Associations of 5HTTLPR polymorphism with major depressive disorder and alcohol dependence: A systematic review and meta-analysis

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ABSTRACT

Objective: The neurotransmitter serotonin is understood to control mood and drug response. Carrying a genetic variant in the serotonin transporter gene (*5HTT*) may increase the risk of major depressive disorder and alcohol dependence. Previous estimates of the association of the S allele of 5HTTLPR polymorphism with major depressive disorder and alcohol dependence have been inconsistent.

Methods: For the systematic review, we used PubMed MEDLINE and Discovery of The University of Melbourne to search for all relevant case-control studies investigating the associations of 5HTTLPR polymorphism with major depressive disorder and alcohol dependence. Summary odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated. To investigate whether year of publication, study population or diagnostic criteria used were potential sources of heterogeneity, we performed meta-regression analyses. Publication bias was assessed using Funnel plots and Egger's statistical tests.

Results: We included 23 studies of major depressive disorder without alcohol dependence containing 3392 cases and 5093 controls, and 11 studies of alcohol dependence without major depressive disorder containing 2079 cases and 2273 controls. The summary OR for homozygote carriers of the S allele of 5HTTLPR polymorphism compared with heterozygote and non-carriers combined (SS vs SL+LL genotype) was 1.33 (95% CI 1.19-1.48) for major depressive disorder and 1.18 (95% CI 1.01-1.38) for alcohol dependence. The summary OR per S allele of 5HTTLPR polymorphism was 1.16 (95% CI 1.08-1.23) for major depressive disorder and 1.12 (95% CI 1.01-1.23) for alcohol dependence. There was no evidence for publication bias of the studies included in our meta-analysis.

Conclusions: Our meta-analysis confirms that individuals with the homozygous S allele of 5HTTLPR polymorphism are at increased risks of major depressive disorder as well as alcohol dependence. Further studies are required to investigate the association between 5HTTLPR polymorphism and the comorbidity of major depressive disorder and alcohol dependence as well as gene $\times$ environmental interactions.

Keywords: Serotonin transporter, 5HTTLPR polymorphism, depression, alcohol dependence, meta-analysis

INTRODUCTION

Depression and alcohol dependence are multi-factorial, complex disorders influenced by both genetic and environmental factors; however, despite intensive research the aetiology remains unclear. Heritability has been estimated to be 37% for major depressive disorder (Sullivan et al., 2000) and 30-36% for alcohol dependence (Pickens et al., 1991). The lifetime prevalence of major depressive disorder in the United States population has been estimated to be 5-12% for men and 10-25% for women (Grant et al., 1994; Kessler et al., 1994; Regier et al., 1990), whereas prevalence of alcohol dependence is 20% for men and 12% for women (Kessler et al., 1994).

There have been several pathophysiological and genetic hypotheses proposed for aetiology of both depression and alcohol dependence. These include dysfunction of the neurotransmitters serotonin (5-hydroxytryptamine (5HT)), a monoamine known to affect anxiety, cognition, reward, emotion, drug responses and stress (Jans et al., 2006). Transport of serotonin from synaptic spaces into presynaptic neurons is regulated by serotonin transporter protein (5HTT), impairment of which has been implicated in susceptibility to many psychiatric disorders including depression and alcohol dependence. The gene encoding the serotonin transporter is called *5HTT* (also known as *SLC6A4*) (Ramamoorthy et al., 1993). Two common variants, thought to affect regulation of the expression of *5HTT*, have been identified in the *5HTT* transporter-linked polymorphic region (5HTTLPR) of this gene (Heinz and Goldman, 2000; Lesch et al., 1996): S allele (484 base pairs long) and L allele (528 base pairs long) (Gelernter et al., 1997). For Caucasians, approximately 0.8-2.3% are homozygous (SS genotype), 16-26% are heterozygous (SL genotype), and 72-83% are wild-type (LL genotype). The corresponding frequencies for African Americans are 6%, 36% and 58% respectively (Hu et al., 2006; Wendland et al., 2008).

Several studies have reported that individuals with depression are more likely to carry the S allele than those without depression (Collier et al., 1996; Gutierrez et al., 1998; Bellivier et al., 1998) while other studies showed no evidence of an association (Hoehe et al., 1998; Furlong et al., 1998; Frisch et al., 1999; Minov et al., 2001; Kunugi et al., 1997). Likewise, several studies have reported that individuals with alcohol dependence are more likely to carry S allele compared to those without alcohol dependence (Gorwood et al., 2000; Hallikainen et al., 1999; Hammoumi et al., 1999) whereas other studies found no evidence of an association (Ishiguro et al., 1999; Johann et al., 2003). Therefore, it is still a matter of controversy whether the S allele of 5HTTLPR polymorphism is associated with depression or alcohol dependence.

We have systematically reviewed all the previously published studies of the association between 5HTTLPR polymorphism with depression and alcohol dependence and conducted a meta-analysis to: (i) investigate separately for the strengths of associations of the S allele of the 5HTTLPR polymorphism with major depressive disorder without alcohol dependence and alcohol dependence without major depressive disorder, and (ii) estimate the proportion of major depressive disorder and alcohol dependence that might be due to the S allele of 5HTTLPR polymorphism.

MATERIALS AND METHODS

A systematic review was performed according to a predetermined protocol and reported in accordance with Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines (Stroup et al., 2000). This review was registered at the International Prospective Register of Systematic Reviews (PROSPERO registration number: CRD42015024911).

Search strategy

We used PubMed MEDLINE and “Discovery” of the University of Melbourne Library to search for all the relevant studies of the association of 5HTTLPR polymorphism with major depressive disorder and alcohol dependence published before April 2014. The following Medical Subject Heading terms and keywords were used for search: “alcohol dependence” OR “alcoholism” OR “alcohol” OR “alcoholics” OR “depression” OR “depressive illness” OR “major depression” OR “depressive disorder” OR “unipolar disorder” AND “serotonin transporter polymorphism” OR “5HTTLPR” OR “SLC6A4”. In addition, the reference lists of all relevant letters, articles, literature reviews and meta-analyses were reviewed to identify any articles not captured by the search engine strategy above. No restriction was imposed on language or year of publication. All articles were triaged for further assessment initially by title alone, then by abstract. Full manuscripts of every article with a relevant title and abstract were then sought for final assessment of eligibility.

Eligibility criteria

We included case-control studies that reported (i) the association of the S allele of 5HTTLPR polymorphism with depression or alcohol dependence, (ii) distribution of S and L alleles of 5HTTLPR polymorphism for both cases and controls and (iii) used one of the following criteria for diagnoses of major depressive disorder (cases with already established diagnosis of depression including recurrent episodes) and/or alcohol dependence: *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* (DSM-IV) (American Psychiatric Association, 1994), *Diagnostic and Statistical Manual of Mental Disorders Third Edition Revised* (DSM-III-R) (American Psychiatric Association, 1987), International Classification of Disease - 10th revision (ICD-10) (World Health Organization, 1992), Center for Epidemiologic

Studies-Depression Scale (CES-D) (Radloff, 1977), Michigan Alcoholism Screening Test (MAST) (Selzer, 1971), or Beck Depression Inventory (BDI) (Beck et al., 1961).

We excluded: (i) studies of cases with both depression and alcohol dependence; (ii) studies of children and adolescents; (iii) reviews or editorial or letters or non-peer-reviewed publications; (iv) studies that were not translated into English; or (v) studies that did not define cases and controls. All studies meeting the eligibility criteria were categorised into two groups: (i) major depressive disorder in the absence of alcohol dependence and (ii) alcohol dependence in the absence of major depressive disorder.

Data extraction

The following data were extracted from each study: title of the study, name of the first author, publication year, country where the study was performed, study design, inclusion and exclusion criteria of cases and controls, case ascertainment methods, number of cases and controls for each genotype and/or for each allele and odds ratios for the association between the S allele of 5HTTLPR polymorphism and the disorder. Cases with bipolar disorder were excluded from this meta-analysis. The literature search, assessment for eligibility, and data extraction were independently undertaken by two investigators (KZO and YKA) using a standardised approach. Any inconsistencies between them was identified by the third investigator (AKW) and resolved by consensus.

Statistical Analysis

The summary odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated for associations of the S allele of 5HTTLPR polymorphism with major depressive disorder and alcohol dependence. Both random effects and fixed effect models were fitted. We estimated associations for different inheritance modes of the S allele: recessive (SS vs. SL and LL);

dominant (SS+SL vs. LL); and codominant (separately for SS and SL vs. LL). We also estimated OR per S allele, i.e. a linear association on the log scale.

To test the influence of individual studies on the summary estimates, we omitted data from each study one at a time and repeated the analysis. To estimate the heterogeneity between studies, the I^2 statistic was used (Higgins and Thompson, 2002). Meta-regression models were conducted including the year of publication (before and in 2000 vs. after 2000), study population (Caucasian vs. others), and diagnostic criteria used (DSM-IV vs. other criteria). To test for publication bias, Begg's funnel plot (Begg and Berlin, 1988) and Egger's statistical test (Egger et al., 1997) were performed. All statistical tests were two-sided, and all analysis was performed using STATA 13.0 (StataCorp, 2013).

Assuming the causal relationship of the homozygous S allele of 5HTTLPR polymorphism with major depressive disorder and/or alcohol dependence, the proportion of the diseases attributable to the homozygous S allele of 5HTTLPR polymorphism for general population was calculated using the formula:

$$PAR = \frac{P (OR - 1)}{P (OR - 1) + 1}$$

where PAR was population attributable risk, P was prevalence of the homozygous S allele of 5HTTLPR polymorphism (assumed to be 26%), and OR was the summary odds ratio estimated from this study.

RESULTS

Of the 1527 papers identified by the literature search, we excluded 1359 based on the title and abstract as they appeared not to be relevant to this systematic review. Of the 168 studies retrieved for full assessment of eligibility, 34 met the eligibility criteria and were included in the meta-analyses (Figure 1). Recruitment methods, selections and ages of cases and controls for each study are summarised in Table 1. Of these studies, 24 were conducted in Europe, two in the USA, and eight in other countries, and all were published between 1996 and 2009. Most of the studies used clinic-based controls or controls via convenient sampling while only a few studies used population-based controls but they did not describe the recruitment methods in details (Minov et al., 2001; Hickie et al., 2007; Gokturk et al., 2008).

5HTTLPR polymorphism and major depressive disorder

We identified 23 case-control studies reporting the association between the S allele of 5HTTLPR polymorphism and major depressive disorder without alcohol dependence. When combined, there were a total of 3392 cases of major depressive disorder of whom 25% ($n = 834$) had SS genotype, 46% ($n = 1568$) had SL genotype, and 29% ($n = 990$) had LL genotype. Of the total 5093 controls, 22% ($n = 1121$) had SS genotype, 48% ($n = 2438$) had SL genotype, and 30% ($n = 1534$) had LL genotype (Table 2). The estimated fixed effect summary OR of major depressive disorder was 1.33 (95% CI 1.19-1.48) for recessive inheritance of the S allele (Supplementary Figure 1), and 1.12 (95% CI 1.01-1.24) for dominant inheritance (Supplementary Figure 2) with no or low heterogeneity between studies (Table 3). Compared with non-carriers of the S allele, the summary OR of major depressive disorder was estimated to be 1.36 (95% CI 1.19-1.56) for homozygous carriers of the S allele (Supplementary Figure 3) and 1.03 (95% CI 0.93-1.15) for heterozygous carriers (Supplementary Figure 4). The

summary OR of major depressive disorder per S allele of 5HTTLPR polymorphism was 1.16 (95% CI 1.08-1.23) (Supplementary Figure 5).

5HTTLPR polymorphism and alcohol dependence

We identified 11 case-control studies reporting the association between the S allele of 5HTTLPR polymorphism and alcohol dependence without major depressive disorder. When combined, there were a total of 2079 cases of alcohol dependence of whom 41% ($n = 854$) had SS genotype, 38% ($n = 793$) had SL genotype, and 21% ($n = 432$) had LL genotype. Of the total 2273 controls, 32% ($n = 720$) had SS genotype, 42% ($n = 949$) had SL genotype, and 21% ($n = 604$) had LL genotype (Table 2). The estimated fixed effect summary OR of alcohol dependence was 1.18 (95% CI 1.01-1.38) for recessive inheritance of the S allele (Supplementary Figure 1) and 1.11 (95% CI 0.94-1.32) for dominant inheritance (Supplementary Figure 2) with low heterogeneity between studies (Table 3). Compared with non-carriers of the S allele, the summary OR of alcohol dependence was estimated to be 1.33 (95% CI 1.06-1.66) for homozygous carriers of the S allele (Supplementary Figure 3) and 1.02 (95% CI 0.86-1.23) for heterozygous carriers (Supplementary Figure 4). The summary OR of alcohol dependence per S allele of 5HTTLPR polymorphism was 1.12 (95% CI 1.01-1.23) (Supplementary Figure 5).

Meta-regression and publication bias

There were no major changes in the direction of summary estimates when any individual study was omitted at a time. Meta-regression models showed that associations of the S allele of 5HTTLPR polymorphism with major depressive disorder or alcohol dependence did not substantially change after adjusting for the year of publication, study population, and diagnostic criteria used (Table 4).

Funnel plots for both major depressive disorder studies and alcohol dependence studies did not provide any evidence of publication bias (Figure 2). Also, there was no statistical evidence of publication bias ($p=0.12$ for major depressive disorder studies and $p=0.10$ for alcohol dependence studies).

Population attributable risk

The population attributable risks were estimated to be 7.7% (95% CI 4.5-11.1%) for major depressive disorder and 4.5% (95% CI 0.3-8.8%) for alcohol dependence.

DISCUSSION

Our meta-analysis has shown that the homozygous and heterozygous carriers of the S allele of 5HTTLPR polymorphism are at increased risk of major depressive disorder and that homozygous carriers are at increased risk of alcohol dependence. There was insufficient evidence to conclude that heterozygous carriers have an increased risk of alcohol dependence. Our estimated summary ORs of disease risk per S allele of 5HTTLPR polymorphism support a dose-response relationship with both major depressive disorder and alcohol dependence.

To our knowledge, there have been five previous meta-analyses investigating the association between 5HTTLPR polymorphism and major depressive disorder (Clarke et al., 2010; Kiyohara and Yoshimasu, 2010; Lasky-Su et al., 2004; Lotrich and Pollock, 2004; Anguelova et al., 2003). Three meta-analyses reported no statistical evidence for an association with major depressive disorder (Lasky-Su et al., 2004; Lotrich and Pollock, 2004; Anguelova et al., 2003). However, Anguelova et al. (2003) suggested that further studies were warranted to investigate variation at *5HTT* at the level of allelic subtypes as different allelic subtypes have different transcriptional activity. Consistent with our results, Clarke et al. (2010) reported an

increased risk of major depressive disorder for carriers of SS genotype compared with carriers of SL or LL genotype (OR=1.16, 95% CI 1.08-1.24) and Kiyohara and Yoshimasu (2010) reported an increased risk of major depressive disorder for Caucasian carriers of SS genotype compared with carriers of LL genotype (OR=1.41, 95% CI 1.15-1.72). However, none of the previous meta-analyses considered that heterozygous carriers of the S allele might be at increased risk because they all compared homozygous carriers of the S allele with heterozygous carriers and/or non-carriers. In our meta-analysis, we observed evidence for both homozygous and heterozygous carriers of the S allele of 5HTTLPR polymorphism associated with an increased risk of major depressive disorder.

A previous meta-analysis (Feinn et al., 2005) studying the association between 5HTTLPR polymorphism and alcohol dependence estimated the odds ratio for homozygous carriers of the S allele to be 1.18 (95% CI 1.03-1.33) but they did not estimate the association for heterozygous carriers. Similar to their result, our meta-analysis showed marginally significant evidence for a positive association between the homozygous carriers of the S allele of 5HTTLPR polymorphism and alcohol dependence.

The population attributable risks suggest that, if the observed associations are due to a causal relationship, then approximately 1 in 20 cases of major depressive disorder in the general population is attributable to the homozygous S allele of 5HTTLPR polymorphism and about 1 in 30 cases of alcohol dependence in the general population is attributable to the homozygous S allele.

A complication in elucidating the role of this polymorphism on the risk of major depressive disorder and alcohol dependence is their comorbidity. Compared with a person without alcohol dependence, a person with prior or current alcohol dependence has a four-fold

increased risk of major depressive disorder (Hasin and Grant, 2002), and longer duration of major depressive disorder (Mueller et al., 1994). Therefore, most previous studies of this polymorphism have studied participants with either depression or alcohol dependence alone. Given that all the studies of major depressive disorder in our current review excluded individuals with alcohol dependence, our findings of an increased risk of depression associated with the SS or SL genotype of 5HTTLPR polymorphism was less likely to be confounded by alcohol dependence. Likewise, given that all studies of alcohol dependence excluded individuals with major depressive disorder, our finding of an increased risk of alcohol dependence associated with the SS genotype of 5HTTLPR polymorphism was less likely to be confounded by major depressive disorder. However, the case-control studies included in this review are unlikely to adequately deal with confounding from lifetime comorbidity between alcohol disorders and major depressive disorder. This is because even if studies attempted to control for or exclude the other condition, lifetime comorbidity is likely to have been underestimated because of inaccurate recall (Moffitt et al., 2010).

Effectiveness studies of selective serotonin reuptake inhibitors (SSRIs) had supported a causal relationship between the S allele and depression (Jones and Blackburn, 2002; Snow et al., 2000; Williams et al., 2000). The effectiveness of SSRIs for treatment of depression was greater in carriers of homozygous L allele when compared with carriers of homozygous S allele (Smeraldi et al., 1998; Zanardi et al., 2000). In another meta-analysis that investigated the association of 5HTTLPR with SSRI efficacy, it was again demonstrated that the L allele of 5HTTLPR polymorphism has association with better response to antidepressant (Serretti et al., 2007). These results of the S allele of 5HTTLPR being associated with poor SSRI efficacy supported the hypothesis that serotonin dysregulation involves in the aetiology of depression (Serretti et al., 2007). Brain-imaging studies have shown association between serotonin deficits

and alcoholism (Cloninger, 1995; Tiihonen et al., 1997), suggesting that 5HTTLPR polymorphism alters the serotonin expression and affects the neurotransmission of serotonin in the nervous system. Moreover, 5HTTLPR polymorphism has been shown to be associated with high alcohol intake (Hinckers et al., 2006; Covault et al., 2007).

Several studies have also shown evidence for gene $\times$ environmental interactions for depression (Caspi et al., 2003) as well as alcohol dependence (Dick and Kendler, 2012; Hines et al., 2015). Individuals with the SL/LL genotype of *5HTTLPR* are more likely to have depression in relation to stressful life events than those with the LL genotype (Kendler et al., 2005; Caspi et al., 2003). Individuals with the SS genotype of *5HTTLPR* are more likely to have frequent drinking and heavy drinking as well as drug use if they experience multiple negative life events (Covault et al., 2007). In contrast, the Mannheim Study of Children at Risk reported that German young adults with the LL genotype who have exposed to early psychosocial adversity are more likely to have hazardous drinking than those with the LL genotype not exposed to early adversity or those with the SL/SS genotype regardless of adversity (Laucht et al., 2009). Accordingly, the genetic model associated with the interaction has been inconsistent across studies, and the primary outcomes and measures of the experience of stress have varied considerably (Young-Wolff et al., 2011). However, our current review was not able to examine 5HTTLPR polymorphism $\times$ environmental interactions.

Our meta-analysis was limited to the fact that unpublished data of association between 5HTTLPR and major depressive disorder and/or alcohol dependence were not included. However, given that the funnel plot and statistical tests showed no evidence of publication bias, the impact of unpublished findings is less likely. Even though the possible potential sources of heterogeneity (year of publication, study populations, and diagnostic criteria used) that we investigated were not found to be significant, other possible residual sources for heterogeneity

such as onset and duration of alcohol dependence, and other comorbid conditions or complications (including suicide/suicidal ideation, aggression and anti-social behaviours) could not be assessed in this meta-analysis. Controls from most of the studies included in this review were not individuals who were randomly recruited from the general population where cases were selected therefore the study results might be biased.

Overall, our review confirmed that individuals with the homozygous S allele of 5HTTLPR polymorphism are at increased risks of major depressive disorder as well as alcohol dependence. The variation seen in each individual study results is consistent with the subtle and environment dependent effect of 5HTTLPR polymorphism on disease risks. Further studies are required to investigate the association between 5HTTLPR polymorphism and the comorbidity of major depressive disorder and alcohol dependence as well as gene $\times$ environmental interactions.

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Table 1 Selection Criteria for cases and controls in the studies included in the meta-analysis for associations of 5HTTLPR polymorphism with (i) major depressive disorder without alcohol dependence, and (ii) alcohol dependence without major depressive disorder.

Study	Year	Country	Sex	Diagnostic Criteria	Selection of Cases	Age of cases, years (mean (SD)/ median (range)/ range)	Selection of Controls	Age of controls, years (mean (SD)/ median (range)/ range)
(i) Studies of major depressive disorder without alcohol dependence								
Collier et al., 1996	1996	England, Germany, Italy	Both	DSM-IV (England, Germany) DSM-III-R (Italy)	Patients from Lithium therapy clinics at the Department of Chemical Pathology, The Institute of Psychiatry, Epsom General Hospital and Northern General Hospital, Sheffield in England (n=86); Department of Psychiatry, University of Wurzburg in Germany (n=47); and inpatient departments of Hospital San Faffaele, Milan in Italy (n=22).	NA	Unrelated Caucasian volunteers with no history of major psychiatric or neurological illness from GP surgery in SE London (n=187) and King's College Hospital (n=83), England or Caucasian blood donors from Germany (n=301) and Italy (n=95).	NA
Kunugi et al., 1997	1997	Japan	Both	DSM-IV	49 (16 M, 33 F) patients from general psychiatric clinics of Teikyo University Hospital, Showa University Hospital and Shiga University of Medical Science Hospital, Tokyo.	58 (13)	212 (99 M, 113 F) healthy volunteers who were medical students and hospital staff.	32 (13)
Rees et al., 1997	1997	England	Males	DSM-IV	54 patients from a Lithium clinic and psychiatric outpatient referrals in South Wales.	57 (13) for Lithium clinic patients 42 (12) for psychiatric outpatients referrals	121 (52 M, 69 F) unrelated Caucasian blood donors from South Wales	44 (11)
Furlong et al., 1998	1998	UK	Both	DSM-IV	125 (47 M, 78 F) Caucasian patients from in-patient and out-patient clinics in East Anglia.	NA (18-65)	Anonymous East Anglian population from the Molecular Genetics Laboratory, Addenbrooke's Hospital DNA bank, matched for age and sex to cases.	NA
Gutierrez et al., 1998	1998	Spain	Both	DSM-IV	74 (17 M, 57 F) patients from the Mental Health Service in the	60.3 (12.5)	84 (32 M, 52 F) unrelated controls, geographically matched, the general	NA

Eixample district of Barcelona.						population of Barcelona.		
Hoehe et al., 1998	1998	France	Both	DSM-IV	36 patients from two hospitals in Rouffach, Alsace, and in Marseilles, West European descent.	NA	Healthy staff and students without a history of mood disorders from Rouffach, Alsace (n=73), and Berlin (n=208), West European descent.	NA
Bellivier et al., 1998	1998	France	Both	DSM-IV	Patients from psychiatric units of the Pitie Salpetriere Hospital, Paris and Charles Perrens Hospital, Bordeaux, French descent.	NA	Blood donors at the Pitie Salpetriere Hospital, French descent.	NA
Ohara et al., 1998	1998	Japan	Both	DSM-IV	41 (18 M, 23 F) patients admitted to Department of Psychiatry, Hamamatsu University School of Medicine Hospital, and affiliated hospitals, Japanese descent.	42.6 (16.0)	92 (37 M, 55 F) healthy unrelated medical staff and students living in the same area as the patients, with no history of psychiatric diseases or psychotic medications.	35.2 (14.5)
Frisch et al., 1999	1999	Israel	Both	DSM-IV	102 patients (63 Ashkenazi and 39 non-Ashkenazi Jews) from three mental health centers.	NA	Mentally and physically healthy controls (112 Ashkenazi and 60 non-Ashkenazi Jews).	NA
Kim et al., 2000	2000	Korea	Both	DSM-III-R	120 patients enrolled in the Clinical Trial Program of the Samsung Medical Center Affective Disorder Clinic, Korean descent.	54.2 (15.9)	252 healthy volunteers with no history of psychiatric illness from Samsung Biomedical Research Institute and Samsung Medical Center.	46.1 (12.6)
Oliveira et al., 2000	2000	Brazil	Both	DSM-IV and/or CID-10	66 patients from the Institute of Psychiatry (Universidade de Sao Paulo) and the Affective Disorder Unit of the hospital (Universidade Federal de Pernambuco), 40% African/Brazilian and 60% Caucasoids.	41.3 (11.4)	Volunteer normal controls with no familial or personal history of psychiatric disorder or treatment working at the Sao Paulo University or normal relatives of muscular dystrophy patients (n=152), and from the Federal University of Pernambuco (n=45).	38.5 (9.8)
Mellerup et al., 2001	2001	Denmark	Both	ICD-10	92 (28 M, 64 F) patients with unipolar major depression who were previously hospitalized at Department of Psychiatry, Copenhagen	53 (18-86)	108 (30 M, 78 F) healthy hospital and university staff and from health retiree club members	46 (20-81)

Minov et al., 2001	2001	Germany	Both	DSM-IV	173 (59 M, 114 F) patients from Department of Psychiatry, Ludwig-Maximilians University of Munich. Patients with coexisting substance abuse or severe organic disorders were excluded.	49.9 (14)	121 (60 M, 61 F) healthy individuals recruited from the general population in southern Germany with no severe physical or psychiatric axis I or axis II disorders.	47.3 (10.5)
Steffens et al., 2002	2002	USA	Both	CES-D	182 (69% F) geriatric patients (inpatients and outpatients) with clinically significant depressive symptoms or previously diagnosed mood disorder, recruited from the Mental Health Clinic Research Centre of the Duke University.	69.6 (59-96)	107 (64% F) controls recruited from the Center for Aging Subject Registry at Duke University. No neurologic or depressive illness.	70.2 (58-92)
Serretti et al., 2002	2002	Italy	Both	DSM-IV	667 (58% F) inpatients admitted to the Department of Psychiatry at the Institute H. San Raffaele. Patients with concomitant diagnoses of mental retardation or drug dependence, together with somatic or neurological illnesses that impaired psychiatric evaluation were excluded.	46 (14.4)	457 (51% F) healthy unrelated individuals recruited from healthy department personnel and people attending the general lab of the Institute H. San Raffaele, only individuals with negative family history included, Italian descent.	46 (15.5)
Arias et al., 2003	2003	Spain	Both	DSM-IV	131 (31 M, 100 F) outpatients from the Centre de Salut Mental Esquerre de l'Eixample (Hospital Clínic i Provincial de Barcelona).	39.9 (12.3)	163 (83 M, 80 F) healthy unrelated controls from the same geographic and ethnic origin as the patients.	NA
Hauser et al., 2003	2003	Poland	Both	DSM-IV	94 (25 M, 68 F) inpatients from the Department of Adult Psychiatry, University of Medical Sciences in Poznan and at the Department of Psychiatry, Pomeranian Academy of Medicine in Szczecin	50 (11)	213 (131 M, 82 F) blood donors who were not psychiatrically screened.	50 (14)
Frodl et al., 2004	2004	Germany	Both	DSM-IV	40 inpatients from the Department of Psychiatry at Ludwig-Maximilians-University, Munich. Patients with previous alcohol or substance abuse or neurologic diseases were excluded.	44.4 (11.7)	40 healthy individuals from the Munich population, who were matched with age, sex, and handedness to cases.	41.7 (12.1)
Hoefgen et al., 2005	2005	Germany	Both	DSM-IV and/or DSM-III-R	466 (161 M, 305 F) patients admitted to the Department of Psychiatry at the University of Bonn	50.9 (14.7)	836 (376 M, 460 F) controls randomly selected from the list of registered inhabitants of Bonn (North Rhine-Westphalia)	54.6 (17.2)

Cervilla et al., 2006	2007	Spain	Both	ICD-10	737 (208 M, 529 F) patients attending primary care centres in the area of Malaga, who involved in a genetic association study called PREDICT study	49.1 (15.2)	European primary-care attendees from PREDICT study (as nested case-control)	NA
Dorado et al., 2007	2007	Spain	Both	DSM-IV	70 (23 M, 47 F) outpatients from a mental health center, Servicio Extremeno de Salud,.	51 (14)	142 (71 M, 71 F) Spanish healthy controls from the same geographical region	30 (11)
Hickie et al., 2007	2007	Australia	Both	DSM-IV	45 (15 M, 30 F) patients from tertiary referral services in South Eastern Sydney. Patients with major depression secondary to a major medical illness were excluded.	52.0 (12.8)	16 (7 M, 9 F) healthy controls recruited from community via local newspaper advertisement	55.8 (10.3)
Wieser et al., 2010	2010	Germany	Both	BDI or CES-D	Patients from three university-based headache clinics (Departments of Neurology of the Universities of Vienna, Münster, and Halle). Patients with either mild or clinically relevant depression were included.	43.3 (12.1)	Age- and sex-matched participants from the headache prevalence study	NA

(ii) Studies of alcohol dependence without major depressive disorder

Sander et al., 1997	1997	Germany	Both	ICD-10	103 severely affected alcoholics selected from 315 alcoholics from three sources: the outpatient unit for addictive disorders at the Department of Psychiatry of the Free University of Berlin, the detoxification unit of the Department of Alcohol Dependence of the Wilhelm-Griesinger Hospital in Berlin, and a meeting point for homeless people in Berlin. Patients were excluded if they had a history of a primary major psychiatric disorder.	42.2 (9.0)	Randomly chosen from blood donors without psychiatric assessment (n=105) and controls who were older than 26 years, without a history of an addictive disorder or previous psychiatric treatment (n=111).	NA
Hallikainen et al., 1999	1999	Finland	Males	DSM-III-R	Type 1 (n=114) alcoholics from detoxification programs from the regions of Turku and Kuopio; and Type 2 alcoholics (n = 51) from	43.8 (8.8) for Type 1 alcoholics 30.1 (8.4) for Type 2 alcoholics	Employees of two industrial corporations in two cities in Southern Finland recruited via occupational health services	44.1 (7.9)

					forensic psychiatric examination in a state mental hospital, Kuopio			
Hammoumi et al., 1999	1999	France	Both	Short MAST criteria	104 unrelated Caucasians of French origin, recruited in hospitals specialized in alcohol therapeutics. Patients with depression disorder were excluded.	43.57 (1.43)	38 blood donors from Centre Régional de Transfusion Sanguine Bois-Guillaume	43.8 (2.65)
Ishiguro et al., 1999	1999	Japan	Both	DSM-IV	166 (153 M, 13 F) unrelated Japanese alcoholic patients with histories of withdrawal seizure or delirium, selected based on hospital records, from patients receiving treatment at one of seven hospitals in Japan. Patients with major psychiatric disorders were excluded.	52.2 (9.4)	213 age- and sex-matched unrelated, healthy Japanese individuals with no documented neuropsychiatric disorders	51.7 (8.2)
(Matsushita et al., 2001)	2001	Japan	Males	DSM-III-R	692 alcoholics patients from the National Institute on Alcoholism	50.5 (9.7)	270 unrelated male non-alcoholic Japanese controls, mainly hospital employee or persons connected with them, age-mated to cases	50.0 (21.3)
Thompson et al., 2000	2000	Canada	Both	DSM-IV	131 patients from The Center for Addiction and Mental Health (CAMH), Toronto, and the City of Hope National Medical Center (COH), Duarte, CA. Patients had no other psychiatric disorder.	30-55	125 controls of Northern European descent from CAMH, Toronto, admitted for medical treatment not related to alcohol dependence and having no history of psychiatric treatment or substance dependence.	30-55
Gorwood et al., 2000	2000	France	Males	DSM-III-R	110 male alcoholics from two general hospitals in Paris, French origin, no comorbid dementia, no schizophrenia, no bipolar manic depressive disorder. Alcoholic patients without major depressive disorders were only included in this meta-analysis (n=83).	35	70 male controls from a blood transfusion center, at least 35 years old and French origin, without any substance dependence	43.6 (10.5)
Preuss et al., 2001	2001	Germany	Both	DSM-IV or ICD-10	163 (131 M, 32 F) Caucasian patients recruited from an addiction treatment ward with diagnosis of alcohol dependence. Alcoholic patients without major depressive disorders were only included in this meta-	41 (8.3)	117 (56 M, 61 F) Caucasian controls from the general population with no severe physical or psychiatric axis I/II disorders.	46 (8.1)

analysis (n=105).

Johann et al., 2003	2003	Germany	Both	DSM-IV or ICD-10	314 (262 M, 52 F) unrelated alcoholics with primary alcoholism recruited from an in-patient abstention program, German descent. Patients with a history of major psychiatric disorders or addictions to drugs were excluded.	43.1 (18-72)	220 unrelated healthy individuals of German descent, residents in the same area as cases, without an addictive of major psychiatric disorder or previous psychiatric treatment.	NA
Konishi et al., 2004	2004	USA	Males	DSM-IV	130 male, Mexican-American alcoholics with extensive history of heavy alcohol use for at least 5 years, living in Los Angeles County.	38.2 (11.8)	251 (105 M, 146 F) healthy volunteer controls, living in Los Angeles County.	32.5 (9.2)
Gokturk et al., 2008	2008	Sweden	Females	ICD-10	Caucasians recruited from a long-term inpatient facility for alcoholism and drug abuse between 18 and 70 years of age. Alcoholic patients without anxiety or depressive disorders were only included in this meta-analysis (n=88).	18-75	631 controls randomly selected from high school students, 80% response rate	17-18

BDI, Beck Depression Inventory; CES-D, Center for Epidemiologic Studies Depression Scale; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders Fourth Edition; DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders Third Edition Revised; ICD-10, International Classification of Disease - 10th revision; MAST, Michigan Alcoholism Screening Test; NA, not available.

Table 2 Distribution of 5HTTLPR genotype for cases and controls in the studies included in the meta-analysis for associations of 5HTTLPR polymorphism with (i) major depressive disorder without alcohol dependence and (ii) alcohol dependence without major depressive disorder.

Study	SS		SL		LL	
	Case	Control	Case	Control	Case	Control
(i) Studies of major depressive disorder without alcohol dependence						
Collier et al., (1996)	41	101	63	278	46	191
Kunugi et al. (1997)	36	132	9	64	4	11
Rees et al. (1997)	13	24	35	59	32	35
Furlong et al. (1998)	26	29	64	81	35	59
Gutierrez et al. (1998)	14	17	41	37	15	29
Hoehe et al. (1998)	5	48	22	138	9	95
Bellivier et al. (1998)	8	12	24	58	13	29
Ohara et al. (1998)	26	54	13	32	2	6
Frisch et al. (1999)	22	39	50	96	30	37
Kim et al. (2000)	68	137	47	103	5	12
Oliveira et al. (2000)	14	32	28	87	24	78
Mellerup et al. (2001)	16	18	40	53	36	37
Minov et al. (2001)	40	17	79	64	53	40
Steffens et al. (2002)	34	12	78	56	70	39
Serretti et al. (2002)	135	75	309	217	223	165
Arias et al. (2003)	27	38	63	84	41	41
Hauser et al. (2003)	26	35	50	98	18	80
Frodl et al. (2004)	8	10	16	17	16	13
Hoefgen et al. (2005)	99	127	222	406	141	294
Cervilla et al. (2006)	133	101	205	246	106	129
Dorado et al. (2007)	17	26	40	71	13	45
Hickie et al. (2007)	12	3	18	5	15	8
Wieser et al. (2010)	14	34	52	88	43	61
TOTAL	834	1121	1568	2438	990	1534

(ii) Studies of alcohol dependence without major depressive disorder						
Sander et al. (1997)	27	35	44	100	32	81
Hallikainen et al. (1999)	45	8	55	20	65	26
Hammoumi et al. (1999)	25	4	43	14	34	20
Ishiguro et al. (1999)	114	201	46	81	6	8
Matsushita et al. (2001)	459	179	212	83	21	8
Thompson et al. (2000)	35	19	53	55	43	51
Gorwood et al. (2000)	15	11	41	26	27	24
Preuss et al. (2001)	18	18	50	58	37	41
Johann et al. (2003)	52	35	154	116	108	69
Konishi et al. (2004)	49	77	62	111	19	63
Gokturk et al. (2008)	15	133	33	285	40	213
TOTAL	854	720	793	949	432	604

Table 3 Meta-analysis summary odds ratios (ORs) and their 95% confidence intervals (CIs) for associations between different genotypes of 5HTTLPR polymorphism and the risk of major depressive disorder without alcohol dependence and the risk of alcohol dependence without major depressive disorder

	Fixed effect	Random effects	Heterogeneity	
	OR (95% CI)	OR (95% CI)	I ² (%)	p-value
Major depressive disorder without alcohol dependence				
Recessive (SS vs. SL+LL)	1.33 (1.19-1.48)	1.33 (1.19-1.48)	0.1	0.46
Dominant (SS+SL vs. LL)	1.12 (1.01-1.24)	1.11 (0.97-1.26)	30.0	0.09
Codominant				
SS vs. LL	1.36 (1.19-1.56)	1.32 (1.10-1.57)	28.5	0.10
SL vs. LL	1.03 (0.93-1.15)	1.03 (0.90-1.17)	20.7	0.18
Per S allele	1.16 (1.08-1.23)	1.14 (1.05-1.24)	29.3	0.09
Alcohol dependence without major depressive disorder				
Recessive (SS vs. SL+LL)	1.18 (1.01-1.38)	1.22 (1.00-1.49)	30.3	0.16
Dominant (SS+SL vs. LL)	1.11 (0.94-1.32)	1.16 (0.91-1.47)	46.0	0.05
Codominant				
SS vs. LL	1.33 (1.06-1.66)	1.36 (0.99-1.87)	47.6	0.04
SL vs. LL	1.02 (0.86-1.23)	1.04 (0.85-1.27)	15.9	0.29
Per S allele	1.12 (1.01-1.23)	1.14 (0.98-1.32)	51.3	0.02

OR: odds ratio; CI confidence interval; I²: % residual variation due to heterogeneity.

Table 4 Meta-regression models for associations between recessive inheritance of the S allele of 5HTTLPR polymorphism (SS vs SL+LL) and the risk of major depressive disorder without alcohol dependence and the risk of alcohol dependence without major depressive disorder

	Number of studies	Fixed effect model Summary OR (95% CI)	I ² (%)	τ ²	Ratio of ORs (95% CI)	p-value
Major depressive disorder without alcohol dependence						
Population						
Caucasians	18	1.36 (1.20, 1.54)	0.29	0.001	1.00	-
Others	5	1.18 (0.90, 1.54)			0.86 (0.63, 1.18)	0.34
Year of publication						
Before and in 2000	11	1.23 (1.02, 1.49)	0.30	0.001	1.00	-
After 2000	12	1.38 (1.20, 1.59)			1.12 (0.87, 1.44)	0.35
Diagnostic Criteria						
DSM-IV criteria	18	1.34 (1.17, 1.53)	4.46	0.01	1.00	
Others	5	1.31 (1.06, 1.61)			0.96 (0.72, 1.29)	0.80
Alcohol dependence without major depressive disorder						
Population						
Caucasians	8	1.33 (1.06, 1.68)	27.6	0.02	1.00	-
Others	3	1.07 (0.86, 1.32)			0.80 (0.50, 1.27)	0.30
Year of publication						
Before and in 2000	6	1.43 (1.11, 1.85)	17.0	0.02	1.00	-
After 2000	5	1.05 (0.86, 1.28)			0.71 (0.46, 1.10)	0.11
Diagnostic Criteria						
DSM-IV criteria	5	1.20 (0.96, 1.50)	37.1	0.04	1.00	
Others	6	1.16 (0.93, 1.44)			1.01 (0.61, 1.68)	0.96

OR: odds ratio; CI confidence interval; DSM IV: *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition*; I²: % residual variation due to heterogeneity; τ², residual maximum likelihood estimate of between-study variance.

Figure 1 Flow diagram of the selection of studies

Figure 2 Begg's funnel plots for publication bias for studies of (A) major depressive disorder without alcohol dependence and (B) alcohol dependence without major depressive disorder. OR, odds ratio, SE, standard error.

Supplementary Figure 1 Meta-analysis of associations between recessive inheritance of the S allele of 5HTTLPR polymorphism (SS vs SL+LL) and the risk of major depressive disorder and alcohol dependence

Supplementary Figure 2 Meta-analysis of associations between dominant inheritance of the S allele of 5HTTLPR polymorphism (SS+SL vs LL) and the risk of major depressive disorder and alcohol dependence

Supplementary Figure 3 Meta-analysis of the risk of major depressive disorder and alcohol dependence for homozygous carriers of the S allele of 5HTTLPR polymorphism compared with non-carriers (SS vs LL)

Supplementary Figure 4 Meta-analysis of the risk of major depressive disorder and alcohol dependence for heterozygous carriers of the S allele of 5HTTLPR polymorphism compared with non-carriers (SL vs LL)

Supplementary Figure 5 Meta-analysis of per allele association between the S allele of 5HTTLPR polymorphism and the risk of major depressive disorder and alcohol dependence