = 1, p > 0.05 \). 5-HTTLPR genotype

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Sociodemographic details of participants included in the present sample.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>Full sample</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Males</td>
<td>312</td>
</tr>
<tr>
<td>Females</td>
<td>338</td>
</tr>
<tr>
<td><strong>SES Quartile</strong></td>
<td>632</td>
</tr>
<tr>
<td>Highest</td>
<td>231</td>
</tr>
<tr>
<td>Medium-High</td>
<td>197</td>
</tr>
<tr>
<td>Medium-Low</td>
<td>129</td>
</tr>
<tr>
<td>Lowest</td>
<td>75</td>
</tr>
<tr>
<td><strong>Parent marital status</strong></td>
<td>645</td>
</tr>
<tr>
<td>Married/De facto</td>
<td>538</td>
</tr>
<tr>
<td>Separated/Divorced</td>
<td>73</td>
</tr>
<tr>
<td>Single/Widowed</td>
<td>22</td>
</tr>
<tr>
<td>Remarried</td>
<td>12</td>
</tr>
<tr>
<td><strong>Father’s occupation</strong></td>
<td>612</td>
</tr>
<tr>
<td>Professional</td>
<td>172</td>
</tr>
<tr>
<td>Managerial</td>
<td>126</td>
</tr>
<tr>
<td>Semi-skilled</td>
<td>200</td>
</tr>
<tr>
<td>Unemployed/Pensioner/House duties</td>
<td>114</td>
</tr>
<tr>
<td><strong>Mother’s occupation</strong></td>
<td>637</td>
</tr>
<tr>
<td>Professional</td>
<td>175</td>
</tr>
<tr>
<td>Managerial</td>
<td>54</td>
</tr>
<tr>
<td>Semi-skilled</td>
<td>193</td>
</tr>
<tr>
<td>Unemployed/Pensioner/House duties</td>
<td>215</td>
</tr>
<tr>
<td><strong>Father’s education</strong></td>
<td>594</td>
</tr>
<tr>
<td>Tertiary</td>
<td>176</td>
</tr>
<tr>
<td>Diploma/Apprenticeship</td>
<td>103</td>
</tr>
<tr>
<td>Year 11/12</td>
<td>167</td>
</tr>
<tr>
<td>Year 10 or less</td>
<td>148</td>
</tr>
<tr>
<td><strong>Mother’s education</strong></td>
<td>633</td>
</tr>
<tr>
<td>Tertiary</td>
<td>133</td>
</tr>
<tr>
<td>Diploma/Apprenticeship</td>
<td>102</td>
</tr>
<tr>
<td>Year 11/12</td>
<td>227</td>
</tr>
<tr>
<td>Year 10 or less</td>
<td>171</td>
</tr>
</tbody>
</table>
distribution was not in Hardy–Weinberg Equilibrium for the subsample providing retrospective reports of sexual and physical stress \((l/l = 139, s/l = 255, \text{ and } s/s = 73; \chi^2 = 6.11, df = 1, p = 0.014)\). Further descriptive statistics are presented in Table 2. Across all regression models, female sex predicted Drive for Thinness and Bulimia scores, while BMI predicted Drive for Thinness (all \(p < 0.001\)). Tables pertaining to each regression model discussed below are contained in the Supplementary Materials.

**Depression**
There was a significant positive association between depressive symptoms and Drive for Thinness \((\beta = 0.24, p < 0.001)\), as well as Bulimia scores \((\beta = 0.41, p < 0.001)\); however, there was no evidence of genetic moderation by \(5-HTTLPR\). There was a significant interaction between depression and sex, with depression associated with greater Drive for Thinness \((\beta = 0.46, p = 0.001)\), and to a lesser extent, Bulimia \((\beta = 0.36, p = 0.018)\), for females only. There were no other significant effects.

**Emotional Control**
Lower emotional control was significantly associated with greater Drive for Thinness \((\beta = -0.22, p < 0.001)\) and Bulimia \((\beta = -0.29, p < 0.001)\) scores; however, there was no evidence of genetic moderation by \(5-HTTLPR\). There was a significant interaction between sex and emotional control, with lower emotional control associated with greater Drive for Thinness \((\beta = -0.45, p = 0.001)\) and Bulimia \((\beta = -0.67, p < 0.001)\) for females to a greater extent than for males. However, amongst those with the highest levels of emotional control, females displayed lower levels of Bulimia than did males.

**Sexual Abuse and Parental Physical Punishment**
Of the 467 participants (59.1% female) who provided data on sexual abuse and parental physical punishment in the 14th survey, 22 (4.7%) reported sexual abuse, 180 (38.5%) reported mild to moderate parental physical punishment, and 27 (5.8%) reported severe parental physical punishment. See Table 3 for further descriptive statistics. Predictor and outcome variables for this sub-sample did not differ from the overall sample \((t\text{-tests all } p > 0.05)\).

**DISCUSSION**
This study affirmed the central relationship between depression, emotional control, and physical abuse and adolescent bulimic behaviors and attitudes regarding thinness. Conversely, \(5-HTTLPR\) did not directly predict any pattern of disordered eating, nor was there conclusive evidence that \(5-HTTLPR\) moderated any risk factor for disordered eating. A statistically significant interaction between \(5-HTTLPR\) and retrospectively reported parental physical punishment was observed; however, genotypes in this subsample were not in Hardy–Weinberg Equilibrium so cautious interpretation and independent replication is needed.

Findings from this study support a key role for depression and compromised emotional control in adolescent drive for thinness and bulimic behaviors. The relationships differed by sex in most cases. Female sex predicted greater overall drive for thinness and bulimia, and sex differences were present in the relationships between depression, emotional control, and sexual abuse with disordered eating symptoms. Parental physical punishment was the only variable that showed no sex differences in its relationship to Drive for Thinness and Bulimia.

**TABLE 2** Descriptive statistics for mean values of continuous predictor and outcome variables in the overall sample \((N = 650)\), in females \((N = 326)\), and in males \((N = 324)\).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Full sample</th>
<th>Females</th>
<th>Males</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.72 (0.16)</td>
<td>15.74 (0.14)</td>
<td>15.72 (0.17)</td>
<td></td>
</tr>
<tr>
<td>EDI-2 Bulimia</td>
<td>1.78 (0.65)</td>
<td>1.94 (0.73)</td>
<td>1.62 (0.53)</td>
<td></td>
</tr>
<tr>
<td>EDI-2 drive for thinness</td>
<td>2.23 (1.15)</td>
<td>2.81 (1.22)</td>
<td>1.64 (0.70)</td>
<td></td>
</tr>
<tr>
<td>Emotional control</td>
<td>3.74 (0.63)</td>
<td>3.62 (0.64)</td>
<td>3.85 (0.59)</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>0.49 (0.34)</td>
<td>0.59 (0.36)</td>
<td>0.39 (0.28)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>21.27 (3.28)</td>
<td>21.20 (3.10)</td>
<td>21.34 (3.44)</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 3** Descriptive statistics for mean values of continuous predictor and outcome variables in the subsample \((N = 467)\), in females \((N = 199)\), and in males \((N = 157)\).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Full sample</th>
<th>Females</th>
<th>Males</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.72 (0.15)</td>
<td>15.73 (0.15)</td>
<td>15.71 (0.14)</td>
<td></td>
</tr>
<tr>
<td>EDI-2 Bulimia</td>
<td>1.81 (0.67)</td>
<td>1.92 (0.68)</td>
<td>1.62 (0.51)</td>
<td></td>
</tr>
<tr>
<td>EDI-2 drive for thinness</td>
<td>2.37 (1.20)</td>
<td>2.79 (1.22)</td>
<td>1.69 (0.74)</td>
<td></td>
</tr>
<tr>
<td>Emotional control</td>
<td>3.74 (0.64)</td>
<td>3.64 (0.65)</td>
<td>3.88 (0.59)</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>0.51 (0.34)</td>
<td>0.58 (0.02)</td>
<td>0.40 (0.28)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>21.19 (3.13)</td>
<td>21.17 (2.97)</td>
<td>21.21 (3.37)</td>
<td></td>
</tr>
</tbody>
</table>
to eating pathology. Results support the notion that correlates of and risk factors for disordered eating symptoms show substantial variation between males and females (Lewinsohn et al., 2002; Striegel-Moore et al., 2009). They also support past studies linking depression (Puccio et al., 2016) and emotional control (Svaldi et al., 2012) to ED symptomatology, and are aligned with theories proposing that individuals may engage in disordered eating in order to better regulate negative affect or other undesirable emotions (Stice, 2001; Haynos and Fruzzetti, 2011; Pearson et al., 2015). Overall, results highlight the importance of psychological factors and the role of the social environment in eating pathology.

The tentative suggestion that 5-HTTLPR may moderate the relationship between severe, but not mild-to-moderate parental physical punishment and bulimic behaviors is reflected in findings from Rozenblat et al. (2017), which reported moderation of both sexual and physical abuse by 5-HTTLPR in predicting bulimia-spectrum pathology, with strongest effects when both types of abuse were experienced. This suggests that further investigation in larger independent samples in Hardy–Weinberg Equilibrium (the expected allele distribution in a given population, deviation from which may compromise validity of results) may well support moderation of more extreme forms of adversity by 5-HTTLPR. Further support for the idea that 5-HTTLPR might moderate more severe forms of risk for disordered eating come from past studies in the ED field that found traumatic life events were associated with bulimic symptoms and disordered eating for individuals with the 5-HTTLPR s-allele (Akkermann et al., 2012; Stoltenberg et al., 2012), and is reflected by the focus on traumatic life events and sexual abuse in the depression field (Nugent et al., 2011).

Lack of genetic moderation of depression or emotional control in predicting drive for thinness and bulimic tendencies is mostly consistent with previous findings (Rozenblat et al., 2017). These results align with the secondary data analysis in Rozenblat et al. (2017), and suggest that the null findings for depression and impulsiveness reported in the secondary data analysis were likely not due to sample heterogeneity or use of the broad impulsiveness variable as opposed to examining a personality construct that is more closely associated with eating pathology, such as emotional control. However, sample size limitations mean that the presence of small effects cannot be entirely ruled out and further investigation in larger samples remains important.

The lack of genetic moderation reported for depression does, however, contradict the significant G×E interactions between depression and 5-HTTLPR identified in two past studies (van Strien et al., 2010; Mata and Gotlib, 2011); however, the sample of Mata and Gotlib (2011) (N = 50) was very low for investigation of genetic association (Duncan and Keller, 2011), while van Strien et al. (2010) examined emotional eating, which differed somewhat from the eating constructs measured in the present study. One possibility is that as psychological factors appear to be strong direct predictors of eating pathology, they may function as risk factors irrespective of 5-HTTLPR genotype. In contrast, certain environmental factors may have a more tenuous association with ED symptoms and thus plausibly could increase risk primarily for individuals with a genetic susceptibility.

The absence of direct genetic association in this study also partly conflicts with previous findings (Calati et al., 2011; Chen et al., 2015). Direct genetic prediction of ED has been investigated in several past studies examining clinical populations with mixed results. Two meta-analyses identified a direct association between 5-HTTLPR and eating pathology (Odds Ratio: 1.35, 95%CI: 1.07–1.71, Calati et al., 2011; Chen et al., 2015), although they examined almost entirely the same group of studies, while the largest and most recent meta-analysis on this topic reported no association (Solmi et al., 2016). Notably, these meta-analyses were limited by substantial heterogeneity, the inclusion of studies with very small sample sizes (N < 100), and omission of tests for publication bias. Publication bias is noted to be a major problem affecting studies of G×E interactions and contributing to false-positive findings (Duncan and Keller, 2011; de Vries et al., 2016), with such issues argued to most strongly affect studies with small sample sizes (Ioannidis et al., 2014).

Strengths and Limitations

Strengths of the present study include use of a homogenous, high-quality data set, with measurement of drive for thinness and bulimic tendencies in a community sample. Results are therefore of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology. A further strength was the fact that the present study constituted the second largest unified sample of key relevance to aiding prevention of the development of clinical-level eating pathology.
model of 5-HTTLPR, with some evidence that the tri-allelic may better represent activity of this polymorphism (Wendland et al., 2006), as well as the use of self-report questionnaires to measure most constructs, with accounts of sexual abuse and parental physical punishment measured retrospectively. Accordingly, the measure of emotional control used in the present study does not have published psychometric properties, although it has been used in previous research (O’Connor et al., 2011; Toumbourou et al., 2011). Finally, 5-HTTLPR is just one of numerous genetic factors that may be involved in the etiology of disordered eating.

**Implications and Future Directions**

Findings from this study suggest that psychological and environmental variables remain central in eating pathology, while evidence for specific candidate genes continues to be tentative at best. Although a statistically significant genetic interaction effect was identified in this study, evidence remains inconclusive because the subsample on which it was based was not in Hardy–Weinberg Equilibrium. It is important to note, however, that the null results reported in this study sit in contrast to the substantial genetic contribution to most psychiatric outcomes estimated in twin study designs (Trace et al., 2013). This suggests that there is still much work to do in the area of eating pathology to adequately explain the variation reported in twin studies. Null findings from this study suggest a more complex picture of genetic determination, one that would benefit from a move to genome-wide approaches, with an emphasis on identifying polygenic effects that emerge from networks of genes, which may better reflect the genetic foundations of complex diseases. Future studies of candidate genes should prioritize increasing statistical power, which may be achieved via data sharing across consortiums of life-course studies. Studies such as the present investigation provide a valuable contribution that should form part of future meta-analytic investigations, and constitute an important step forward in progressing investigation of how psychosocial and genetic factors may be related to eating pathology.

**AUTHOR CONTRIBUTIONS**

VR was responsible for conducting all analyses and preparing all sections of the manuscript. IK, JR, EW, RK, and CO were involved in collecting data and revising the manuscript for important intellectual content. All authors contributed to and approved the final manuscript.

**FUNDING**

This work was supported by an Early Career Researcher Grant (1350035), an Australian Research Council Senior Research Fellowship (DP 130101459), and the Australian Postgraduate Award. None of these institutions had any role in the study design, collection, analysis and interpretation of data, preparation of the manuscript, or decision to submit the manuscript for publication.

**ACKNOWLEDGMENTS**

The ATP study is located at The Royal Children’s Hospital Melbourne and is a collaboration between Deakin University, The University of Melbourne, the Australian Institute of Family Studies, The University of New South Wales, The University of Otago (NZ), and The Royal Children’s Hospital; further information available at www.aifs.gov.au/atp. The views expressed in this paper are those of the authors and may not reflect those of their organizational affiliations, nor of other collaborating individuals or organizations. We acknowledge all collaborators who have contributed to the Australian Temperament Project, especially Professors Ann Sanson, Margot Prior, Frank Oberklaid, John Toumbourou and Ms. Diana Smart. We would also like to sincerely thank the participating families for their time and invaluable contribution to the study. This paper forms part of VR’s Ph.D. with publication undertaken at The University of Melbourne.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpsyg.2017.01361/full#supplementary-material

**REFERENCES**


**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2017 Rozenblat, Ryan, Wertheim, Olsson and Krug. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.
RESEARCH ARTICLE

Relationships Between Self-Reported and Observed Parenting Behaviour, Adolescent Disordered Eating Attitudes and Behaviours, and the 5-HTTLPR Polymorphism: Data From the Australian Temperament Project

Vanja Rozenblat1* 1Psychological Sciences, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, VIC, Australia
Joanne Ryan2, Eleanor Wertheim3, Ross King4, Craig A. Olsson2,4,5, Primrose Letcher5 & Isabel Krug1

1Murdock Children’s Research Institute, Royal Children’s Hospital Melbourne, Parkville, VIC, Australia
2School of Psychology and Public Health, Faculty of Health, La Trobe University, Bundoora, VIC, Australia
3Department of Paediatrics, The Royal Children’s Hospital Melbourne, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, VIC, Australia
4Centre for Social and Early Emotional Development, School of Psychology, Faculty of Health, Deakin University, Geelong, VIC, Australia
5School of Psychology, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, VIC, Australia

Abstract

This study examined whether self-reported and observationally measured parental behaviours were associated with disordered eating, and investigated possible moderation by a serotonin-transporter polymorphism (5-HTTLPR). Study 1 included 650 adolescents from the Australian Temperament Project who completed the Eating Disorder Inventory-2 Drive for Thinness and Bulimia scales at 15/16 years and were genotyped for 5-HTTLPR. Parents completed an Australian Temperament Project-devised measure of parental warmth and harsh punishment. Study 2 included a subgroup of 304 participants who also engaged in a video-recorded family interaction, with observed parental warmth and hostility coded by the Iowa Family Interaction Rating Scale. Greater self-reported parental warmth associated with lower bulimia scores. Conversely, observationally measured parental warmth was associated with lower drive for thinness, but not bulimia. Self-reported parental harsh punishment was associated with bulimia only, with observed parental hostility associated with neither outcome. 5-HTTLPR genotype did not moderate the relationship between parent behaviours and adolescent disordered eating. Copyright © 2017 John Wiley & Sons, Ltd and Eating Disorders Association.

Received 21 February 2017; Revised 18 May 2017; Accepted 19 May 2017

Keywords

disordered eating; gene environment interactions; 5-HTTLPR; parenting behaviours; observational measurement

*Correspondence

Vanja Rozenblat, School of Psychological Sciences, The University of Melbourne, Level 12 Redmond Barry Building, Parkville 3010, Australia.
Email: vanja@rozenblat.net

Published online in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/erv.2530

The biopsychosocial approach to eating disorder (ED) aetiology proposes that risk factors for EDs range from those relating to the individual, such as genes and psychological traits, to those that form part of the environment, including relationships with parents and peers, significant life events, exposure to the ‘thin ideal’, and numerous other factors (Culbert, Racine, & Klump, 2015; Stice, 2002; Trace, Baker, Penas-Lledo, & Bulik, 2013). Risk of developing an ED is believed to be associated with an interplay between these factors. For example, individuals exposed to ‘risky’ environments may develop ED symptoms only if they also carry certain ‘risky’ psychological or genetic factors (Stice, Marti, & Durant, 2011). In particular, following a lack of findings of direct genetic effects (Root et al., 2011), a growing area of ED research involves examining how individual genetic differences may moderate the impact of certain environmental variables on ED symptoms (Rozenblat et al., 2017).

Most research to date has approached investigation of gene × environment (GxE) interactions through the diathesis-stress lens, investigating how genetic ‘vulnerability’ may increase susceptibility to stressful environments (e.g. Stoltenberg, Anderson, Nag, & Anagnopoulos, 2012). However, GxE investigations across other fields increasingly support an alternative model, under which certain genetic factors conceptualised as conferring ‘risk’ may be better conceptualised as ‘plasticity’ factors that are associated with better outcomes under positive or neutral environmental conditions (Belsky et al., 2009). The current study will examine possible genetic plasticity in disordered eating attitudes and behaviours by examining adolescent exposure to both ‘positive’ and ‘negative’ parenting behaviours, using a multimethod multisource approach.

The concept of genetic plasticity has received some early support in the depression field (Belsky & Pluess, 2009; Uher &
Parenting, 5-HTTLPR, and Disordered Eating

V. Rozenblat et al.

McGuffin, 2008). Belsky and Pluess (2009) identified a number of studies in which participants with the short (s) allele of the serotonin transporter 5-HTTLPR polymorphism, typically considered a ‘risk’ allele, exhibited lower depression under conditions of few or no stressful life events, compared with those homozygous for the long (l) allele (Brummett et al., 2008; Eley et al., 2004). However, recent meta-analyses cast some doubt over these earlier findings of plasticity (e.g. Culverhouse et al., 2017).

5-HTTLPR has been the most heavily studied polymorphism in investigations of GxE interactions, with one or two copies of the s-allele resulting in reduced serotonin transcription (Heils et al., 2004). The s-allele has been implicated in changes in appetite, mood, and the stress-response system (Gotlib, Joormann, Minor, & Hallmayer, 2008; Leibowitz & Alexander, 1998; Ruhé, Mason, & Schene, 2007) and thus is of direct relevance to ED aetiology. As such, most GxE investigations in eating pathology have focussed on 5-HTTLPR, with a recent meta-analysis finding significant interactions between 5-HTTLPR and traumatic life events, as well as sexual and physical abuse, in predicting EDs and bulimia nervosa (BN), respectively (Rozenblat et al., 2017). However, no study in the ED field has examined GxE ‘risk’ from a plasticity perspective.

Parenting factors, such as parental behaviour and child–parent relationship quality, have been implicated in EDs (Pamies, Botella, & Treasure, 2013; Tetzlaff, Schmidt, Brauhardt, & Hilbert, 2016). As parenting practices range from more positive to more negative, they provide an excellent opportunity for investigating the plasticity hypothesis of GxE interactions in relation to eating pathology. This may involve analysing two dimensionally opposite parenting behaviours, such as warmth and hostility (Schaefer, 1959), which have some theoretical and empirical support for a role in disordered eating. For example, compared with controls, questionnaire-based studies investigating families of ED patients have found that families tend to be characterised by lower warmth and fewer positive family bonds (Calam, Waller, Slade, & Newton, 1990; Cunha, Relvas, & Soares, 2009; Vidovic, Juresa, Begovac, Mahnik, & Tocilj, 2004) but greater family conflict and parental control (Calam et al., 1990; Canetti, Kanyas, Lerer, Latzer, & Bachar, 2008). Furthermore, parenting styles (Baumrind, 1971) conceptualised as low on warmth and high on control and hostility (i.e. authoritarian parenting) have been associated with greater disordered eating compared with parenting styles not low on warmth (Lobera, Rios, & Casals, 2011; Zubatsky, Berge, & Neumark-Sztainer, 2015). Parental warmth is therefore a possible protective factor for disordered eating, while hostility or harsh punishment may constitute risk factors, although limited research specifically investigates the latter.

Given the importance of accurate measurement of environmental stimuli in GxE research (Moffitt, Caspi, & Rutter, 2005), a second approach may involve investigating parenting behaviours by using observational techniques. Unlike idiographic self-report measurement (Margolin et al., 1998), observational measures assess families by using the same metric and may overcome issues such as limitations in self-awareness (Aspland & Gardner, 2003). Other than studies specifically examined parental expressed emotion (Vaughn & Leff, 1976), very few studies have thus far investigated the relationship between (non-mealtime) parental behaviours and eating pathology by using observational techniques, and most have not analysed parental warmth or hostility (these include Blair, Freeman, & Cull, 1995; Humphrey, 1989; Kog & Vandreycsken, 1989; Lattimore, Wagner, & Gowers, 2000; Ratti, Humphrey, & Lyons, 1996; Stasch & Reich, 2000; Thomas, Hoste, & Le Grange, 2012). Across these studies, findings regarding parental warmth and hostility have been mixed (e.g. Humphrey, 1989, cf. Lattimore et al., 2000). This inconsistency is unsurprising given that most existing observational studies are limited by use of poor, unestablished, or inappropriate instruments to code the observational data (e.g. Kog & Vandreycsken, 1989; Lattimore et al., 2000), have used small to medium sample sizes [largest N=74 in Humphrey (1989)], and have analysed female-only samples. Furthermore, no studies have examined the relationship between observed parent behaviours and sub-clinical eating pathology. There remains a need to establish solid conclusions regarding how parental warmth and hostility are related to disordered eating by using reliable and consistent observational approaches, alongside self-report measures and use of large sample sizes. One further possibility is that genetics may, to an extent, account for some discrepancies in the literature.

To our knowledge, two studies (Karwautz et al., 2011; van Strien, Snoek, van der Zwaluw, & Engels, 2010) have analysed parenting within a GxE framework in the ED field. One study (N=265) found an interaction between problematic parenting styles, in particular parental control, and the s-allele of 5-HTTLPR in predicting anorexia nervosa (AN) diagnosis in a female-only discordant AN sister-pair sample (Karwautz et al., 2011). The second study (N=279) found an interaction between parental psychological control and the A1 allele of the Taq1A polymorphism on DRD2 in predicting greater emotional eating in a mixed-gender sample (van Strien et al., 2010). However, neither study explicitly tested for genetic plasticity. Furthermore, both studies focused largely on parental control and did not shed light on the role of other parenting styles, including positive parent behaviours.

The present investigation aimed to assess the relationship between self-reported and observed parental behaviours and adolescent disordered eating and to investigate whether 5-HTTLPR moderated this relationship, using the largest sample to date. This is an important improvement on previous ED research, given the critical nature of sample size in genetic research (Duncan & Keller, 2011). The first study included self-reported measures of parenting behaviour to examine the direct effects of parental warmth and use of harsh punishment on disordered eating attitudes and behaviours, as well as possible moderation by 5-HTTLPR. The second study aimed to replicate these analyses with the use of observationally measured parenting behaviours, to provide insight into how observed parenting behaviours are related to adolescent disordered eating by using a sample size multiple times larger than previously assessed, as well as aiming to provide further support for any GxEs identified in Study 1. These studies represent an advancement both in research relating to the role of parenting factors in EDs, which has included predominantly self-report measures, as well as GxE interactions in the ED field, which has focussed on presence or absence of negative
outcomes only. This is the first investigation of observed parenting within a GxE framework in the ED field, and inclusion of a mixed-gender community sample allows for results to inform ED prevention initiatives.

Study 1

Method

Participants

Australian Temperament Project participants were initially recruited in infancy (4–8 months) in Victoria in 1983 via maternal and child health centres in urban and rural locations. The first survey included 2443 infants (48.0% female), with 16 surveys completed to date. The present study involved a subset of 650 participants (50.2% female), who had completed the 11th survey at age 15–16 years and self-identified as Caucasian. The participants provided information on disordered eating attitudes and behaviours, as well as a saliva sample for genotyping. One parent of each participant completed a parent-reported measure of parenting behaviours. To increase sample size for the present study, in 2015, 196 participants who had completed the survey but had not provided saliva for DNA extraction were invited to provide a saliva sample. Of 196 participants, 107 participants were successfully contacted and 83 individuals agreed to provide a sample. The parents and adolescents provided written informed consent for each survey wave and for the collection of saliva samples. The data collection was approved by the Australian Institute of Family Studies Ethics Review and carried out in accordance with the latest version of the Declaration of Helsinki. The final sample included a greater proportion of participants from the highest socioeconomic status (SES) quartile compared with the original sample in 1983 (see Table 1 for participant characteristics).

Measures

Disordered eating attitudes and behaviours. The Eating Disorder Inventory-2 (EDI-2; Garner, 1991) Drive for Thinness and Bulimia subscales assessed adolescent disordered eating. The Drive for Thinness subscale consists of seven items measuring the participants’ desire to lose weight or fear of weight gain (e.g. ‘I am preoccupied with the desire to be thinner’) with Cronbach’s α = .92 in the current sample. The Bulimia subscale consists of eight items measuring bulimic behaviours, including binging and purging (e.g. ‘I stuff myself with food’), with a somewhat lower internal consistency of α = .74 in the present sample. Responses ranged from Never to Always and were scored on the original 1–6 scale, as recommended for nonclinical samples (Schoemaker, van Strien, & van der Staa, 1994), and then averaged with higher mean scores reflecting greater disordered eating attitudes and behaviours. Wording of the EDI-2 was modified somewhat for an Australian audience, as detailed in Krug et al. (2016).

Parent behaviours. The ATP-devised Parenting Practices Scale (Prior, Sanson, Smart, & Oberklaid, 2000) was used to assess parental behaviours, with parents responding to five questions investigating parental warmth (e.g. ‘In general, how easy is it to spend time with your teenager?’), with α = .77, and 10 questions regarding parental punishment (e.g. ‘I use threats of punishment to control him/her’), also α = .77. Responses were recorded on a 5-point scale from Always/Almost Always to Never and recoded so higher scores reflected greater warmth or greater punishment.

5-HTTLPR genotype. DNA was isolated by using Qiagen QIAamp kits from buccal epithelial cells via cotton swabs and genotyping performed as described previously in Jorm et al. (2000). The additional 83 saliva samples obtained for Study 2 were collected via Oragene saliva tubes, with DNA extracted and genotyped at the Australian Genomics Research Facility, Adelaide, Australia. In both cases, 5-HTTLPR genotype was coded into s-present (s/s or s/l genotype) or s-absent (l/l genotype) groups.

Sociodemographics. Adolescent age, height in centimetres, and weight in kilograms were self-reported, allowing for calculation of participant body mass index (BMI; kg/m²). SES was calculated on the basis of parent-reported maternal and paternal education and employment status.
Data analyses

Using IBM SPSS version 21, four multiple linear regression models separately assessed the direct effects of parental warmth and parental harsh discipline on participant EDI-2 Drive for Thinness and Bulimia scores, controlling for BMI and gender. Additional models tested for moderation by the 5-HTTLPR polymorphism by including the GxE interaction terms. These models also controlled for the potential confounding effects of gender and BMI as per Keller (2014), by including all the covariate x gene and covariate x parenting style interaction terms in the regression models. Prior to analyses, missing data (23.5%) for the BMI variable were imputed by using multiple imputation, with no systematic patterns of missingness observed.

Power analysis

Power analysis was conducted by using Quanto (http://biostats.usc.edu/Quanto.html), with the following specifications: continuous outcome, independent individual design, and a dominant model with s-allele frequency of 43% (based on allele frequencies in the succeeding texts). To detect a large-sized to medium-sized GxE interaction ($R^2 = 0.02$) at an alpha level of .05, a sample of 389 was required, compared with the present sample of 650. However, this sample was not powered to detect small effects (i.e. $R^2 < 0.01$).

Results

Descriptive statistics for participants in Study 1 are featured in Table 2. Participant 5-HTTLPR genotype distribution ($l/l = 197$, $s/l = 341$, and $s/s = 112$) met the Hardy–Weinberg equilibrium, $\chi^2 = 2.96$, $df = 1$, $p > .05$. Tables featuring the results of all regression models are in the supporting information.

Across all regression models examining the main effects, there was a direct effect of female gender and BMI on Drive for Thinness scores, with the former also predicting Bulimia scores (all $p < .001$). There was a significant relationship between Bulimia, but not Drive for Thinness, and self-reported parental warmth ($\beta = .11$, $p = .005$) and harsh punishment ($\beta = .11$, $p = .003$). There was also an interaction between gender and harsh punishment in predicting drive for thinness ($\beta = .30$, $p = .038$), with scores for men lower under conditions of higher harsh punishment, while they remained largely unchanged for women. There were no main effects of 5-HTTLPR or any significant interaction effects between 5-HTTLPR and parent behaviours.

Table 2 Descriptive statistics for key variables investigated in Study 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full sample</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Age (years)</td>
<td>15.72 (0.16)</td>
<td>15.74 (0.14)</td>
<td>15.72 (0.17)</td>
</tr>
<tr>
<td>Parental warmth</td>
<td>4.22 (0.63)</td>
<td>4.25 (0.65)</td>
<td>4.19 (0.61)</td>
</tr>
<tr>
<td>Parental punishment</td>
<td>2.00 (0.56)</td>
<td>1.94 (0.55)</td>
<td>2.06 (0.57)</td>
</tr>
<tr>
<td>EDI-2 bulimia</td>
<td>1.78 (0.65)</td>
<td>1.94 (0.73)</td>
<td>1.62 (0.53)</td>
</tr>
<tr>
<td>EDI-2 drive for thinness</td>
<td>2.23 (1.15)</td>
<td>2.81 (1.22)</td>
<td>1.64 (0.70)</td>
</tr>
<tr>
<td>BMI</td>
<td>21.27 (3.28)</td>
<td>21.20 (3.10)</td>
<td>21.34 (3.44)</td>
</tr>
</tbody>
</table>

Study 2

Method

Participants

Study 2 consisted of a subsample of 304 participants (53.0% female) from Study 1 who were selected to participate in an observational family interaction task based on responses to the 11th survey, as part of an earlier investigation into factors contributing to risk and resilience for adolescent adjustment. Approximately three-quarters of those invited consented to participate and were characterised into a problem (16.7%), high-risk (21.7%), or low-risk (55.2%) group. The participants in the problem group either exhibited elevated levels of depressed mood, as indicated by endorsing five or more Diagnostic and Statistical Manual of Mental Disorders, Third Edition (APA, 1980) symptoms of depression, reported frequent substance use, or exhibited antisocial behaviour, determined by endorsing four or more delinquent acts (e.g. stealing, fighting, and driving a car without permission). Based on a number of factors that differentiated the problem group from the main ATP sample (such as earlier behaviour problems, school adjustment difficulties, and peer relationships), a group of problem-free yet ‘high-risk’ participants was identified. A gender-balanced low-risk group was drawn randomly from the remaining sample. The participants completed the EDI-2 and provided saliva samples as described in Study 1. The final sample for Study 2 included a lower proportion of participants from the lowest SES quartile compared with the original sample. Demographic information is featured in Table 1.

Measures

Disordered eating attitudes and behaviours, BMI, and age were measured as in Study 1.

Observational measure of parent behaviours. Trained interviewers performed home visits during which the participants and one of their parents (usually the mother) engaged in a video-recorded 15-min discussion based on a set of cards containing 14 questions about family life (e.g. teenager’s accomplishments and disappointments and parental rules and fairness). Parenting behaviours were coded by a team who had undergone extensive training and were blind to adolescent group membership, using the Iowa Family Interaction Rating Scale (Melby et al., 1998), a macro-level observational coding system that was designed to measure behaviours and emotions in family discussions with adolescents. This is considered a valid tool for measuring behaviours in a variety of dyadic interactions and has been validated against self and other reports from family members (Melby & Conger, 2001). Two scales were selected for the present study, parental warmth and parental hostility.

Warmth measured parental expressions of care, concern, and support directed at their child, including expressions of approval, affectionate physical contact, and building upon or reciprocating warmth displayed by their child. It was rated on a 9-point scale from no warmth to frequent warmth. Average intraclass correlation to assess inter-rater reliability for this scale was 0.80, based on cross examination of XX% of the videos.
Hostility measured the extent to which parents engaged in hostile behaviours directed to their child, including rejection, active ignoring, showing contempt, or expressing complaints or critical remarks. It was rated on a 9-point scale from no hostility to frequent hostility. The average intraclass correlation for this scale was 0.75, which compares favourably with previous studies using the Iowa Family Interaction Rating Scale and is deemed acceptable (Ge, Best, Conger, & Simons, 1996).

Data analyses

Data were analysed by using IBM SPSS version 21, as in Study 1, via four linear regression models examining the direct effects of observed parental warmth and observed parental hostility on EDI-2 Drive for Thinness and Bulimia scales. Additional models examined possible moderation by 5-HTTLPR, again controlling for gender and BMI. In the analyses investigating parental warmth and 5-HTTLPR, covariate x gene and covariate x environment contrast terms were included, as recommended by Keller (2014). However, this was not possible in the investigation of parental hostility and 5-HTTLPR due to issues of excessive collinearity (further discussed in the Results section). Finally, to ensure that any differences between Studies 1 and 2 reflected measurement effects as opposed to sample effects, the direct effects of self-reported parental warmth and self-reported use of harsh punishment were also tested in the Study 2 subsample. GxE interactions were not included in this analysis to avoid multiple testing issues. Missing data for BMI (28.29%) were imputed via multiple imputation, with no systematic patterns of missingness observed.

Power analysis

Power analysis was conducted by using Quanto (http://biostats.usc.edu/Quanto.html), as for Study 1, with s-allele frequency of 43% (based on allele frequencies for Study 2 participants). The current sample was powered to detect a large-sized to medium-sized GxE interaction, $R^2 = 0.03$, which requires 258 participants at an alpha level of .05 but was not powered to detect effects smaller than this.

Results

Descriptive statistics for the participants in Study 2 are featured in Table 3. 5-HTTLPR genotype distribution ($+/l = 90, s/l = 154$, and $s/s = 60$) met the Hardy–Weinberg equilibrium, $\chi^2 = 0.16$, $df = 1$, $p > .05$. The results from all regression models are located in the supporting information.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.73 (0.15)</td>
<td>15.73 (0.13)</td>
<td>15.72 (0.10)</td>
</tr>
<tr>
<td>Parental warmth</td>
<td>4.38 (1.93)</td>
<td>4.57 (1.92)</td>
<td>4.16 (1.92)</td>
</tr>
<tr>
<td>Parental hostility</td>
<td>1.92 (1.53)</td>
<td>2.06 (1.65)</td>
<td>1.76 (1.37)</td>
</tr>
<tr>
<td>EDI-2 bulimia</td>
<td>1.89 (0.73)</td>
<td>2.08 (0.80)</td>
<td>1.68 (0.58)</td>
</tr>
<tr>
<td>EDI-2 drive for thinness</td>
<td>2.34 (1.23)</td>
<td>2.99 (1.28)</td>
<td>1.61 (0.67)</td>
</tr>
<tr>
<td>BMI</td>
<td>21.55 (3.59)</td>
<td>21.65 (3.40)</td>
<td>21.45 (3.77)</td>
</tr>
</tbody>
</table>

Female gender predicted greater Drive for Thinness and Bulimia scores (all $p < .001$), while BMI was associated with the former only. There were also significant direct effects of greater observed parental warmth on reduced Drive for Thinness ($\beta = -.10, p = .029$), but not Bulimia, and no direct effects of observed parental hostility. No significant main effects of 5-HTTLPR or interaction between 5-HTTLPR and observed parenting behaviours were observed.

The models investigating moderation by 5-HTTLPR in the relationship between observed parental hostility and disordered eating do not include gene × covariate and environment × covariate contrast terms due to issues with collinearity. Although Keller (2014) argues collinearity functions to control for alternate explanations of any GxE interaction, the GxE interaction showed minimal change regardless of whether the additional contrast terms were included in the models. Therefore, the simpler model less affected by collinearity is presented.

The follow-up analysis investigating self-reported parental warmth and use of harsh punishment in the present subsample revealed main effects of the warmth and punishment on bulimia scores ($\beta = -.14, p = .013$ and $\beta = .14, p = .011$ respectively) but not drive for thinness scores. These results mirrored those of the larger sample in Study 1.

Discussion

We adopted a multimethod assessment approach in two studies to investigate the relationship between parental behaviours (warmth, harsh punishment, and hostility) and disordered eating attitudes and behaviours. We also investigated whether the relationship between parenting factors and eating pathology was moderated by the serotonin transporter 5-HTTLPR polymorphism. The results indicated that greater parent-reported warmth was associated with lower bulimia symptoms, and in concordance, parent-reported use of harsh punishment techniques was associated with greater bulimia symptoms. Conversely, greater observed parental warmth was associated with lower adolescent drive for thinness, while greater observed parental hostility did not predict either disordered eating outcome. No moderation by 5-HTTLPR was identified in any of these relationships.

Parental warmth and harsh punishment

Findings of a relationship between parental warmth and use of harsh punishment techniques with disordered eating attitudes and behaviours support the results of past studies that have assessed these parental behaviours via questionnaires. For example, parental warmth and care have been identified as lower in families of patients with AN and BN compared with controls (Tetley, Moghaddam, Dawson, & Rennoldson, 2014), while parental harsh punishment has been associated with BN in a number of studies (Rorty, Yager, & Rossotto, 1995; Stuart, Laraia, & Weinberg, 2007; Yap, Pilkington, Ryan, & Jorm, 2014), while parental harsh punishment has been associated with BN in a number of studies (Rorty, Yager, & Rossotto, 1995; Stuart, Laraia, & Weinberg, 2007; Yap, Pilkington, Ryan, & Jorm, 2014). However, few studies have investigated these associations by using observed parenting behaviours, as we have here, and a novel pattern of results was identified compared with research based on questionnaires. Across both samples, the two self-reported measures of parenting behaviours were clearly associated with
adolescent bulimia symptoms, but neither was related to drive for thinness. Conversely, the observed parenting behaviours tended to be associated with drive for thinness but not bulimia. Other studies have also found limited correspondence between parent responses on questionnaires and their actual observed behaviours (e.g. Kallstrom-Fuqua, 2004). Such discrepancies are not unexpected for a number of reasons, including socially desirable or idiosyncratic responding to a questionnaire or issues of ecological validity during observational tasks. It is also possible that the two measures of parental warmth used in the present study tapped into slightly different constructs. The observational measurement may have had a stronger focus on caring and warm behaviour displayed by the parent, irrespective of the child’s behaviour, while the questionnaire measure perhaps reflected more greatly the level of general positive interaction between parent and child, albeit from the parent’s perspective. This may suggest that the precise relationship between parental behaviours and adolescent disordered eating is highly nuanced and varies according to the specific element of parenting or disordered eating measured.

Gene x environment interactions

The lack of significant GxE interactions identified in the present study contrasts with Karwautz et al. (2011) (N = 256), who found that problematic parenting styles interacted with the s-allele of 5-HTTLPR to predict AN diagnosis in a discordant sister-pair sample. One possibility is that the influence of 5-HTTLPR may only be evident when clinical-level disordered eating pathology, such as AN, is considered. Another consideration is that Karwautz et al. (2011) examined parental control, a different parental behaviour to those examined in the present study. The other study identifying a possible interaction between parenting and genes (p = .06) in the ED field also investigated parental control (van Strien et al., 2010) (N = 276). One possibility, therefore, is that parental control but not other parenting behaviours may interact with genetic factors to predict eating pathology. However, it remains possible that discrepant findings between the current study and previous investigations are due to low power in both Karwautz et al. (2011) and van Strien et al. (2010), which increases the chances of false positive findings (Duncan, Pollastri, & Smoller, 2014). Sample sizes required for genetic association studies are very large due to the small effect sizes purportedly involved, and the present sample size (N = 650) was more than double that of the previous two studies identifying GxE interactions involving parenting in EDs. Similarly, numerous past studies that have identified plasticity related to the 5-HTTLPR s-allele in other fields, and indeed have been cited as early evidence of this effect, have been vastly underpowered. A large portion of these studies included fewer than 150 participants (e.g. Kim, 2010; Pluess, Belsky, Way, & Taylor, 2010; Taylor et al., 2006; Wilhelm et al., 2006).

The lack of moderation by 5-HTTLPR in the relationship between parental warmth and bulimic behaviours and drive for thinness supports the view that this polymorphism does not act as a plasticity allele in the presence of family environments typically considered ‘protective’. This is also reflected in the fact that, to the authors’ knowledge, no prior study investigating GxEs in the ED field has reported differential susceptibility in response to positive environments (distinct from absence of negative stressors). In other fields, parental warmth and 5-HTTLPR have not been heavily studied within a GxE framework, with the few existing studies presenting a mix of results (e.g. Hankin et al., 2011; Kochanska, Kim, Barry, & Philibert, 2011). Given the large-scale publication bias that purportedly affects studies of GxE interactions (Duncan & Keller, 2011), it cannot be ascertained how many other unpublished investigations have failed to find GxE effects involving parental warmth.

Strengths and limitations

The present paper contributes to research examining parenting factors in disordered eating by analysing a large-scale community sample, with a focus on replication across two samples using multistudy multisource assessment. In contrast, past studies have largely adopted questionnaire-based measures, often retrospective, with limited investigation using observational measurement of parenting behaviours. A few limitations of the present study must also be noted. The present study did not examine comorbid psychopathology, so was unable to assess whether this factor may possibly mediate the identified relationships between particular parenting behaviours and disordered eating outcomes. The present study also examined the biallelic model of 5-HTTLPR. There is some evidence to suggest that examination of a triallelic model may better reflect 5-HTTLPR activity (Wendland, Martin, Kruse, Lesch, & Murphy, 2006), with support from some studies in the ED field (Steiger et al., 2009; Stoltenberg et al., 2012 but not Richardson et al., 2008), although more broadly findings have been tentative (e.g. Uher & McGuffin, 2008). In the present study, data were not available to examine the triallelic model, which may yet have resulted in identification of a GxE interaction in the present sample. Furthermore, the sample size, particularly in Study 2, was relatively underpowered for genetic association studies (although it was above the mean N = 288 of GxE studies in the ED field; Rozenblat et al., 2017). Conversely, the sample in Study 1 constitutes the third largest investigation of its kind in the field (following Akkermann et al., 2012, N = 765, and the combined-samples analyses in Rozenblat et al., 2017, N > 1000). This is an important strength. Furthermore, assessing disordered eating attitudes and behaviours dimensionally increases power and sensitivity compared with case-control designs, in which the ‘clinical’ group may represent heterogeneous underlying patterns of eating pathology or genetic influences (Abbott, 2008). Assessing disordered eating symptoms in a population-based sample also increases generalisability of results and utility for informing prevention and early intervention initiatives, although it may limit the applicability of results to clinical populations. Finally, as with all studies of candidate genes, 5-HTTLPR represents only one polymorphism in a system of genetic factors likely to be involved in ED pathology, and thus, such investigations constitute stepping stones towards increasing our understanding of the genetic underpinnings of EDs.

Clinical implications and future directions

The results support a clear relationship between parent behaviours and adolescent disordered eating, across both genders, independent of individual genetic predisposition. The findings can inform parenting programmes targeting ED prevention or psychoeducation for ‘at risk’ families. For example, one systematic review of parent-focussed ED prevention initiatives stated that
early positive findings required support from additional research regarding the family setting (Hart, Cornel, Damiano, & Paxton, 2015). Adoption of longitudinal designs would also help establish whether parental behaviours constitute protective/risk factors or are better conceptualised as correlates of eating pathology. Furthermore, investigations into genetic plasticity could also expand beyond 5-HTTLPR to examine cumulative plasticity across numerous polymorphisms testing multiple positive environmental factors. Selection of positive eating-related outcomes, such as positive body image (Webb, Wood-Barcalow, & Tylka, 2015), as opposed to absence of eating-related pathology, may also be more appropriate for reflecting the relationship between genetic plasticity and environmental factors. However, it is also possible that regardless of precise measurement techniques, the role of 5-HTTLPR in EDs may prove to be very minor at best, such that continued investigation into parenting and other environmental factors may constitute a more fruitful approach to untangling the complex aetiology of eating pathology.

Acknowledgements

The ATP study is located at The Royal Children’s Hospital Melbourne and is a collaboration between Deakin University, The University of Melbourne, the Australian Institute of Family Studies, The University of New South Wales, The University of Otago (NZ), and the Royal Children’s Hospital; further information is available at www.aifs.gov.au/atp. The views expressed in this paper are those of the authors and may not reflect those of their organisational affiliations nor of other collaborating individuals or organisations. We acknowledge all collaborators who have contributed to the Australian Temperament Project, especially Professors Ann Sanson, Margot Prior, Frank Oberklaid, and John Toubourou and Ms Diana Smart. We would also like to sincerely thank the participating families for their time and invaluable contribution to the study. This paper forms part of Vanja Rozenblat’s PhD with publication undertaken at The University of Melbourne.

This work was supported by an Early Career Researcher Grant (1350035), an Australian Research Council Senior Research Fellowship (DP 130101459), and the Australian Postgraduate Award.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

Hankin, B., Nederhof, E., Oppeneheimer, C., Jenness, J., Young, I., Abela, J., et al. (2011). Differential susceptibility in youth: Evidence that 5-HTTLPR x positive parenting is associated with positive affect ‘for better and worse’. Translational Psychiatry, 1(10), e44.


Steiger, H., Richardson, J., Schmitz, N., Joob, R., Israel, M., Bruce, K. R., et al. (2009). Association of trait-defined, eating-disorder sub-phenotypes with (biallelic and triallelic) 5-HTTLPR polymorphisms and environmental adversity, the serotonin transporter promoter polymorphism, and environmental adversity in the aetiology of bulimia nervosa. Psychological Medicine, 49(6), 620–630.


Supporting information
Additional Supporting Information may be found online in the supporting information tab for this article.
Minerva Access is the Institutional Repository of The University of Melbourne

Author/s: 
Rozenblat, Vanja

Title: 
The role of candidate gene x environment interactions in eating pathology: an investigation of the 5-HTTLPR polymorphism

Date: 
2017

Persistent Link: 
http://hdl.handle.net/11343/197975

File Description: 
The role of candidate gene X environment interactions in eating pathology: an investigation of the 5-HTTLPR polymorphism

Terms and Conditions: 
Terms and Conditions: Copyright in works deposited in Minerva Access is retained by the copyright owner. The work may not be altered without permission from the copyright owner. Readers may only download, print and save electronic copies of whole works for their own personal non-commercial use. Any use that exceeds these limits requires permission from the copyright owner. Attribution is essential when quoting or paraphrasing from these works.