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Investigating psychiatric, cognitive, and genetic factors

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Towards a biobehavioral understanding of methamphetamine use disorder: Investigating psychiatric, cognitive, and genetic factors

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Abstract

Methamphetamine use is a major health concern globally, with ever-expanding market and an increasing number of users worldwide. In Australia, the number of people with methamphetamine use disorder has increased over the past 10 years, specifically amongst adolescents and young adults. This is a particular concern, as the age of onset of substance use predicts the severity of substance use disorder later in life. In addition, young people are more resistant to treatment. While it is still poorly understood why methamphetamine dependence is increasing in adolescents, literature suggests that methamphetamine use is associated with other psychiatric disorders, deficits in cognition, and certain genes involved in the neurocircuitry of addiction. Therefore, the aim of this thesis was to explore psychiatric, cognitive, and genetic factors associated with early onset of methamphetamine use to gain a holistic understanding of methamphetamine use disorder.

To investigate these factors, I conducted a cross-sectional two-group study, recruiting people with a current diagnosis of stimulant use disorder, methamphetamine-type, and controls with no history of substance use disorder. All participants were administered a clinical interview to collect demographic and drug use characteristics. Psychiatric comorbidities and psychotic symptoms were also assessed. Following the interview, participants completed a cognitive task battery assessing attention, speed of processing, cognitive flexibility, working memory, and inhibitory control. Inhibitory control was also assessed using a cue reactivity task that I specifically developed for this project. It consisted of the pseudorandomized presentation of methamphetamine-related cues counterbalanced with control, food-related cues. Upon completion of the study session, whole blood was collected for single nucleotide polymorphism analysis in genes of interest. Genes were selected based on robust preclinical data and results from the meta-analysis presented in this thesis.

Poor inhibitory control was identified as an age-dependent risk factor in this thesis, with an earlier age of onset associated with greater deficits. In addition, poor inhibition was associated with an increase in craving upon exposure to methamphetamine-related cues. This is critical as cue-induced craving may lead to relapse after abstinence, and therefore poor treatment outcome. Results from this thesis therefore suggest that pre-existing reduced inhibitory control in adolescence is a risk factor for developing methamphetamine use disorder when methamphetamine is first taken early in life, potentially by perpetuating methamphetamine use and inducing repeated relapses.

This thesis also identified comorbid antisocial personality disorder as a strong predictor for age of onset of methamphetamine use. This highlights the need to treat co-occurring mental disorders in young people who use drugs to prevent them from transitioning into problematic use. Lastly, a mutation in the neuregulin-1 gene was associated with early onset methamphetamine use, suggesting that people carrying the gene variant are more likely to develop methamphetamine use disorder when exposed to methamphetamine earlier in life.

Taken together, this thesis identified a range of psychiatric, cognitive, and genetic factors associated with early onset methamphetamine use. Findings from this work will contribute to the development of larger studies and clinical trials investigating new early interventions to prevent young people who casually use methamphetamine transitioning into a formal methamphetamine use disorder, thus alleviating the rising burden of disease.

Declaration

This is to certify that:

- (i) the thesis comprises only my original work towards the Doctor of Philosophy except where indicated in the preface;
- (ii) due acknowledgement has been made in the text to all other material used; and
- (iii) the thesis is fewer than 100,000 words in length, exclusive of tables, figures, and references.

Signed,

Preface

I acknowledge Dr. Wei Lin Toh and Dr. Andrea Phillipou from Swinburne University of Technology for critical training in administration of PANSS and cognitive tasks battery, respectively. I acknowledge Dr. Kiymet Bozaoglu from the Murdoch Children's Research Institute for conducting genotyping of single nucleotide polymorphisms. I acknowledge A/Prof Yvonne Bonomo, Amanda Norman, Helen O'Neill, Oonagh McNamara, and Angela Fazio from the Department of Addiction Medicine and DePaul House, St Vincent's Hospital Melbourne, for their contribution to recruitment, and supervision of study sessions.

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Publication in Chapter 4 was accepted for publication by Neuroscience and Biobehavioral Reviews on 05/11/2020. For this publication, Alexandre Arthur Guerin and A/Prof. Jee Hyun Kim were responsible for the study concept and design, with support from Prof. Susan Rossell, Prof. Andrew Lawrence, and Prof. Eric Nestler. Alexandre Arthur Guerin and Jee Hyun Kim performed full-text screens and risk of bias analysis. Alexandre

Arthur Guerin extracted all data and performed all analyses. Alexandre Arthur Guerin wrote the initial version of the manuscript, with subsequent contribution from A/Prof. Jee Hyun Kim. Prof. Susan Rossell, Prof. Andrew Lawrence, Prof. Eric Nestler, and Prof. Michael Berk provided critical revision of the manuscript for important intellectual content. I, Alexandre Arthur Guerin, contributed to 70% of the work towards this publication. All work in this publication was carried out during enrolment in the degree.

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Abbreviations

ACC	Anterior cingulate cortex
Ala	Alanine
AMY	Amygdala
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ANT	Attention networks task
Arg	Arginine
ASPD	Antisocial personality disorder
ASSIST	Alcohol, Smoking and Substance Involvement Screening Test
AUDIT	Alcohol Use Disorder Identification test
BDNF	Brain-Derived Neurotrophic Factor
BIS	Barratt impulsiveness scale
BP	Blood pressure
CBT	Cognitive behavioral therapy
CI	Confidence interval
COMT	Catechol-O-Methyltransferase
CPT	Continuous performance test
CRT	Cognitive remediation therapy
CTQ	Childhood trauma questionnaire
DDT	Delayed discounting task
DRD2	Dopamine Receptor D2
FAAH	Fatty Acid Amide Hydrolase
GHB	Gamma-hydroxybutyric acid
GWAS	Genome-wide association study
HIPP	Hippocampus
HR	Heart rate
Ile	Isoleucine
I.V.	Intravenous
Met	Methionine
NAc	Nucleus accumbens
NPAS3	Neuronal PAS domain protein 3
NRG1	Neuregulin-1
OR	Odds ratios
PCR	Polymerase chain reaction
PFC	Prefrontal cortex
Phe	Phenylalanine
Pro	Proline
RM	Repeated measures
rTMS	Repetitive transcranial magnetic stimulation
SCR	Skin conductance response
SD	Standard deviation
SEM	Standard error of the mean
Ser	Serine
SGCZ	Sarcoglycan Zeta
SLC18A1	Solute carrier family 18 member 1
SLC18A2	Solute carrier family 18 member 2
SNP	Single-nucleotide polymorphisms
SSRT	Stop-signal reaction time

tDCS	Transcranial direct current stimulation
Thr	Threonine
TMT	Trail Making test
Val	Valine
VMAT1	Vesicular monoamine transporter 1
VMAT2	Vesicular monoamine transporter 2
VR	Virtual reality
VTA	Ventral tegmental area
WCST	Wisconsin card sorting test
WTAR	Wechsler test of adult reading
ΔSCR	Differential skin conductance response

Chapter 1. Introduction

Substance use disorders are chronic relapsing disorders characterized by compulsive drug-taking and -seeking despite serious negative consequences (American Psychiatric Association, 2013). It is estimated that ~29.5 million people, or 0.6% of the global adult population, experience a substance use disorder (United Nations Office on Drugs and Crime, 2017a). In Australia, lifetime prevalence of substance use disorder is 25% (ABS, 2008), and illicit drug use costs \$7 billion annually (Collins, 2008). In 2015, there were at least 190,000 preventable drug-related deaths world-wide, with North America experiencing the highest drug-related mortality rate, followed by Australia and New Zealand (United Nations Office on Drugs and Crime, 2017b). With fewer than one in six people with substance use disorder receiving treatment each year (United Nations Office on Drugs and Crime, 2017a), illicit drug use remains a significant concern.

Amphetamines, a class of psychostimulant drugs made in varied forms, is the second most abused illicit drug in the world, second only to cannabis (United Nations Office on Drugs and Crime, 2010). Among amphetamines, methamphetamine represents the greatest global health threat, with an ever-expanding market and an increasing amount of users worldwide (United Nations Office on Drugs and Crime, 2017c). Chronic and repeated use can lead to the development of stimulant use disorder, methamphetamine-type (henceforth referred to as methamphetamine use disorder). The 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) defines methamphetamine use disorder as a pattern of methamphetamine use leading to clinically significant impairment or distress, as manifested by a range of factors (American Psychiatric Association, 2013). These include, but are not limited to, inability to cut down on use, craving, strong desire or urge to use methamphetamine, continued use despite social, physical and/or psychological problems, and development of tolerance and withdrawal symptoms. Methamphetamine use disorder

can be mild (presence of two to three symptoms in a 12-month period), moderate (four to five symptoms), or severe (six or more symptoms). Individuals may initiate use to control weight or to enhance academic, work, or athletics performance. Methamphetamine use disorder develop more rapidly when used intravenously or smoked. Patterns of methamphetamine use may be episodic (each use separated by two or more days) or chronic (almost daily use). Chronic use often leads to a diminution in pleasurable effects of methamphetamine due to tolerance, and in turn to an increase in dose over (American Psychiatric Association, 2013).

Methamphetamine use is a major concern in Australia, with methamphetamine-related deaths doubling from 2009 to 2015 (Darke et al., 2017), and annual costs estimated to exceed \$AU55 billion (Tait et al., 2018). In addition, the number of regular and dependent methamphetamine users in Australia has increased between 2002 and 2014, specifically amongst adolescents and young adults aged 15-34 (Degenhardt et al., 2016). In fact, over 5% of Australians aged 14-19 have reported using methamphetamine in the last 12 months (AIHW, 2020), making it one of the highest rates of use in the world. This is a particular concern, as numerous studies have highlighted a link between the age of onset of substance use and severity of substance use disorder later in life (Anthony and Petronis, 1995; Brown et al., 2009; Chen et al., 2009; Dawson et al., 2008; Grant and Dawson, 1998; King et al., 2007; Robins and Przybeck, 1985). In fact, evidence from large datasets ($n = 1567$) suggests that adolescent-onset stimulant users are 1.7 times more likely to develop clinical features of substance use disorders within two years following onset compared to adult-onset users (Chen et al., 2009). Further, a large twin study ($n = 1733$) revealed that experimentation with drug in adolescence can impact the development of substance use disorders later in life (Palmer et al., 2009). Another twin studies found that early onset of substance use was associated with development of dependence (Grant et al., 2006). Notably, this association was not explained by genetic or shared family environmental

factors. More recently, a study found that people who started using substances earlier in life (age ≤ 17) were more likely to use drugs more frequently, have a diagnosis of substance use disorder, and relapse after abstinence compared to late-onset users (age > 18) (Poudel and Gautam, 2017). Importantly, adolescents are also more resistant to treatments (Catalano et al., 1991; Perepletchikova et al., 2008; Spear, 2016). Indeed, rodent studies from our laboratory suggest that the age of onset of substance use is a critical factor associated with both changes in addiction-related behaviors and neurobiology (Guerin et al., 2021b; Luikinga et al., 2018; Zbukvic et al., 2016; Zbukvic and Kim, 2018)

It is poorly understood why methamphetamine dependence is increasing in adolescents. Very few studies have focused on adolescent use, which may be contributing to the delayed progress in finding effective therapeutics for addiction (Insel and Gogtay, 2014). Methamphetamine use is highly comorbid with other psychiatric disorders (McKetin et al., 2019), with adolescents particularly affected by anxiety and personality disorders (Embry et al., 2009; Kuitunen-Paul et al., 2020; Rawson et al., 2005; Russell et al., 2008). Evidence suggests that the latter may be associated with earlier onset of methamphetamine use (Kramer et al., 2009). In fact, results from large epidemiological studies suggest that adverse childhood events may be implicated in maintenance of substance use disorder, with personality disorders a key mediator of this mechanism (Sarvet and Hasin, 2016). In addition, adolescents with conduct disorders are more at risk to have comorbid substance use disorders compared to other psychiatric disorders (Masroor et al., 2019), and more likely to initiate substance use earlier in life (Hopfer et al., 2013).

Our laboratory recently observed in rats that inhibitory control may be impaired following methamphetamine use in adolescent, but not in adults (Luikinga et al., unpublished observations). This is consistent with findings in humans showing that adolescent methamphetamine use is associated with impaired inhibitory control (Kim et al., 2016; King et al., 2010; Lyoo et al., 2015). Further we found that certain genes involved in addiction

neurocircuitry may be differentially expressed in adolescent compared with adult rats following methamphetamine use (Luikinga et al., unpublished observations). This is particularly interesting as family studies have highlighted a strong link between genetic heritability and substance use disorder (Agrawal and Lynskey, 2008), though this has yet to be demonstrated in people with methamphetamine use disorder. It is a clear that a better understanding of risks and/or consequences of methamphetamine use disorder is critical to reduce harm and to develop early interventions preventing adolescent users becoming dependent, thus alleviating burden of disease (Chen et al., 2009; Insel, 2014). Therefore, the overall aim of my PhD was to explore psychiatric, cognitive, and genetic factors associated with early onset of methamphetamine use to gain a holistic understanding of methamphetamine use disorder.

1.1. The neural circuitry of substance use disorders: a summary

Rodent and human studies indicate that the development of substance use disorders primarily involves the mesocorticolimbic dopamine system (Kalivas and Volkow, 2005), commonly known as the 'reward pathway'. This pathway comprises of the ventral tegmental area (VTA) dopaminergic neurons projecting to the nucleus accumbens, the amygdala, and the prefrontal cortex (PFC) (**Figure 1.1**). This system is responsible for the acute rewarding effects of food, social interaction and sex, which are crucial for our survival. Dopamine release throughout the system in response to rewarding events leads the circuit to establish learned associations to better respond to the event in the future (Nestler, 2005).

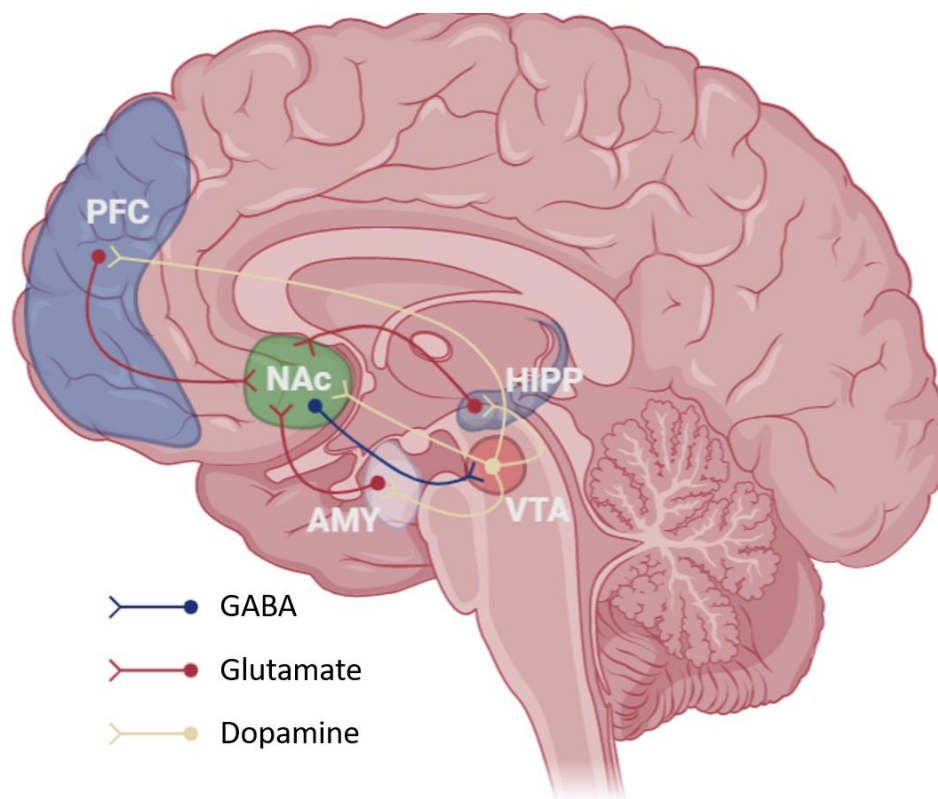


Figure 1.1. Simplified diagram of the structures and circuitry of the mesocorticolimbic dopamine system. Substances of abuse trigger release of dopamine (yellow) from the VTA. Dopaminergic activity is modulated by excitatory glutamatergic projections (red), and inhibitory GABAergic projections (blue). AMY: amygdala; HIP: hippocampus; NAc: nucleus accumbens; PFC: prefrontal cortex; VTA: ventral tegmental area. Credit: GeorgeVKach, distributed under a CC BY-SA 4.0

All substances of abuse acutely increase dopamine release within the mesocorticolimbic system via different mechanisms (Nestler, 2005). Methamphetamine specifically affects dopamine release by disrupting cytosolic pH, which impairs the reuptake of dopamine at the presynaptic membrane, and reversing the function of vesicular transporters (Cruickshank and Dyer, 2009; Riddle et al., 2008; Sulzer et al., 2005). These events lead to excess dopamine concentration in the synaptic cleft. When such an increase in dopamine signaling is continued, neuroplasticity within the system can occur, which can contribute towards compulsive drug-taking and -seeking (Kalivas and Volkow, 2005). Further, reversing the action of vesicular transporters can cause excess dopamine in the cytoplasm, causing cell damage and toxicity (Kousik et al., 2014), which can lead to cognitive and psychomotor impairments (Moszczynska and Callan, 2017). It should be noted

that while dopamine plays a key role, it has also been suggested that other neurotransmitters such as serotonin contribute to the development of substance use disorders (Rocha et al., 1998).

Cortical regions are known to undergo maturation and reorganization during adolescence (Kolb et al., 2012; Spear, 2000), with dopaminergic innervation in these regions maturing very slowly (Kalsbeek et al., 1988; Naneix et al., 2012), thus methamphetamine use may cause more alterations in adolescents compared to their adult counterparts. For example, adolescent-onset of methamphetamine use causes greater grey and white matter alterations in the frontostriatal system compared to adult-onset (Lyoo et al., 2015). In addition, earlier onset of methamphetamine use is associated with disruption of cortical metabolism in adolescents who use methamphetamine (Kim et al., 2016). Taken together, chronic methamphetamine use causes neuroadaptation in the mesocorticolimbic system, leading to the development of methamphetamine use disorder. Ongoing cortical maturation in adolescence may increase vulnerability to develop compulsive drug-seeking and -taking when methamphetamine is first used early in life.

1.2. Theory of addiction: a brief overview

There are numerous theories underpinning the field of substance use disorder, including psychological, biological, sociological, economic theories, and biopsychosocial theories (West and Brown, 2013). I will briefly describe 1) the disease; 2) the Pavlovian conditioning; and 3) the impaired response inhibition and salience attribution models of addiction, which are the most pertinent to this thesis.

The disease model of addiction was first presented by Jellinek (1960). It proposes that development of substance use disorders involve abnormal brain structure and functions leading to failure to exercise control, and development of overpowering desire (i.e. craving) for the drug (Edwards and Gross, 1976; Jellinek, 1960). In the Pavlovian conditioning model

of addiction, drug craving is believed to be a natural result of Pavlovian conditioning that occurs during drug-taking (Drummond et al., 1995; Perry et al., 2014), where repeated pairings between drug use and surrounding cues (i.e. places, people and objects associated with the drug-taking experience) result in the cues alone to elicit craving. This is referred to as a conditioned response (Pavlov, 1927). Notably, cue-elicited craving may predict relapse behavior (Drummond, 2000). More recently, evidence from neuroimaging studies led to the development of the impaired response inhibition and salience attribution model (Goldstein and Volkow, 2011, 2002). This model proposes that impairment in executive function and salience networks may contribute to the development of symptoms, including craving and withdrawal (Zilverstand and Goldstein, 2020). Heightened salience network activity to drug-related cues has been observed in people with a range of substance use disorders compared to controls (Zilverstand et al., 2018). Likewise, executive network activity also increases when presented with such cues, which can lead to poor response inhibition, and reduced impulse control (Zilverstand et al., 2018).

Taken together, the theories discussed above highlight a critical role for lack of control, poor inhibition and impulse control, and abnormal response to cues in the development of the clinical symptomatology of substance use disorders. Therefore, this thesis will focus on these processes in the context of methamphetamine use disorder.

1.3. Methamphetamine use disorder and psychotic symptoms

Methamphetamine use is highly comorbid with other psychiatric disorders. A recent meta-analysis on 59 studies found that any amphetamine use was associated with increased odds of psychosis (odds ratio (OR) = 2.0), suicidality (OR = 4.4), and depression (OR = 1.6) (McKetin et al., 2019). When investigating studies in people with a formal amphetamine use disorder diagnosis, the odds of psychosis were considerably higher (OR = 3.0) and suicidality was lower (OR = 2.3) than recreational use (McKetin et al., 2019). Amphetamine

use disorder was not associated with depression and anxiety. I will, therefore, focus on the association between methamphetamine use and psychosis.

Psychotic symptoms refer to symptoms that are excess or distortion of normal functions (e.g., delusions, hallucinations) (Kay et al., 1987). Early laboratory studies in healthy participants found that administration of amphetamines resulted in the development of psychotic symptoms, specifically paranoia and hallucinations (Angrist and Gershon, 1970; Bell, 1973). This is thought to occur due to excessive methamphetamine-induced release of dopamine and striatal dopamine receptor stimulation (Cruickshank and Dyer, 2009). In Australia, the reports on the prevalence of methamphetamine-induced psychotic symptoms vary considerably, from 15-60% (McKetin et al., 2013, 2010, 2006). Notably, people diagnosed with methamphetamine dependence are three time more likely to experience symptoms compared to recreational users (McKetin et al., 2006). Importantly, 22% of people experiencing methamphetamine-induced psychosis transition to a primary psychotic disorder such as schizophrenia (Murrie et al., 2020), possibly due to sensitization from repeated use (Rognli and Bramness, 2015).

A recent systematic review revealed that frequency and severity of methamphetamine use are the risk factors most consistently associated with methamphetamine-induced psychotic symptoms (Arunogiri et al., 2018). In contrast, evidence for the role of age of onset is inconsistent. A study in people of Thai descent reported that participants experiencing certain psychotic symptoms (i.e., paranoia) had started using methamphetamine earlier in life compared to those did not report any symptoms (Kalayasiri et al., 2010). It should be noted that the difference between the two groups did not reach statistical significance. A different report, this time in people of Taiwanese descent, also found that the age of onset of methamphetamine use in participants with a formal diagnosis of methamphetamine-induced psychosis was lower than methamphetamine users with no lifetime experience of psychosis (Chen et al., 2003). This

is consistent with an Australian study showing that people who started using methamphetamine earlier were more likely to have a substance-induced psychosis (Hides et al., 2015). However, other studies reported no differences in age of onset of methamphetamine use between methamphetamine users with or without psychotic symptoms (McKetin et al., 2010, 2006; Salo et al., 2013b). Discrepancies between studies may be due to the variability in measures of psychosis; studies that investigated methamphetamine-induced psychotic disorders found an association with early onset methamphetamine use, whereas studies that only looked at specific symptoms found no association. Taken together, early methamphetamine use may be a risk factor for formal psychotic disorders, rather than transient psychotic symptoms. A recent study found a bidirectional relationship between methamphetamine use and psychotic symptoms, with psychotic symptoms predicting an increase in methamphetamine intake in the next 30 days, and vice-versa (Hides et al., 2019). Notably, the magnitude of the relationship was similar for each direction. Hence, psychotic symptoms may be a risk factor for methamphetamine use.

1.4. Methamphetamine use disorder and cognition

1.4.1. Inhibition and impulsivity

Importantly, methamphetamine use and psychosis are both associated with development of cognitive deficits (Bramness and Rognli, 2016; Wearne and Cornish, 2018), potentially highlighting a shared risk. A meta-analysis of 44 studies revealed that people who use methamphetamine display cognitive deficits of medium effect size (Potvin et al., 2018). Notably, impairments were most consistently observed in inhibitory and impulse control. Parts of the next section are from my first author review *“Cognition and Related Neural Findings on Methamphetamine Use Disorder: Insights and Treatment Implications from Schizophrenia Research”* (Guerin et al., 2019).

Inhibitory control, which is also known as response inhibition, is the process of suppressing impulses and behavioral response to stimuli (Diamond, 2013). It can be measured using different tests, including the Wisconsin Card Sorting, Delayed Discounting, and Stroop tasks. The latter is one of the most used tests in neuropsychiatric populations. It consists of two trials: in the first trial (baseline), participants are presented with colored square and are instructed to name the color of the squares as fast as they can. In the second trial (inhibition) participants are required to suppress a habitual response (i.e., read the words), and instead name the ink color of incongruently colored words. The extent of delay between naming the color of an incongruent color word compared to a colored square is known as Stroop interference (Lansbergen et al., 2007).

Chronic methamphetamine use is associated with poorer performance in the Stroop task in both adolescents (Kim et al., 2016; King et al., 2010; Lyoo et al., 2015) and adults with methamphetamine use disorder (Dean et al., 2018, 2011; Kalechstein et al., 2003; Nestor et al., 2011; Salo et al., 2013a, 2011, 2009b, 2007, 2002; Simon et al., 2000, 2002; Stock et al., 2018) (**Table 1.1**). Effects in adolescents suggest that reduced inhibitory control may be a predisposing factor to developing methamphetamine use disorder rather than a consequence of use. Lifetime use of methamphetamine in adolescent is likely to be lower than adult users (Kim et al., 2016). Therefore, poor inhibitory control in adolescents is unlikely to be consequence of chronic methamphetamine use. In fact, poor inhibition contribute to the development of substance use disorders (Goldstein and Volkow, 2011). In addition, while several cross-sectional studies suggest that inhibitory control deficits may recover with abstinence (Cherner et al., 2019, 2010; Hoffman et al., 2006; Stock et al., 2018) indicating that methamphetamine use is causing these deficits, two recent longitudinal studies revealed no improvement in Stroop performance and response inhibition even after abstinence ranging from six weeks to six months (Fitzpatrick et al., 2021; Proebstl et al.,

2019). This further suggests that inhibitory control deficits may not be a consequence of methamphetamine use, but rather a predisposing factor.

Table 1.1. Summary of studies that have investigated inhibitory and impulse control deficits in methamphetamine use disorder. Studies are ordered in ascending order of shortest abstinence length reported in the sample. Percentage of female participant is specified when reported. ANT: attention networks task; BIS: Barratt impulsiveness scale; CPT: continuous performance test; DDT: delayed discounting task; Meth: methamphetamine; SSRT: stop-signal reaction time; WCST: Wisconsin card sorting test.

Study	Sample	n (% females)	Inhibition & Impulse Control	
			Types (measures)	Impaired in meth?
Dean et al., 2011	Controls	17 (53%)	Inhibition (Stroop color-word; CPT; ANT)	Yes
	Chronic meth users (current)	24 (50%)		
Lyyo et al., 2015	Controls	120 (20%)	Inhibition (Stroop color-word)	Yes
	Chronic meth users (current)	106 (21%)		
Mahoney et al., 2015	Controls	31 (45%)	Impulsivity (BIS)	Yes
	Chronic meth users (current)	31 (29%)		
Kim et al., 2016	Controls	53 (23%)	Inhibition (Stroop color-word)	Yes
	Chronic meth users (current)	44 (20%)		
Andres et al., 2016	Controls	34 (38%)	Impulsivity (BIS)	Yes, but only in current users
	Chronic meth users	27 (44%)		
	Chronic meth users (abstinent 1 month - 24 years)	32 (37%)		
Dean et al., 2018	Controls	41	Inhibition (Stroop color-word and WCST)	Yes
	Chronic meth users (current)	37		
Simon et al., 2000	Controls	65 (60%)	Inhibition (Stroop color-word)	Yes
	Chronic meth users (last use <3 days)	65 (45%)		
Simon et al., 2002	Controls	40 (65%)	Inhibition (Stroop color-word)	Yes
	Chronic meth users (last use <3 days)	40 (50%)		
Monterosso et al., 2005	Controls (smokers)	14 (28%)	Inhibition (SSRT)	Yes.
	Controls (non-smokers)	29 (67%)		
	Chronic meth users (last use 5-7 days)	11 (36%)		
Nestor et al., 2011	Controls	18 (39%)	Inhibition (Stroop color-word)	Yes.
	Chronic meth users (abstinent 4-7 days)	10 (50%)		

Simon et al., 2010	Controls	Baseline: 28 (50%) Follow-up: 21 (43%)	Inhibition (Stroop color-word)	Baseline & follow-up: No
	Chronic meth users (baseline: abstinent 4-9 days; follow-up: abstinent 1 month)	Baseline: 27 (37%) Follow-up: 18 (28%)		
Kalechstein et al., 2003	Controls	18 (17%)	Inhibition (Stroop color-word)	Yes
	Chronic meth users (abstinent 5-14 days)	27 (30%)		
Schwartz et al., 2010	Controls	44 (50%)	Impulsivity (DDT)	Yes
	Chronic meth users (abstinent 14-160 days)	61 (49%)		
Hoffman et al., 2006	Controls	41 (27%)	Inhibition (Stroop color-word)	No
	Chronic meth users (abstinent 0.5-6 months)	41 (24%)		
Uhlmann et al., 2016	Controls	40 (28%)	Impulsivity (UPPS-P impulsive behavior scale)	Yes
	Chronic meth users (abstinent ~21 days)	39 (28%)		
Salo et al., 2011	Controls	38 (45%)	Inhibition (Stroop color-word)	Yes, but only in early abstinence
	Chronic meth users (abstinent 3 weeks-6 months)	41 (54%)		
	Chronic meth users (abstinent > 1 year)	28 (68%)		
Cherner et al., 2019	Controls	64 (0%)	Inhibition (Stroop color-word)	No
	Chronic meth users (abstinent 1-8 months)	85 (0%)		
Salo et al., 2002	Controls	12 (0%)	Inhibition (Stroop color-word)	Yes
	Chronic meth users (abstinent 2-4 months)	8 (0%)		
Salo et al., 2009b	Controls	16 (50%)	Inhibition (Stroop color-word)	Yes
	Chronic meth users (abstinent 2-12 months)	12 (58%)		
Kim et al., 2006	Controls	20 (25%)	Inhibition (WCST)	Yes
	Chronic meth users (abstinent 2.6-30.6 months)	29 (7%)		
Cherner et al., 2010	Controls	46	Inhibition (Stroop color-word)	No
	Chronic meth users (abstinent ~4 months)	54 (26%)		
King et al., 2010	Controls	74 (50%)	Inhibition (Stroop color-word)	Yes
	Chronic meth users (abstinent ~252 days)	54 (68%)		
Stock et al., 2018	Controls	32	Inhibition (Stroop color-word)	Yes, but only in early abstinence
	Chronic meth users (abstinent ~9.9 months)	13 (38%)		

	Chronic meth users (abstinent ~47.6 months)	14 (43%)		
Salo et al., 2013a	Controls	30 (43%)	Inhibition (Stroop color- word)	Yes
	Chronic meth users (abstinent ~13.7 months)	30 (50%)		
Salo et al., 2007	Controls	16 (50%)	Inhibition (Stroop color- word)	Yes
	Chronic meth users (abstinent ~20 months)	36 (64%)		
Salo et al., 2009a	Controls	17 (47%)	Inhibition (Stroop color- word)	Yes
	Chronic meth users (abstinent ~20.98 months)	37 (65%)		

There is a strong association between inhibitory control and impulsivity (Logan et al., 1997). It is therefore not surprising that people with methamphetamine use disorder also have poor impulse control (Andres et al., 2016; Hoffman et al., 2008; Mahoney et al., 2015; Monterosso et al., 2005; Schwartz et al., 2010; Uhlmann et al., 2016). Notably, impulsivity is regarded as one key predisposing factor to developing substance use disorder (Everitt, 2014). Monterosso et al. (2005) found that reaction time in an impulse control task positively correlated with grams of methamphetamine used per week, highlighting the relationship between poor impulse control performance and the extent of recent methamphetamine use. These deficits have important clinical implications, as poor inhibition and impulse control are linked with unemployment, treatment non-completion and higher rates of relapse in this population (Pattij and De Vries, 2013; Weber et al., 2012; Winhusen et al., 2013)

Inhibitory control deficits and heightened impulsivity in people with methamphetamine use disorder are associated with gray and white matter abnormalities (Andres et al., 2016; Kim et al., 2009, 2006; Lederer et al., 2016; Lyoo et al., 2015; Salo et al., 2009a; Tobias et al., 2010; Uhlmann et al., 2016). For more detailed description of these findings, see Guerin et al. (2019). In addition to structural abnormalities, people with methamphetamine use disorder display reduced activation of the PFC when performing the Stroop task (Salo et al., 2013a, 2009c), and increased activation in the right inferior frontal gyrus and anterior cingulate cortex (ACC) (Nestor et al., 2011). There is evidence for age-related alterations in

ACC metabolism, which is measured by *N*-acetylaspartate, a marker of neuronal integrity (Bates et al., 1996). Reduced *N*-acetylaspartate levels in the ACC are observed in people with methamphetamine use disorder (Howells et al., 2014). *N*-acetylaspartate levels correlate with poorer attention and inhibition in adult (Salo et al., 2007) and adolescent who use methamphetamine (Kim et al., 2016). Notably, reduced *N*-acetylaspartate in the ACC is more dramatic with early onset of methamphetamine use (Kim et al., 2016).

Taken together, poor neuronal integrity and associated inhibitory deficits seem to be more pronounced in adolescents who use methamphetamine. However, it is unclear whether adolescence is a naturally risky period of mental problems and immature cognition, or if methamphetamine is more harmful during adolescence.

1.4.2. Cue-induced craving, relapse, and cue exposure

Reduced cognition in methamphetamine use disorder is important because it can interfere with therapy, leading to a cycle of relapse. There is currently no government-approved medication to treat methamphetamine use disorder (Baker and Lee, 2003). Psychological interventions such as cognitive behavioral therapy (CBT) are one of the most common approaches to treat substance use disorders (National Institute on Drug Abuse, 2020). Notably, the current evidence for CBT efficacy in methamphetamine use disorder is low quality (Harada et al., 2018), with only two studies investigating its efficacy. Nonetheless, evidence supports the use of CBT to reduce substance use in other drugs (Gates et al., 2016; McHugh et al., 2010). A promising approach to treat methamphetamine use disorder is to combine CBT with cue exposure. Cue exposure aims to reduce craving, which is defined as an intense urge to acquire and consume the drug. Common craving triggers include exposure to cues related to the drug-taking experience, stress, or the drug itself (Carter and Tiffany, 1999; de Wit and Stewart, 1981; Drummond, 2000; Wemm and Sinha, 2019). Exposure to cues associated with the drug-taking experience is one of the strongest

predictor for relapse (Davis and Smith, 1976). Cue-induced craving is observed following the use of a variety of drugs, such as nicotine (Conklin et al., 2008; LaRowe et al., 2007), alcohol (Courtney et al., 2016; Groefsema et al., 2016), heroin and other opioids (McHugh et al., 2016; B. Su et al., 2017), cocaine (Childress et al., 1999, 1986; Modesto-Lowe and Kranzler, 1999), and methamphetamine (reviewed in Seow et al., 2020).

Based on the Pavlovian conditioning model of addiction, response to drug-related cues is believed to be a natural result of conditioning that occurring during drug-taking (Drummond et al., 1995; Perry et al., 2014). After repeated pairing of the drug-taking experience and the cues, presentation of the cues alone can elicit an emotional, conditioned response (Pavlov, 1927). The aim of cue exposure is to reduce such conditioned response to reduce or prevent relapse. It relies on the principle of cue extinction (Pavlov, 1927), which is the reduction of cue-induced craving due to the repeated exposure to drug-associated cues without any drug availability (Biferno and Dawson, 1977; Perry et al., 2014). Extinction involves cognitive control (Lovibond, 2004) and may form part of CBT to treat addiction (Louie et al., 2018). Clinical studies have shown that cue extinction can reduce craving in participants with methamphetamine use disorder (DeSantis et al., 2009; Price et al., 2010).

Craving can be measured in several ways, including subjective self-report (Childress et al., 1986), and objective physiological responses, such as heart rate (HR), blood pressure (BP) and skin conductance response (SCR; McHugh et al., 2016). The magnitude of physiological changes when presented with drug-associated cues is referred to as 'cue reactivity'. Using these measures, researchers have shown that craving can be elicited using a range of cues associated with previous drug experience, including but not limited to visual, verbal, gustatory and olfactory (Mehrjerdi et al., 2011).

There is a growing body of methamphetamine cue reactivity literature (**Table 1.2**). Liang and colleagues (2019) investigated cue reactivity using a paradigm consisting of six methamphetamine-related pictures. 52 people with methamphetamine use disorder were

asked to rate their craving following presentation of each picture. They observed that over half of participants reported no craving following cue presentation (Liang et al., 2019), suggesting that cue reactivity may not reliably be elicited when too few cues are presented. Tolliver and colleagues (2010) used a longer paradigm. It consisted of a 20-min sequence of methamphetamine cue presentation, including 30 to 35 photographs of people purchasing and using methamphetamine, a 7-8 min long video of people using methamphetamine in different locations, and *in vivo* paraphernalia presented directly in front of the participant (Tolliver et al., 2010). Their study revealed that all three types of cues (photos, video and paraphernalia) similarly increased subjective craving compared to baseline craving levels. They also observed an increase in SCR following cue presentation compared to baseline, as well as a decrease in HR following picture cues.

Table 1.2. Methodology of methamphetamine cue reactivity studies. BP: blood pressure; HR: heart rate; I.V.: intravenous; Meth: methamphetamine; SCR = skin conductance response; VR: virtual reality.

Study	Participants		Cues		Measure	
	Control	Meth	Control	Meth	Objective	Subjective
DeSantis et al., 2009	None	25	None	Photos; video; <i>in vivo</i> paraphernalia	HR	Self-reported craving
Price et al., 2010	None	24	None	Photos; video; <i>in vivo</i> paraphernalia	SCR; HR	Self-reported craving
Tolliver et al., 2010	None	43	None	Photos; video; <i>in vivo</i> paraphernalia	SCR;HR	Self-reported craving
Culberston et al., 2010	None	17	Neutral-VR fish video; <i>in vivo</i> objects (e.g. feather and pencil)	Meth-VR; meth-video; <i>in vivo</i> paraphernalia	HR	Self-reported mood and urge to use meth
Tolliver et al., 2012	30	30	Nature video	Meth video	SCR; HR	Self-reported craving
Malcolm et al., 2016	9	9	Visual neutral cues	Pictures (I.V., nasal, smoking)	None	Self-reported craving
Wang et al., 2013	None	139	Neutral pictures; nature video	Photos; video; <i>in vivo</i> paraphernalia	HR BP	Self-reported craving

Lopez et al., 2015	None	21	Food photos	Photos Video	None	Self-reported craving
Wang et al., 2018	45	61	None	Meth-VR	HR	Self-reported craving
Liang et al., 2019	None	52	None	Photos	None	Self-reported craving
Tan et al., 2019	None	60	Neutral-VR	Meth-VR	SCR HR	Self-reported craving

Price and colleagues (2010) then used the same paradigm from Tolliver et al. (2010) to investigate cue extinction of craving for methamphetamine. Participants were administered cue exposure across two sessions, each session involving three 20-min block of methamphetamine cue presentations. They found that subjective craving response significantly decreased across blocks. This was consistent with a previous study showing that people with methamphetamine use disorder exhibiting high use behavior displayed complete abstinence in the two weeks following a cue exposure session (DeSantis et al., 2009). DeSantis and colleagues employed the same paradigm as Price et al. (2010), except they conducted a follow-up interview two-weeks following cue exposure to assess their methamphetamine use. Of the 25 participants whom completed follow-up assessments, none reported using methamphetamine in the two-weeks following the first session (DeSantis et al., 2009), providing preliminary evidence for a protective role of cue exposure against craving and relapse. It should be noted that the study lacked a non-exposed control group, and reduction in methamphetamine use could have been caused by other factors not controlled for. In addition, the study had a high attrition rate, with only 60% of participants completing follow-up assessments.

Notably, neither Tolliver et al. (2010) nor Price et al. (2010) found correlation between self-reported craving and HR or SCR. This is surprising, as SCR is a good predictor of craving for other drugs (Bordnick et al., 2005; Kaag et al., 2016). Discrepancies could be

explained by the lack of inter-trial intervals between cues in Price et al. (2010) and Tolliver et al. (2010). SCR is known to have a post stimulus-onset latency period lasting between 1 and 3 seconds (**Figure 1.2**) (Cacioppo et al., 2007). Peaks in SCR, representing response to the cue, will only be elicited several seconds after presentation of the stimulus. Similarly, cue-induced HR changes can only be detected seconds post stimulus presentation. It is crucial to include periods of rest between cues when designing such paradigm to obtain reliable physiological measures (Boucsein et al., 2012).

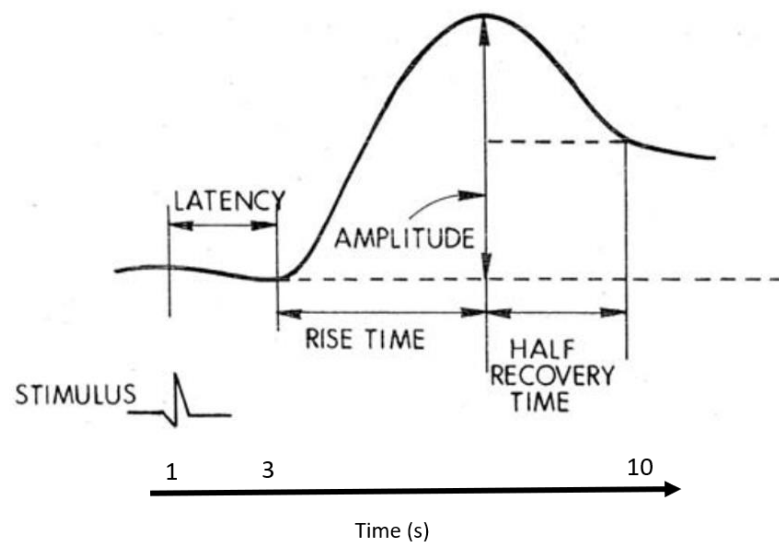


Figure 1.2. A typical skin conductance response. Post stimulus-onset latency period lasts between 1 and 3 s. SCR peaks within 10 s of cue onset, before returning to baseline. Adapted from *The Handbook of Psychophysiology* (Cacioppo et al., 2007).

Discrepancies between subjective and physiological measures in these studies could also be explained by the fact that the studies lacked non-drug control cues. This means that any increase in craving to cues may be independent of their association with methamphetamine. Non-drug cues serve as a critical control stimulus to understand drug-specific cue reactivity. Rigorous studies measuring cue-induced SCR should include control cues (Ganella et al., 2018; Savage et al., 2020).

A number of studies used a range of control cues to overcome this issue. In one study, participants were exposed to a methamphetamine-associated virtual environment

using virtual reality (VR) tools, including avatars administering methamphetamine, methamphetamine paraphernalia, and contextual “methamphetamine house” characteristics (Culbertson et al., 2010). The neutral (control) virtual environment depicted a modern apartment devoid of methamphetamine cues. The methamphetamine virtual environment elicited significantly greater increase in subjective craving compared to the neutral environment. Tan and colleagues (2019) replicated this finding, and additionally reported that skin conductance levels were elevated in response to the methamphetamine-related environment. In a different study, Wang and colleagues (2013) counterbalanced methamphetamine pictures and videos with neutral pictures and videos depicting nature. They found that craving was significantly higher in response to methamphetamine cues compared to control cues, regardless of the length of abstinence (Wang et al., 2013). This suggests that subjective response to methamphetamine-related cues can be elicited long after methamphetamine use has been discontinued. Notably, there were no differences in objective response (HR and BP) between methamphetamine and control cues (Wang et al., 2013). These studies (Culbertson et al., 2010; Lopez et al., 2015; Tan et al., 2019; Wang et al., 2013) suggest that cue-induced craving in methamphetamine users are in fact specific to methamphetamine-related cues.

It should be noted that most studies discussed thus far did not include control participants (**Table 1.2**). That is, it is unclear whether cue-induced craving is due to their history of methamphetamine use, and not to the potentially stimulating nature of methamphetamine cues compared to control cues. Tolliver and colleagues (2012) added a control group to their experimental design. Both groups were asked to perform a simple Go-No-Go task, in which they were instructed to click a computer mouse as quickly as possible each time they heard a “low” frequency (1000 Hz) tone, and not to respond each time they heard a “high” frequency (2000 Hz) tone. They were asked to watch two 3-min silent videos while performing the task: one depicting people procuring and using methamphetamine, and

the other depicting nature landscapes (Tolliver et al., 2012). Control participants reported no craving at any time, whereas people with methamphetamine use disorder reported significantly more craving after drug cue presentations compared to neutral cues. A more recent study observed significantly higher levels of craving for methamphetamine cues compared to neutral cues in people with methamphetamine use disorder, but not in controls (Malcolm et al., 2016). While these two studies provide evidence that subjective response to methamphetamine cues is of Pavlovian nature (i.e. experience specific), only one study to date has reported an objective physiological measurement but without any control cues (Wang et al., 2018). Taken together, I propose that an ideal methamphetamine cue reactivity paradigm should include methamphetamine-related cues interweaved with control cues to ensure the specificity of the craving response, and measure both subjective and objective conditioned responding.

Cue exposure does not always successfully reduce craving in every individual (Conklin and Tiffany, 2002), as they fail to consider important factors that may have an impact on the treatment outcome. Such factors include age of onset of drug use, which may have an effect on cognitive control (Kim et al., 2016; King et al., 2010; Lyoo et al., 2015). Adolescents may also be more resistant to psychosocial treatments. In fact, evidence suggests that age affects treatment retention in outpatient settings, with younger clients less likely to reduce their drug use and complete the program (Catalano et al., 1991). Adolescents also display high relapse rates (Perepletchikova et al., 2008). Younger adolescents appear to be more resistant to CBT and functional family therapy compared to older adolescents (Hendriks et al., 2012; Slesnick and Prestopnik, 2009). It is hypothesized that younger adolescents may not have the cognitive skills required to successfully complete treatment relying on cognitive control (Bachrach and Chung, 2020). On the other hand, Greenbaum and colleagues (2015) found that age was not related to treatment outcome for

family therapy. While evidence points towards a role for age in CBT outcome, there is a critical need to better understand how age potentially mediates treatment success.

In addition to age, it is also widely theorized that a genetic predisposition interacts with drug exposure to result in dependence (Ducci and Goldman, 2012). Indeed, genetic factors have also been highlighted to potentially predispose an individual to develop methamphetamine use disorder (Bousman et al., 2009; Guerin et al., 2021a).

1.5. Methamphetamine use disorder and genes

1.5.1. Heritability of methamphetamine use disorder

Evidence from twin and family studies suggests that substance use disorders may be highly heritable (Ducci and Goldman, 2012; Goldman et al., 2005; Vanyukov and Tarter, 2000). Notably, the heritability of stimulant use disorders is estimated to be close to 40% (Goldman et al., 2005). However, no twin or family studies have been conducted in people with methamphetamine use disorder, making it challenging to uncover the heritability of risk for this particular drug.

Another approach to investigate the heritability of disorders are genome-wide association studies (GWASs). To date, only three GWASs have been conducted in people with methamphetamine use disorder (Ikeda et al., 2013; Sun et al., 2019; Uhl et al., 2008). While Uhl et al. (2008) and Ikeda et al. (2013) did not detect significant genome-wide single nucleotide polymorphism (SNP) associations, Sun and colleagues (2019) more recently reported an association between methamphetamine use disorder and three single-nucleotide polymorphisms (SNPs). SNPs are single base mutation in certain genes, which can lead to changes in gene expression, as well as structural and functional changes on the protein level. It should be noted that all three studies present some major limitations. The first two studies used overlapping samples, and all three studies were gravely under-powered for GWASs. Specifically, the study with the largest sample size included 1750 people with methamphetamine use disorder, compared to the tens or even hundreds of

thousands of participants required for reliable genetic findings for similarly polygenic complex psychiatric disorders.

While twin studies have been lacking and GWAS results have been inconclusive, there has been an accumulation of candidate gene association studies in people with methamphetamine use disorder over the past two decades, which I systematically reviewed as a part of this PhD (see Chapter 4; Guerin et al., 2021a). Though not without important limitations, candidate gene studies can identify specific sequence variations in genes of interest and point towards potential molecular pathways affected to inform future research.

1.5.2. Genes and adolescent-onset methamphetamine use

While gene polymorphisms may contribute to the development of methamphetamine use disorder, it is unclear whether they play a role in adolescent vulnerability. Of the 80 studies reviewed in Guerin et al. (2021a), only nine investigated the association between SNPs and age of onset of methamphetamine use (Kotaka et al., 2009; Morita et al., 2005a, 2005b; Nomura et al., 2006b, 2006a; Okahisa et al., 2009; Yoshimura et al., 2011). A variant of the glial cell line-derived neurotrophic factor was associated with a later (> 20 years old) onset of methamphetamine use (Yoshimura et al., 2011), but the study was highly underpowered (power = 0.07).

While gene-association studies in humans are lacking, we have compelling preclinical evidence for the role of adolescent methamphetamine use on gene expression. Using genome-wide transcriptome analyses (RNA-sequencing), our laboratory discovered 30 genes that are significantly altered in adolescent, but not adult, methamphetamine self-administering rats (Luikinga et al. unpublished observations). Ingenuity Pathway Analysis identified solute carrier family 18 member 1 (*SLC18A1*) gene as a main node, with nine of the genes identified being involved in the same pathway. *SLC18A1* codes for the vesicular monoamine transporter 1 (VMAT1) protein (Eiden et al., 2004). While studies have

highlighted an association between variants in this gene and a range of neuropsychiatric disorders in humans (Bly, 2005; Chen et al., 2007; Dutta et al., 2016; Lohoff et al., 2008b, 2008a, 2006; Need et al., 2009; Noroozi et al., 2017; Richards et al., 2006; Vaht et al., 2016; Zhu et al., 2015), our transcriptome study is the first one to observe *SLC18A1* to be linked with methamphetamine use. Downregulation of this gene measured by RNA-seq only in adolescents (**Figure 1.3a**) has been validated in a new set of rats at the mRNA (**Figure 1.3b**) and then at the protein level by Western Blot (**Figure 1.3c**), showing that VMAT1 expressing was lower in the dorsal striatum of adolescent rats that received methamphetamine compared to saline. Further investigation of the role of *SLC18A1* and VMAT1 in methamphetamine use disorder is clearly necessary and may provide important clues in understanding the disease in its relationship with age of onset of methamphetamine use.

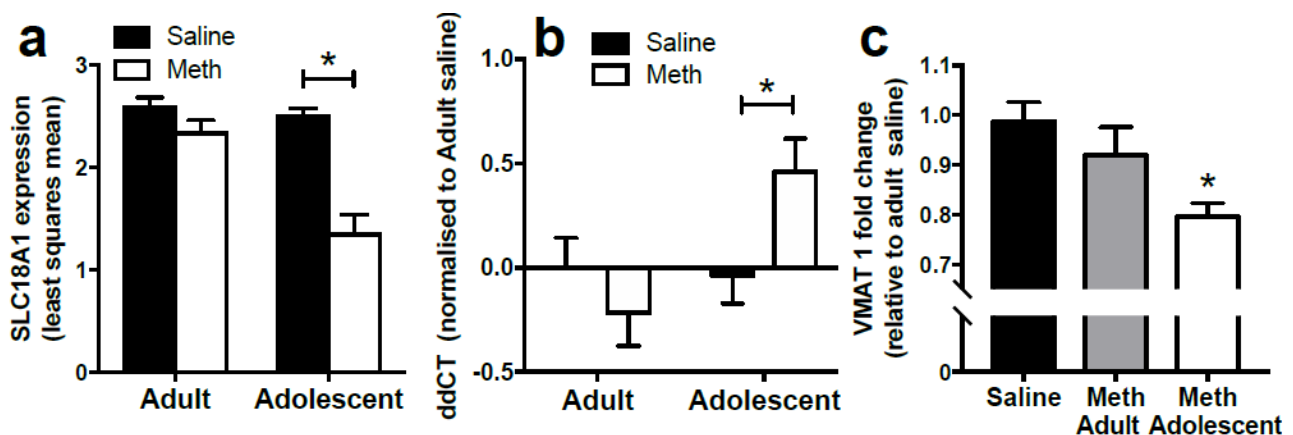


Figure 1.3. *SLC18A1*/VMAT1 and adolescent methamphetamine use **a.** RNA-seq data showed *SLC18A1* expression is significantly lower in the dorsal striatum following methamphetamine self-administration compared to saline in adolescent rats. Adult rats showed no difference in *SLC18A1* expression between groups. **b.** Longer qPCR cycles are needed to detect *SLC18A1* mRNA, indicating that there is less mRNA in the adolescent methamphetamine group. **c.** VMAT1 protein expression is lower in the dorsal striatum following methamphetamine self-administration compared to saline in adolescent rats. Unpublished observations. *p < 0.05

1.5.2.1. Vesicular Monoamine Transporter 1 (VMAT1)

VMATs manage the uptake of cytoplasmic monoamines neurotransmitters such as dopamine into presynaptic storage vesicles (Schuldiner et al, 1995). Those vesicles then release their contents into the synaptic cleft upon an action potential (Liu et al., 1992). Two distinct VMATs, encoded by two different genes, have been identified: VMAT1 (encoded by gene *SLC18A1*) located on chromosome 8p21.3, and VMAT2 (encoded by gene *SLC18A2*) located on chromosome 10q25 (Peter et al., 1993). While similar in structure, VMAT1 and VMAT2 have distinct properties (Erickson et al., 1996; Multani et al., 2013). For example, VMAT1 has a lower turnover number and lower affinity to monoamines compared to VMAT2. In addition, Hansson and colleagues (1998) have highlighted that VMATs are differentially expressed in the brain during the embryonic developments in rats (Hansson et al., 1998).

Historically, VMAT2 has received more attention in the context of methamphetamine use (Eiden and Weihe, 2011; Fleckenstein et al., 2007; Fumagalli et al., 1999; McFadden et al., 2012; Nickell et al., 2014; Uhl et al., 2000) and no published studies examined VMAT1's role in methamphetamine use. For example, *in vitro* studies observed that methamphetamine reduced dopamine uptake by VMAT2, increasing cellular and synaptic dopamine (reviewed in Fleckenstein et al., 2007). Methamphetamine is also believed to redistribute VMAT2 away from the pre-synaptic nerve terminal (Riddle et al., 2002). The relative neglect in VMAT1 research is most likely due to the fact that it was initially undetected in the rodent brain, although it was robustly shown to be expressed in the human brain (Erickson et al., 1996; Peter et al., 1995). However, recent studies have shown the presence of both *SLC18A1* mRNA and VMAT1 protein in the rat brain (Multani et al., 2013; Nickell et al., 2014; Luikinga et al., unpublished observations). Importantly, mutations in *SLC18A1* in humans are associated with several neuropsychiatric disorders, including bipolar disorder, anxiety-related personality traits, autism spectrum disorder, major depressive disorder, alcohol use disorder, and schizophrenia (**Table 1.3**; Bly, 2005; Chen

et al., 2007; Dutta et al., 2016; Lohoff et al., 2008a, 2008b, 2006; Need et al., 2009; Noroozi et al., 2017; Richards et al., 2006). The latter two are especially important, as they share many similarities with methamphetamine use disorder.

Table 1.3. Summary of candidate-gene studies investigating *SLC18A1* SNPs

Disorder	Study	Single Nucleotide Polymorphism
Schizophrenia	Bly, 2005	Thr4Pro associated with risk of schizophrenia
	Richards et al., 2006	Ser98Thr associated with risk of schizophrenia
Alcohol Use Disorder	Vaht et al., 2016	Thr136Ile found to be protective in clinical sample
	Dutta et al., 2016	Thr136Ile, rs952859 and rs3779672 associated with lower withdrawal severity in clinical sample
	Zhu et al., 2015	Thr136Ile found to be protective in clinical sample
Autism Spectrum Disorder (ASD)	Noroozi et al., 2017	Thr136Ile associated with risk of ASD
Bipolar Disorder Type 1	Lohoff et al., 2006	Thr136Ile found to be protective in clinical sample
Anxiety-like personality traits	Lohoff et al., 2008	Thr136Ile found to be protective in clinical sample
Major Depressive Disorder (MDD)	Won et al., 2017	Thr136Ile associated with white matter integrity in MDD participants

Schizophrenia and methamphetamine use disorder are often co-morbid and share key features, such as underlying monoamine dysregulation, psychotic symptoms, cognitive deficits and emergence during adolescence (Gordon, 2008; Guerin et al., 2019; Lecomte et al., 2010). A GWAS highlighted that methamphetamine-induced psychosis and schizophrenia share genetic features (Ikeda et al., 2013). In addition, a recent meta-analysis revealed that 22% of people who experience methamphetamine-induced psychosis go on to develop a diagnosis of schizophrenia (Murrie et al., 2020). Our laboratory's recent discovery of *SLC18A1* downregulation following methamphetamine self-administration in adolescent rats and this gene's contribution to schizophrenia strongly suggest biological significance of *SLC18A1* in methamphetamine use in adolescence.

Bly (2005) used postmortem brain tissues from 28 unrelated Caucasian patients with schizophrenia to test for allele frequencies in *SLC18A1*. He found that the missense mutation A277C (resulting in the substitution of the 4th amino acid residue from a threonine to a proline, which is noted as Thr4Pro, **Figure 1.3**) occurred in 21.4% of the clinical group, compared to only 2.6% of the healthy controls (Bly, 2005). This finding was replicated in a sample of 188 people with schizophrenia vs 197 healthy Russians (Galaktionova et al., 2014). Very little is known on the effect of the Thr4Pro variant on VMAT1 protein expression, structure, and function. In addition, Bly's findings were not replicated in Richards et al. (2006), which examined 354 Japanese patients and 365 healthy controls. Discrepancy between results may be due to differences between the Caucasian and Asian samples (Richards et al., 2006), suggesting that ethnicity may play a role in *SLC18A1* mutations. Such ethnic divergence has been observed in other gene polymorphisms in methamphetamine use disorder (Bousman et al., 2010b).

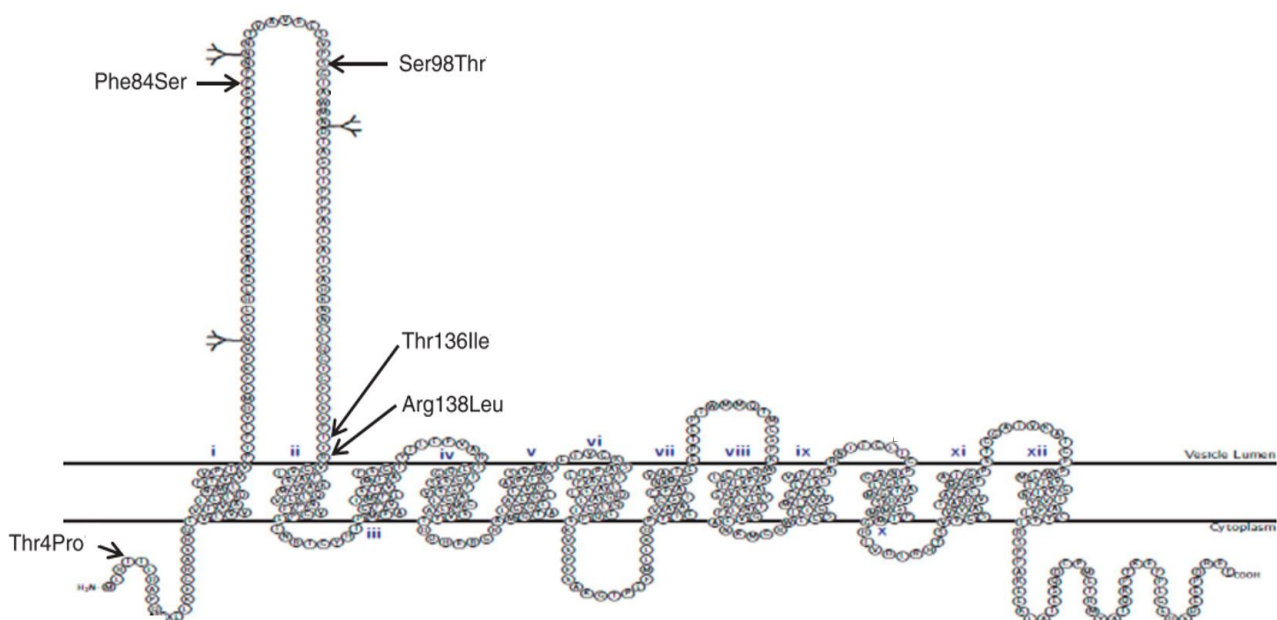


Figure 1.4. Schematic of the VMAT1 protein and its common variants. The Ser98Thr variant may affect VMAT1 protein trafficking. Thr136Ile variant increases VMAT1 activity and monoamine uptake. Phe84Ser and Arg138Leu significantly increase and decrease dopamine reuptake in the vesicle, respectively. Function of the Thr4Pro variant is unknown. Adapted from Lohoff et al. (2014)

Richards et al. (2006) further reported a significant difference in Ser98Thr distribution between cases and controls in their female sample, whereas there was no difference in males. The Thr98Ser variant results in the substitution of the 98th amino acid residue from a serine to a threonine. It is located on the first luminal loop between two transmembrane domains (**Figure 1.3**), which is thought to be involved in targeting VMAT1 to the pre-synaptic terminal (Erickson et al., 1996). Richards and colleagues speculate that Ser98Thr may therefore *reduce* the processing and overall function of VMAT1 through alteration of its trafficking pathway (Richards et al., 2006). This may lead to monoamine imbalance, and potentially increasing the risk of developing schizophrenia.

Alcohol and methamphetamine use disorders are both substance use disorders characterized by predominant adolescent age of onset (Hingson and Zha, 2009). Two studies investigating the link between *SLC18A1* polymorphisms and alcohol use disorder found an association between the Thr136Ile variant of the protein and reduced risk (Dutta et al., 2016; Vaht et al., 2016). Thr136Ile results in substitution of the 136th amino acid isoleucine by a threonine (**Figure 1.3**). The first study investigated 609 participants with alcohol dependence (Dutta et al., 2016) and the second study examined an Estonian database of over 1200 participants (Vaht et al., 2016). A functional MRI study looking at the brain of 68 people with alcohol dependence and 72 controls reported that the variant was associated with reduced connectivity in brain networks that usually show an increased connectivity in alcohol users (Zhu et al., 2015). Taken together, these results strongly suggest a potential protective role for the Thr136Ile VMAT1 variant against alcohol use disorder. The variant is located first luminal loop of VMAT1 (**Figure 1.3**), which mediates G-protein dependent regulation of neurotransmitter uptake (Brunk et al., 2006). *In vitro* studies have demonstrated that it leads to *increased* VMAT1 activity, and increased monoamine transport into the presynaptic vesicles (Khalifa et al., 2012; Lohoff et al., 2014), which could explain the protective effect of the allele in alcohol use disorder.

In their *in vitro* study, Lohoff et al. (2014) found that two *SLC18A1* variants, Phe84Ser and Arg138Leu (**Figure 1.3**), played a role in dopamine transport. Phe84Ser significantly increased uptake, whereas the Arg138Leu decreased uptake of dopamine (Lohoff et al., 2014). Although those two polymorphisms have not yet been identified to be different in clinical populations, they may be of importance in the role of VMAT1 in substance use disorder, as extensive literature highlights the role of dopamine in addiction (Riddle et al., 2008). It should be noted that both variants are extremely rare and are only detected in less than 0.1% of the population.

In summary, there is a growing body of literature suggesting an association between *SLC18A1* polymorphisms and neuropsychiatric disorders. This indicated that it may play a role also in vulnerability to methamphetamine use disorder. Because of the features shared between some of these disorders and methamphetamine use, in addition to our findings in rats, it is clear that the distribution and function of *SLC18A1* variations need to be further investigated.

1.5.2.2. Vesicular Monoamine Transporter 2 (VMAT2)

Although our main target is *SLC18A1*, it is important to assess *SLC18A2* polymorphisms due to the known interaction between methamphetamine and VMAT2 (Eiden and Weihe, 2011; Fleckenstein et al., 2007; Fumagalli et al., 1999; Luikinga et al., 2018; McFadden et al., 2012; Uhl et al., 2000). Using positron emission tomography, a reduction of 10% of VMAT2 binding availability in the striatal subregions was observed in methamphetamine users (Johanson et al., 2006). This suggest that methamphetamine use may downregulate *SLC18A2* expression. In addition, they found no correlation between this reduced VMAT2 levels and years of abstinence from methamphetamine, which suggests that methamphetamine can cause irreversible damage. Alternatively, pre-existing reduced levels of VMAT2 may be a risk factor for developing methamphetamine use disorder. Similar to

SLC18A1, associations between *SLC18A2* polymorphisms and a range of psychiatric disorders have been observed (**Table 1.4**). I will highlight *SLC18A2* SNPs associated with schizophrenia and substance use disorders.

Table 1.4. Summary of candidate-gene studies investigating *SLC18A2* SNPs

Disorder	Study	SNP
Schizophrenia	Simons and Van Winkel, 2013	rs363227 : T-allele associated with poorer cognitive function in clinical sample
	Zai et al., 2013	rs363224 : A-allele protective against involuntary movements in clinical sample
	Kukshal et al., 2013	rs363285 : C-allele associated with poorer executive function in clinical sample rs10082463 : A-allele associated with psychomotor performance in clinical sample
Alcohol Use Disorder	Schwab et al., 2005	rs363333 : C-allele associated with risk of alcohol dependence rs363387 : T-allele associated with risk of alcohol dependence
	Fehr et al., 2013	rs363387 : T-allele associated with risk of alcohol dependence; G-allele found to be protective
Opioid and cocaine dependence	Levrán et al., 2015	rs363276 : T-allele more common in clinical samples
	Randesi et al., 2019	rs3785143 : C-allele more common in clinical sample (opioid dependence)
Post-traumatic stress disorder (PTSD)	Bharadwaj et al., 2018	rs363276 : T-allele associated with risk of PTSD
	Solovieff et al., 2014	rs363276 : T-allele associated with of risk of PTSD
Cognitive impairment	Myrga et al., 2016	rs363226 : C- and G-alleles associated with risk of cognitive impairment in traumatic brain injury sample
	Markos et al., 2017	rs363226 : C- and G-alleles associated with risk of cognitive impairment in traumatic brain injury sample
Risk-taking behavior	Roe et al., 2009	rs363333 : C-allele associated with risk-taking behaviour rs1860404 : T-allele associated with risk-taking behavior
Bipolar Disorder Type I	Harley et al., 2011	rs363420 : T-allele associated with risk of bipolar disorder

		rs363272: G-allele associated with risk of bipolar disorder
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In a large family study of 1120 participants and their siblings, there was a significant association between the *SLC18A2* polymorphism rs363227 and cognitive function in schizophrenia participants, with the T-allele correlating with poorer cognitive function (Simons and Van Winkel, 2013). Another study identified rs363285 and rs10082463 to be associated with poorer cognition in schizophrenia patients (Kukshal et al., 2013).

In a study including 131 families with alcohol use disorder, Schwab and colleagues (2005) found an association between increased alcohol dependence and the *SLC18A2* variants rs363333 and rs363387. This is consistent with the findings of another study (Fehr et al., 2013), which observed an increased risk of alcohol dependence of the participants possessing the T-allele in the rs363387 polymorphism, whereas the G-allele seemed protective. The allele frequency of rs363333 did not significantly differ between the clinical and control population in Fehr's study. In a study involving over 1800 participants, Levran et al. (2015) found an association between the variant rs363276 and both opioid and cocaine dependence in a cohort of African ancestry (Levran et al., 2015). In contrast, a more recent study comparing 281 Caucasians with opioid dependence and 153 controls did not find any association between the disorder and rs363276 (Randesi et al., 2019). The frequency of the rs363276 variant in the general Caucasian population is 15% compared to 37% in people of African descent, explaining why the smaller study in Caucasians did not detect significant differences (Randesi et al., 2019). Nevertheless, Randesi and colleagues (2019) found an association between the rs3785143 polymorphism and opioid dependence. This further suggests a link between *SLC18A2* variations and substance use disorders.

It is important to note that all of the *SLC18A2* variants discussed above are either located in an intron or in the 3' untranslated region of the gene, and therefore may have little

effect on the structure and function of VMAT2. In addition, there is a clear lack of replication between studies. More research on the exact role of *SLC18A2* variants is warranted.

1.6. Summary

Extensive research has demonstrated a link between age of onset of substance use and severity of addiction later in life (Anthony and Petronis, 1995; Brown et al., 2009; Chen et al., 2009; Dawson et al., 2008; Grant and Dawson, 1998; King et al., 2007; Robins and Przybeck, 1985). It is also widely accepted that adolescents are more resistant to treatment, and more likely to relapse following cue exposure therapy (Catalano et al., 1991; Perepletchikova et al., 2008; Spear, 2000). While it is currently poorly understood why adolescents are more vulnerable, there is growing evidence that psychiatric comorbidities and poor inhibitory and impulse control may be predisposing factors to developing methamphetamine use disorder. Well-controlled and validated cue reactivity paradigms are needed to assess the effect of age of onset of methamphetamine use on cue extinction behavior in this population. In addition, I have compelling evidence that certain gene variants may confer risk to developing methamphetamine use disorder (Guerin et al., 2021a). We have also previously showed that the age of onset of drug use may be linked to changes in gene expression in rats (Luikinga et al, unpublished observations). *SLC18A1* and *SLC18A2*, coding for VMAT1 and VMAT2 respectively, are promising candidates. Variants in those genes have been found to be associated with neuropsychiatric disorders including other substance use disorders, and schizophrenia. The latter is especially interesting, as methamphetamine use and schizophrenia are often comorbid and share a similar symptom profile (Guerin et al., 2019). It is clear that to gain a holistic understanding of methamphetamine use disorder, we must investigate psychiatric, cognitive and genetic factors associated with the age of onset of methamphetamine use.

1.7. Project aims

The overall aim of this thesis was to investigate the association between psychiatric, cognitive, and genetic factors and age of onset of methamphetamine use in people with methamphetamine use disorder. In Chapter 2, I developed and validated a novel cue reactivity paradigm to assess subjective and physiological responses to methamphetamine-related cues and their extinction. In Chapter 3, I investigated the association between comorbid psychiatric symptoms, performance in cognitive tasks, and age of onset of methamphetamine use. In Chapter 4, I systematically reviewed gene-association studies in people with methamphetamine use disorder. In Chapter 5, I investigated the association between key gene polymorphisms and expression and methamphetamine use disorder.

**Chapter 2. Assessing subjective and physiological
methamphetamine-related cue reactivity in people with
methamphetamine use disorder relative to controls**

Under Review in Addictive Behaviors

ASSESSING METHAMPHETAMINE-RELATED CUE REACTIVITY IN PEOPLE WITH METHAMPHETAMINE USE DISORDER RELATIVE TO CONTROLS

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ABSTRACT

Methamphetamine use disorder involves methamphetamine-related cues invoking intense craving leading to relapse. Such cue reactivity is thought to arise from Pavlovian conditioning that occurs during the drug-taking experience. Cue reactivity then should be selective to methamphetamine cues (and not other cues), and not observed in people who have never experienced methamphetamine. However, these premises are not tested and reported using objective measures such as skin conductance response (SCR). The primary aim of this study was to test these premises using a cue reactivity paradigm we developed using control cues. The secondary aim was to explore the relationship between cue reactivity, drug use parameters, and cognition. Thirty people with a current diagnosis of methamphetamine use disorder and 30 matched controls with no history of substance use disorder were recruited. We observed higher overall subjective reactivity ($F=62.810$; $p<0.001$) and cue-selective physiological reactivity ($F=5.160$; $p=0.026$) in people with methamphetamine use disorder but not in controls. Co-morbid sedative use was associated with higher subjective craving ($r=0.521$; $p=0.003$). People who use methamphetamine intravenously had higher cue-selective SCR than smokers ($t=3.750$; $p<0.001$). Low inhibitory control measured by the Stroop task was associated with increased craving across the cue paradigm ($r=-0.494$; $p=0.006$). Overall, these results support that cue reactivity in people with methamphetamine use disorder is due to Pavlovian conditioning. Its association with drug use and cognition highlights cue reactivity paradigm's utility in understanding methamphetamine use disorder to develop new treatments targeting cue-induced craving.

Keywords: Cognition; craving; cue reactivity; methamphetamine use disorder; skin conductance response.

Abbreviations¹

¹ ASSIST = Alcohol, Smoking and Substance Involvement Screening Test; AUDIT = Alcohol Use Disorder Identification Test; GHB = Gamma-Hydroxybutyric Acid; ITI = Intertrial Interval; M.I.N.I. = MINI Neuropsychiatric Interview; PFC = Prefrontal Cortex; SCR = Skin Conductance Response; TMT = Trail Making Task; Δ SCR = Differential SCR

INTRODUCTION

Methamphetamine use disorder is a chronic relapsing disorder characterized by compulsive methamphetamine taking and seeking despite serious negative consequences (1). A major feature of methamphetamine use disorder is intense craving and repeated relapses. Several factors contribute to relapse, including exposure to cues associated with the drug taking experience (2). Salience attribution to drug-related cues in chronic methamphetamine use has biological significance, potentially signaling neuroadaptations (3). Response to drug-related cues is believed to be a natural result of Pavlovian conditioning that occurs during drug taking (4,5), whereby repeated pairings between the cues and drug use result in the cues alone to elicit an emotional response.

This putative conditioned association can be examined by presenting drug-related cues to people who use drugs, and measuring their cue reactivity by subjective self-reports and/or objective physiological measures such as heart rate variability, blood pressure, and skin conductance response (SCR) (4). SCR in particular is an autonomic response that is widely used in the laboratory to study biological bases of conditioned reflexes, arousal, and emotion (6). Cue reactivity under laboratory conditions may be a predictor of treatment outcomes in other drugs (4), and it can be used as a tool to assess the efficacy of potential therapies (7). Such importance of objective cue reactivity may be because it represents Pavlovian conditioned reflex that can be inhibited by cognitive control (4,6).

While cue reactivity in people with methamphetamine use disorder has been reported (reviewed in (9)), its Pavlovian nature remains ambiguous. Only a few studies have included both control participants and control cues in their design (**Table 1**). This is important because Pavlovian conditioning should be indicated by reactivity specific to methamphetamine cues and not to control cues unrelated to methamphetamine experience. Such cue specificity should also not be observed in people who have never experienced methamphetamine. Without control participants and cues, the response to cues may be due to the stimulating

nature of methamphetamine cues (e.g., needles) and not associated with the drug taking experience. Importantly, of those few studies that did include control participants and cues (**Table 1**), only Huang et al. (2018) and Chen et al. (2020) analyzed and reported objective reactivity measured by functional magnetic resonance imaging (10,11). Physiological reactivity provides high temporal resolution compared to subjective self-reports to distinguish reactivity to methamphetamine or control cues, and is less affected by demand characteristics. While self-reported craving offers a quick way to measure reactivity, they may be unreliable (2). However, all participants were abstinent from methamphetamine use in Huang et al. (2018) and Chen et al. (2020), which may be a confounding factor, given that length of abstinence can affect craving for methamphetamine (12).

Therefore, our primary aim was to assess subjective and physiological responses to methamphetamine-related cues in people with a current diagnosis of methamphetamine use disorder and drug-naïve control participants, using a cue reactivity paradigm we developed. Considering the paucity of data on factors modulating cue reactivity in people who use methamphetamine, our secondary aim was to explore the relationship between cue reactivity, drug use characteristics (e.g., duration and severity of drug use), and potential cognitive deficits in people with methamphetamine use disorder. Methamphetamine use characteristics and related cognition contribute towards treatment outcomes (13–15), and their potential relationship with cue reactivity would provide important insights into the relevance of cue reactivity in methamphetamine use disorder. We hypothesized that people with methamphetamine use disorder will physiologically respond more to methamphetamine-related cues over and above control cues. This would not be observed in control participants. In addition, we hypothesized that drug use parameters and deficits in certain cognitive domains would modulate the magnitude of cue reactivity in people with methamphetamine use disorder, based on previous reports on other drugs (16).

METHODS

Participants

Thirty people with methamphetamine use disorder and 30 controls were recruited between March 2018 and March 2020 through referrals at the Department of Addiction Medicine at St Vincent's Hospital Melbourne, and from local community groups in Melbourne metropolitan area. All participants provided written informed consent before any assessment.

People with methamphetamine use disorder were recruited if they were 1) aged 18 to 50 years-old; and 2) met DSM-5 criteria for stimulant use disorder, methamphetamine-type (moderate or severe) in the past six months as assessed by the MINI International Neuropsychiatric Interview for the DSM-5 (M.I.N.I 7.0.2.) (17). They were excluded if they 1) had a major neurological or medical illness; 2) a history of substance use disorder where methamphetamine was not the primary substance of abuse; or 3) if they were not able to provide informed consent (i.e., if they were under the influence of methamphetamine or other drugs on the day of testing and if they had an IQ < 70). Every participant in the methamphetamine use group were actively using (last use < 7 days).

Age-, sex- and IQ-matched controls were recruited via advertisements on social media, university, and hospital notice boards in Melbourne metropolitan. Controls were eligible to take part in the study if they 1) were aged between 18 and 50 years; and 2) had no history of substance use disorder. They were excluded if they had 1) a major neurological or medical illness; 2) a dependence on any substance; or 3) if they were not able to provide informed consent (i.e. if they had an IQ < 70). Controls were recruited to ensure response to cues was specific to the methamphetamine-taking experience.

All participants provided a urine sample for qualitative urinalysis (Pathtech, Melbourne, Australia), to test for amphetamines, cocaine, methamphetamine, opiates, benzodiazepines, and cannabis (THC). The calibrators and cut-offs for each drug are shown

in **Supplementary Table S1**. People who use methamphetamine were excluded if they tested positive for any drug other than methamphetamine on the day of testing, with the exception of cannabis that can be detected in urine up to a month after consumption (18). Controls were excluded if they tested positive for any illicit drug. All participants received reimbursement for their time and travel. People with methamphetamine use disorder were invited to discuss their craving with a clinician upon completion of the study.

Study design and procedure

The study was a cross-sectional two-group study conducted at the Department of Addiction Medicine at St Vincent's Hospital Melbourne, Fitzroy, Australia. All procedures were in accordance with the Declaration of Helsinki (World Medical Association, 2013) and received ethical approval from St Vincent's Hospital Melbourne Human Research Ethics Committee (HREC/17/SVHM/169). All participants were administered a clinical interview, a cognitive task battery, and a cue reactivity task by a trained assessor.

Background Measures

Wechsler Test of Adult Reading: assesses premorbid verbal IQ (19). Participants read out loud 50 irregularly spelled words. Each correct pronunciation is given a score of 1, with 50 as the maximum raw score. The raw score is then standardized by age (range 50-129).

Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): assesses severity, frequency, and high-risk substance use (20). It consists of eight items, and assesses alcohol, tobacco, cannabis, cocaine, amphetamine-type stimulants (including methamphetamine), sedatives, hallucinogens, inhalants and opioids use. A score of 4 or above reflects problem use, and a score of 27 or above reflects high-risk use. It also collects information about mode of drug use.

Alcohol Use Disorder Identification Test (AUDIT): assesses excessive drinking (21). It has 10 items rated 0-4. A score of 7 and above indicates an excessive alcohol use.

MINI International Neuropsychiatric Interview (M.I.N.I): The M.I.N.I English Version 7.0.2 for the DSM-5 (17) was administered to all participants to assess neuropsychiatric symptoms

and confirm stimulant use disorder diagnosis.

Cognitive measures

All participants were administered a brief cognitive task battery given in a random sequence, adapted from the MATRICS Consensus Cognitive Battery (22) and the Delis-Kaplan Executive Function System (23). Description of all tasks is in **Table 2**.

Cue reactivity paradigm

The cue reactivity paradigm was developed in-house. Cues were obtained from publicly available images/videos or purchased from stock websites. Methamphetamine cues were validated to induce craving with a focus group consisting of inpatients with methamphetamine use disorder. The final cue reactivity paradigm consisted of 35 methamphetamine-related cues (20 pictures and 15 videos with no sound) depicting methamphetamine, methamphetamine-related paraphernalia (e.g. glass pipes, syringes, lighters), and people consuming methamphetamine (orally, intranasally, and intravenously) counterbalanced with 35 control cues (20 pictures and 15 videos), depicting food, food-related paraphernalia (e.g. cutlery), people preparing food, and people consuming food. Food-related cues were chosen as control cues because food consumption and methamphetamine use both involve motor activity, and cues share characteristics (object alone, object being handled, object being used etc.). Similar criteria have been used to select control cues in cue reactivity studies of other drugs (24). In addition, previous studies have used either nature (i.e. landscapes) or sexual control cues (reviewed in (9)), which may either be too relaxing or too arousing even for control participants. Food-related cues were matched to methamphetamine cues for background color, quality, and hue. Each cue was presented in a pseudorandomized fashion for 5 s, to ensure first 4-minute block, middle 4-minute block, and final 4-minute block of the paradigm had the same number of drug and control cues. Cues differing on the route of use were also balanced for those blocks (e.g. same number of pipes vs needles cues). Presentation was followed by an inter-trial interval

(ITI), consisting of a black cross on a white background (**Figure 1A**). ITIs were jittered for 8–12 s (i.e., 13-17 s between each cue presentation) to differentiate SCRs to consecutive cues (6) because peak skin conductance responding is typically within 10 s of cue onset (25) (**Figure 1B**).

Measure of subjective response: Participants were prompted to rate their craving for methamphetamine verbally immediately before, in the middle, and immediately after the task. Craving were rated on a 0-10 Likert scale adapted from the Within Session Rating Scale (26), with 0 = “no craving at all”, and 10 = “extreme craving”. To assess the change across the task, craving index was calculated as craving after the task minus craving before the task, with a positive score indicating an increase and a negative score indicating a decrease.

Measure of physiological response: Skin conductance response (SCR) was collected throughout the cue reactivity paradigm using a galvanic skin response amplifier (ADInstruments, Milford, MA). Two silver/silver chloride (Ag-AgCl) contoured finger magnetic resonance safe electrodes were strapped with a Velcro strap to the palmar surfaces of the index and middle fingers of the non-dominant hand, as described elsewhere (27). SCR was acquired at a sampling rate of 1 kHz/s using PowerLab software (ADInstruments) and analyzed offline using MATLAB (script available upon request). Maximum amplitude (highest reading – lowest reading) for each trial in a 10 s window following cue onset was analyzed. SCR has a post stimulus-onset latency period lasting between 1 and 3 s, and peaks well within 10 s (25). Example data from a trial are shown in **Figure 1B**. Maximum amplitude was considered cue-related if it was greater or equal to 0.02 μ S (6), with any lower value coded as zero for that trial in as reported in other studies (28). Maximum amplitudes were square root transformed to normalize the distribution (6). Maximum amplitudes were blocked into first 4 minutes (early), middle 4 minutes (middle), and last 4 minutes (late) as cues were balanced for those blocks as described above. All

analyses were performed on the differential SCR (Δ SCR) scores (29,30), which were calculated as methamphetamine-related cue response minus food-related cue response. Therefore, a positive score indicates reactivity towards methamphetamine-related cues, whereas a negative score indicates reactivity towards food-related cues. To assess the change in response across the task, Δ SCR index was calculated as Δ SCR in the late block minus Δ SCR in the early block, with a positive score indicating an increase and a negative score indicating a decrease.

Statistical analyses and sample size calculation

All statistical analyses were performed using IBM SPSS Statistics 26 (IBM Corp., NY, USA). Independent t-tests and Chi-square analyses with Fisher's exact test were used to compare methamphetamine use disorder and control groups for all demographics and drug use characteristics. Cognitive tasks, self-reported craving and SCR were analyzed using repeated measure analysis of variance (ANOVA) or analysis of covariance (ANCOVA). Self-report analyses were conducted on craving at three time points (before, in the middle, and after the task). SCR analyses were conducted on response in three blocks (early, middle, and late). Response to picture and video cues were combined in the analysis. Following significant interactions, post-hoc independent t-tests with Bonferroni corrections were conducted. Associations between methamphetamine cue reactivity, drug use characteristics (total years of methamphetamine use and ASSIST score for amphetamines and other drugs), and cognitive performance were also examined using Pearson's correlation analyses, only in people with methamphetamine use disorder. For this, Bonferroni correction was employed for each measure ($p < 0.0125$) to account for multiple comparisons as recommended (62,63). It should be noted that Bonferroni correction should be for multiple tests per measure, and not for the whole study (62,63).

Power calculations were conducted in G*Power 3.1 (31), using a type 1 error rate (α) = 0.05, power ($1 - \beta$) = 0.80, and large effect size ($f = 0.40$; based on pilot data), which

recommended a total sample size of 52 (26 in each group). Calculations were based on ANCOVA as it requires the most power.

RESULTS

Demographic characteristics, drug use parameters, and cognitive performance

Demographic characteristics, drug use parameters, and cognitive performance of people with methamphetamine use disorder and controls are described in **Table 3**. Some co-morbidities with other substances were observed amongst the methamphetamine use disorder group, nevertheless, methamphetamine was their primary substance of use.

There were no demographic differences between groups, except people who use methamphetamine reported fewer years of education than controls ($p < 0.001$). Therefore, education years were used as covariate for all subsequent group comparisons. Controls performed better than the methamphetamine group in all cognitive tests (**Table 3**). Controlling for education years did not change any outcomes except for performance in the TMT-A, which was significant with ANOVA but not with ANCOVA.

Cue reactivity

We report ANOVA values here because controlling for education did not change any between-subjects outcomes for the cue reactivity data (education as covariate: $F = 0.439$; $p = 0.510$ for subjective reactivity, and $F = 0.473$; $p = 0.494$ for physiological reactivity).

Self-reported craving. Two-way ANOVA [within-subjects factor: Block (before, middle, and after); between-subjects factor: Group (controls vs. methamphetamine)] revealed main effect of Group [$F(1,58) = 62.810$, $p < 0.001$], showing significantly higher average craving levels in people with methamphetamine use disorder compared to controls who reported no craving at any time point. Block [$F(2, 116) = 8.689$, $p < 0.001$], and Block x Group interaction [$F(2, 116) = 8.689$, $p < 0.001$] were also significant (**Figure 2A**). Block x Group interaction was driven by a linear increase in craving in the methamphetamine group over time [$F(1,29) = 9.436$, $p = 0.005$], whereas self-reported craving remained the same across blocks in the

control group. Consistent with these results, subjective rating index was also significantly higher in the methamphetamine group compared to controls [$F(1,58) = 9.436, p = 0.003$] (**Figure 2B**).

Differential skin conductance response (ΔSCR). ΔSCR was calculated as response to methamphetamine cues minus response to food cues. Therefore, a positive score indicates reactivity towards methamphetamine cues, whereas a negative score indicates reactivity towards food cues. Three-way ANOVA [within-subjects factors: Cue Modality (pictures vs. videos) and Block (early, middle, and late); between-subjects factor: Group (controls vs. methamphetamine)] revealed no effect of Group [$F < 1; p > 0.05$], indicating that on average, ΔSCR was not different in people with methamphetamine use disorder compared to controls. However, there were significant interactions between Group x Block [$F(2, 116) = 4.609, p = 0.012$], and Group x Cue Modality [$F(1, 58) = 4.727, p = 0.034$]. These results indicate that methamphetamine and control groups showed significantly different ΔSCR depending on when SCR was assessed (**Figure 3A**) and the modality of cue (picture vs video) presented (**Figure 3B**). Post hoc tests showed that Group x Block interaction was driven by a linear decrease in response over time in people with methamphetamine use disorder [$F(1,29) = 6.817, p = 0.014$], while there was no change in controls [$F < 1; p > 0.05$]. Post hoc tests also showed that Group x Cue Modality interaction was driven by significantly higher average response to picture cues in people with methamphetamine use disorder compared to controls [$F(1, 58) = 5.216, p = 0.026$] (**Figure 3B**). There was no difference between groups when presented with video cues [$F < 1$]. This suggests that people who use methamphetamine overall showed more differential response to methamphetamine-related pictures than controls.

Further, ΔSCR index was lower in the methamphetamine compared to control group [$F(1, 58) = 6.484, p = 0.014$], showing decrease in ΔSCR over time in methamphetamine compared to control (**Figure 3C**).

Factors associated with cue reactivity

Results so far evidence the idea that methamphetamine cue reactivity is due to Pavlovian conditioning (i.e., dependent on and specific to methamphetamine use). To understand potential factors modulating cue reactivity in people with methamphetamine use disorder, we conducted exploratory analyses on average response and indices, both subjective and physiological.

Drug use characteristics. Pearson's correlation analyses revealed a positive correlation between the ASSIST score for sedatives use and average subjective craving ($r = 0.521$, $p = 0.003$), suggesting that people who reported using more sedatives also reported more overall craving for methamphetamine (**Figure 4A; Table 4**). Notably, cue reactivity was not driven by cannabis use (**Table 4**). There was also a positive correlation between total years of methamphetamine use and average Δ SCR ($r = 0.379$; $p = 0.039$), which was no longer significant after correcting for multiple comparisons (**Figure 4B; Table 4**). There were no other significant correlations.

Independent-samples t-tests revealed no differences in either subjective or physiological response between treatment seeking and non-treatment participants (lowest $p = 0.319$). People who reported using methamphetamine intravenously displayed significantly higher average Δ SCR compared to smokers [$t(27) = 3.750$; $p < 0.001$] (**Figure 4C**). There were no other significant differences (lowest $p = 0.780$).

Cognitive performance. There was a negative correlation between subjective response index and Stroop Inhibition performance ($r = -0.494$, $p = 0.006$), suggesting that people with poorer inhibitory control were more likely to report increased craving for methamphetamine at the end of the cue reactivity task (**Figure 4D; Table 5**). There were no other significant correlations.

DISCUSSION

In the present study, methamphetamine-related cues elicited subjective and physiological reactivity specifically in people with a current diagnosis of stimulant use disorder, methamphetamine-type. Positive associations between physiological reactivity to methamphetamine cues and total years of methamphetamine use, and between sedative use and craving were also observed. People who use methamphetamine intravenously displayed higher physiological reactivity than people who smoke methamphetamine. Lastly, there was a negative association between performance on the Stroop task and change in craving before and after the task.

Consistent with previous studies (10,33,34), people with methamphetamine use disorder reported higher levels of subjective craving for methamphetamine at all time points compared to controls. In addition, increased Δ SCR levels were only observed in people with methamphetamine use disorder and not in controls. Previous studies in the field lacked control cues and/or control participants (Table 1). Therefore, this study provides comprehensive evidence that people who currently use methamphetamine show Pavlovian conditioning reflex to cues that can be measured by physiological cue reactivity. Further, this study shows that food cues serve as good control cues against methamphetamine cues with control participants showing average Δ SCR levels close to zero, indicating similar objective reactivity to food and methamphetamine cues. We chose food cues because previous studies have used either nature (i.e. landscapes) or sexual control cues (reviewed in (9)), which may either be too relaxing or too arousing even for control participants.

Notably, elevated Δ SCR in people with methamphetamine use disorder was limited to picture cues in our study. No group differences were observed in response to video cues. This finding is in contrast with a previous study reporting that the two modalities of cues elicit similar responses (38), however, control cues were not included in that study. It may be that videos of all types are similarly stimulating, regardless of drug use status.

Across the task, Δ SCR linearly decreased in the methamphetamine group. While this is consistent with a previous study (37), no control cues were used in that study to rule out habituation. While SCR can decrease over time in a non-associative process of habituation (6,25), this is unlikely to be the case in the present study because habituation would occur for both methamphetamine- and food-related cues, and in control participants. It is possible that repeated presentation of methamphetamine-related cues led to extinction of cue reactivity in physiological measurement but not in subjective measurement. This is interesting because repeated exposure to methamphetamine-related cues may be protective against relapse. For example, a study showed that all participants with methamphetamine use disorder, most of whom used methamphetamine daily or weekly before the study, reported abstinence from methamphetamine use for at least 2 weeks following the completion of a cue reactivity session (36).

While predictors of drug-related cue reactivity have been thoroughly investigated for alcohol and other drugs (16) due to their importance in clinical outcomes (4,9,10,11), predictors of methamphetamine-related cue reactivity are poorly understood. A previous study found no correlation between demographic characteristics, treatment status, or recent methamphetamine use and subjective reactivity (38). In this study, we explored a range of potential factors, and recognize that any correlation discussed in this report does not indicate causation. We found that Δ SCR was on average higher in people who used methamphetamine intravenously compared to those who smoked methamphetamine. This may indicate a dose-related effect because injecting methamphetamine is associated with higher bioavailability of the drug compared to smoking (43). Such dose-related effect further strengthens that Δ SCR observed in people with methamphetamine use disorder in the present study is due to Pavlovian conditioning. Importantly, intravenous use is associated with repeated relapses and poor treatment outcome (44). It appears that people who use methamphetamine intravenously are more sensitive to methamphetamine-related cues than

people who smoke methamphetamine, which may result in poorer response to treatment. It should be noted that there were no other differences between people who intravenously use vs smoke methamphetamine in cognition or other drug use characteristics in this study.

We also observed a positive correlation between total years of methamphetamine use and Δ SCR response to methamphetamine cues, suggesting that the longer the use, the stronger the physiological cue reactivity. This is the first evidence of an association between length of use and physiological response to methamphetamine cues. Other drugs have shown similar findings (16). Together they highlight the potential utility of physiological cue reactivity that is over and above control cues to understand drug use history. Notably, a recent study found that when presented with amphetamine-related video cues, a longer history of amphetamine use resulted in greater activation the prefrontal cortex (PFC) in people with severe amphetamine dependence (45). PFC activation is involved in drug-cue processing (3,16), and in light of present findings, longer use of drugs may cause neuroadaptations in this brain region to result in greater physiological cue reactivity. Importantly, correlation is not causation, hence it is also possible that greater physiological cue reactivity may lead to longer duration of methamphetamine use. The significant correlation between Δ SCR and total years of use did not survive correction for multiple comparisons and should be replicated in a larger sample.

A surprising finding was the strong association between sedative use severity and average subjective craving. People who use methamphetamine have reported to also use sedatives such as gamma-hydroxybutyric acid (GHB) and benzodiazepines to manage stimulant withdrawal symptoms (46,47). People who use who use more sedatives were possibly attempting to manage heightened withdrawal symptoms and were likely to report withdrawal-related craving for methamphetamine. This is likely considering that subjective craving levels were high right before the cue reactivity task (**Figure 2A**).

Extending previous literature (reviewed in (49,50)), we found that people with methamphetamine use disorder displayed cognitive deficits in a range of domains compared to controls. Notably, response inhibition (measured by the Stroop task) was one of the most impaired domains. The increase in craving following the cue reactivity task as indicated by positive craving index was also associated with poorer performance in the Stroop task. This finding is consistent with a previous study that used a different measure of inhibitory control, which found that number of response errors in a Go-No Go task positively correlated with subjective craving for methamphetamine at baseline and after cue exposure (33). Another study reported that people with methamphetamine use disorder who performed better in a Stop-Signal task also reported lower craving for methamphetamine (51), though this was not in response to cues. Our results support the impaired Response Inhibition and Salience Attribution model of addiction, which proposes that response inhibition deficit contributes to the symptomology of addiction, including craving for the drug (52). It is also possible that heightened craving may interfere with inhibitory control. There are currently no approved pharmacotherapy to treat craving for methamphetamine. Given craving can be elicited by methamphetamine-related cues, reducing cue reactivity with medication may be a promising avenue to reduce methamphetamine use. In fact, preliminary evidence from methamphetamine cue reactivity studies suggest a beneficial role for bupropion or naltrexone compared to placebo in reducing cue-induced craving (7,54).

This study is not without limitations. Cues used in this paradigm were all visual. While virtual reality has proven to be a promising avenue to investigate cue reactivity in methamphetamine use disorder (35,41), research in other psychostimulants suggests that tactile cues such as paraphernalia may be more salient (55), and multi-sensory cues may be more ecologically valid (56). Secondly, due to the cross-sectional nature of the present study, we could not assess the link between cue reactivity and future methamphetamine use, or determine the directionality of the significant correlations. Future studies should aim

to follow-up participants at several time points and re-assess methamphetamine use parameters. Lastly, our sample was exclusively recruited from metropolitan Melbourne, Australia, and findings in the present study may not be reliably extrapolated to other populations.

In conclusion, we have established a cue reactivity paradigm that can elicit methamphetamine cue-specific objective responses in people with methamphetamine use disorder and not in controls. Our paradigm showed that cue reactivity in people who use methamphetamine is due to Pavlovian conditioning from methamphetamine-taking experience. Subjective and objective reactivity were significantly associated with mode and length of use, use of other drugs, and deficits in response inhibition. These results highlight the utility of cue reactivity in understanding methamphetamine use disorder, which may help in the development of novel therapeutics to address the global methamphetamine crisis.

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TABLES

Table 1. Summary of the design of methamphetamine cue reactivity studies, listed in the chronological order of publication. Studies that measured but did not report any cue reactivity analyses are not included in this table. *indicate studies with control participants and cues. Ticks (✓) indicate the presence of the feature, whereas crosses (X) indicate the absence of the feature.

Study	Participants		Cues		Measure		Notes
	Control (n)	Meth-amphetamine (n)	Control	Meth-amphetamine	Objective	Subjective	
Newton et al., 2006 (54)	X	20	X	✓	X	✓	
Newton et al., 2008 (57)	X	16	✓	✓	✓	✓	
DeSantis et al., 2009 (36)	X	25	X	✓	✓	✓	
Price et al., 2010 (37)	X	24	X	✓	✓	✓	
Tolliver et al., 2010 (38)	X	43	X	✓	✓	✓	
Culberston et al., 2010 (41)	X	17	✓	✓	✓	✓	
Ekhtiari et al., 2010 (58)	X	50	X	✓	X	✓	
Saladin et al., 2012 (39)	X	40	X	✓	X	✓	
Tolliver et al., 2012* (33)	30	30	✓	✓	✓	✓	Physiological findings unreported.
Li et al., 2013* (59)	8	10	✓	✓	X	✓	
Wang et al., 2013 (12)	X	139	✓	✓	✓	✓	
Shahbabaie et al., 2014 (60)	X	32	✓	✓	X	✓	
Lopez et al., 2015 (40)	X	21	✓	✓	X	✓	
Ray et al., 2015 (7)	X	30	✓	✓	X	✓	
Courtney et al., 2016 (61)	X	30	✓	✓	✓	✓	
Malcolm et al., 2016* (34)	9	9	✓	✓	X	✓	
Shahmohammadi et al., 2016* (62)	10	10	✓	✓	✓	X	Comparison between control and methamphetamine cues unreported.
Liu et al., 2017 (63)	X	50	X	✓	X	✓	
Roche et al., 2017 (64)	X	30	✓	✓	X	✓	
Su et al., 2017 (65)	X	30	X	✓	X	✓	
Huang et al., 2018* (10)	28	27	✓	✓	✓	✓	All participants were abstinent for > 16 months
Wang et al., 2018 (35)	45	61	X	✓	✓	✓	
Dean et al., 2019 (66)	X	42	✓	✓	✓	✓	

Running head: Methamphetamine cue reactivity

Liang et al., 2019 (67)	X	52	X	✓	X	✓	
Tan et al., 2019 (42)	X	60	✓	✓	✓	✓	
Chen et al., 2020* (11)	47	99	✓	✓	✓	X	All participants were abstinent for > 1 month
Su et al., 2020 (68)	X	126	X	✓	X	✓	

Table 2. Cognitive task battery description.

Task	Domain	Task Description	Performance index
Trail Making Part A (TMT-A)	Visual attention (69)	Participants are required to connect circles numbered from 1 to 25 in numerical order.	Inverse score of the time to completion was calculated as $100/(\text{raw performance score})$. Thus higher index = better performance.
Trail Making Part B (TMT-B)	Task switching (69)	Circles either have a number (1 to 13) or a letter (A to L), and participants are required to connect circles while alternating between numbers and letters in alphanumeric order.	Inverse score of the time to completion was calculated as $100/(\text{raw performance score})$. Thus higher index = better performance.
Symbol Coding	Attention and speed of information processing (70)	Participants are required to match numbers to abstract symbols using a key guide.	Number of correct responses produced in 90 seconds. Ranges from 0-110
Spatial Span	Visual memory span and visual working (19)	Participants are required to repeat sequences pointed out by the experimenter on a 10-cube array, first forward and then backward.	Number of correct responses. Ranges from 0-32.
Digit Span	Verbal memory span and verbal working memory (19)	Participants are required to repeat sequences of digits read out by the experimenter, first forward and then backward.	Number of correct responses. Ranges from 0-32.
Word-Color Stroop Task (Inhibition)	Selective attention, processing speed and inhibitory control (71)	It consists of two trials. In the first trial (baseline), participants are presented with colored square and are instructed to name the color of the squares as fast as they can. In the second trial (inhibition) participants are required to suppress a habitual response to read the words and instead name the ink color of incongruently colored words.	The raw score is calculated as the time taken to complete the inhibition trial minus the time taken to complete the baseline trial. The main performance index is the inverse scores, calculated as $100/(\text{raw performance score} + 1)$. The final score ranges from 0 to 100.

Word-Color Stroop Task (Switching)	Selective attention, processing speed, inhibitory control, and cognitive flexibility (71)	It consists of two trials. In the first trial (baseline), participants are presented with colored square and are instructed to name the color of the squares as fast as they can. In the second trial (switching), participants are presented with two different rules and must switch between the rules.	The raw score is calculated as the time taken to complete the switching trial minus the time taken to complete the baseline trial. The main performance index is the inverse scores, calculated as $100/(\text{raw performance score} + 1)$. The final score ranges from 0 to 100.
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Table 3. Demographic characteristics, drug use characteristics, and cognitive performance. *indicates a dependent variable with a significant group difference. ^aDigit Span data are missing for four people in the methamphetamine group who declined taking the test, and ^bStroop (Inhibition and Switching) data are missing for one person in the methamphetamine group who reported being colorblind and unable to complete the task. [&]indicate a dependent variable with a significant group difference before covariate analysis.

	Methamphetamine (n = 30)	Control (n = 30)	Test; p-value
Demographic:			
Age – years (±SD)	33.70 (±7.27)	30.67 (±5.05)	t=-1.876; p=0.066
Females – no. (%)	12 (40)	15 (50)	$\chi^2=0.606$; p=0.436
Premorbid IQ (±SD)	103.47 (±10.36)	108.67 (±8.75)	t=1.922; p=0.060
Ethnicity – no. (%)			
Caucasian	24 (80)	22 (73)	
Asian	4 (13.3)	8 (27)	$\chi^2=3.905$;
Aboriginal/Torres Strait Islander	1 (3.3)	0 (0)	p=0.419
Pacific Islander	1 (3.3)	0 (0)	
Education years (±SD)*	13.22 (±3.02)	16.46 (±2.60)	t=4.887; p<0.001
AUDIT score (±SD)	5.67 (±5.80)	4.04 (±3.25)	t=-1.346; p=0.184
Drug Use:			
Total years of use (±SD)	11.03 (±6.49)	-	-
ASSIST Amphetamine score (±SD)	31.70 (±5.80)	0 (±0)	-
Treatment seeking – no (%)	12 (40)	-	-
Main mode of meth use – no (%)			
Smoking	16 (53.3)		
I.V.	13 (43.3)	-	-
Intranasal	1 (3.3)		

Cannabis Use Disorder – no (%)	6 (20)	-	-
Tobacco Use Disorder – no (%)	6 (20)	-	-
Sedative Use Disorder – no (%)	1 (3.3)	-	-
Opioid Use Disorder – no (%)	1 (3.3)	-	-
Cognitive Performance:			
TMT-A ($\pm$ SD) ^{&}	3.15 ($\pm$ 0.98)	4.31 ($\pm$ 1.50)	F=3.366; p=0.072
TMT-B ($\pm$ SD) [*]	1.48 ($\pm$ 0.48)	1.99 ($\pm$ 0.59)	F=5.010; p=0.029
Symbol Coding ($\pm$ SD) [*]	48.83 ($\pm$ 10.64)	64.90 ($\pm$ 11.24)	F=16.59; p<0.001
Spatial Span ($\pm$ SD) [*]	16.2 ($\pm$ 2.89)	18.93 ($\pm$ 3.62)	F=6.408; p=0.014
Digit Span ($\pm$ SD) ^{*,a}	15.38 ($\pm$ 3.38)	18.52 ($\pm$ 3.91)	F=6.114; p=0.017
Stroop Inhibition ($\pm$ SD) ^{*,b}	11.01 ($\pm$ 5.56)	19.49 ($\pm$ 17.97)	F=10.384 p=0.002
Stroop Switching ($\pm$ SD) ^{*,b}	2.51 ($\pm$ 1.43)	4.00 ($\pm$ 1.32)	F=12.499; p=0.001

Table 4: Pearson's correlation analyses between index and average response to methamphetamine-related cues, and drug use characteristics in people with methamphetamine use disorder. ASSIST = Alcohol Smoking and Substance Involvement Screening Test; AUDIT = Alcohol Use Disorder Identification Test. Values in *italics* are significant at p = 0.05. Values in **bold** are significant at p = 0.0125 (Bonferroni corrected).

Drug Use Characteristics	Average Subjective Rating		Subjective Rating Index		Average Δ SCR		Δ SCR Index	
	r	p	r	p	r	p	r	p
Total Years of Use	0.215	0.254	-0.061	0.749	0.379	0.039	0.162	0.392
ASSIST Tobacco	0.099	0.603	-0.149	0.432	0.042	0.825	-0.221	0.241
ASSIST Alcohol	-0.092	0.628	-0.171	0.366	-0.019	0.920	-0.126	0.506
ASSIST Cannabis	0.064	0.735	0.214	0.255	-0.045	0.813	0.110	0.564
ASSIST Cocaine	-0.087	0.646	-0.028	0.883	0.195	0.302	0.037	0.846
ASSIST Amphetamine	-0.043	0.822	0.068	0.722	-0.119	0.530	-0.149	0.433
ASSIST Inhalants	0.058	0.759	0.187	0.324	0.196	0.298	-0.137	0.470

ASSIST Sedatives	0.521	0.003	0.196	0.298	0.179	0.343	-0.109	0.566
ASSIST Hallucinogens	-0.001	0.995	0.062	0.744	-0.091	0.633	0.088	0.645
ASSIST Opioids	0.292	0.117	-0.056	0.768	0.202	0.283	-0.295	0.114
AUDIT	-0.047	0.804	-0.069	0.716	0.227	0.228	-0.237	0.208

Table 5: Pearson's correlation analyses between index and average response to methamphetamine-related cues, and cognitive performance in people with methamphetamine use disorder. TMT-A and TMT-B = Trail Making Task part A and part B. Values in **bold** are significant at $p = 0.0125$ (Bonferroni corrected)

Cognitive Test	Average Subjective Rating		Subjective Rating Index		Average Δ SCR		Δ SCR Index	
	r	p	r	p	r	p	r	p
TMT-A	-0.36	0.051	0.074	0.697	0.014	0.943	0.167	0.377
TMT-B	-0.16	0.398	0.031	0.872	-0.016	0.934	0.057	0.764
Symbol Coding	-0.255	0.174	0.094	0.62	-0.183	0.332	-0.042	0.828
Spatial Span	-0.292	0.118	0.012	0.951	-0.138	0.466	-0.035	0.853
Digit Span	-0.047	0.818	-0.294	0.145	0.142	0.490	-0.160	0.434
Stroop Inhibition	-0.089	0.645	-0.494	0.006	0.095	0.623	0.308	0.104
Stroop Switching	-0.077	0.697	-0.349	0.069	-0.148	0.453	0.113	0.567

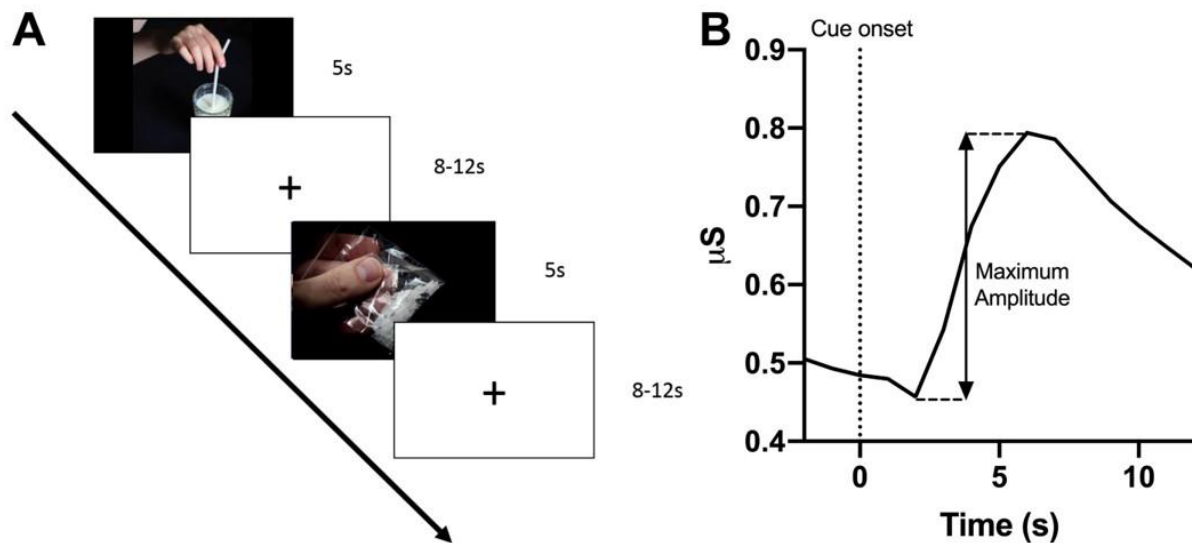


Figure 1. Schematic of the cue reactivity paradigm and typical skin conductance response. **A.** Cues were presented for 5 s each, with an inter-trial interval (ITI) jittered between 8-12 s. Methamphetamine and control cues were presented in a pseudorandomized fashion. **B.** An example of skin conductance response (SCR) to a cue trial from a participant. SCR peaks within 10 s of cue onset, before returning to baseline. Maximum amplitude (highest reading – lowest reading) for each trial in a 10 s window following cue onset was measured. Maximum amplitudes were then square root transformed to normalize the distribution for analyses (7)

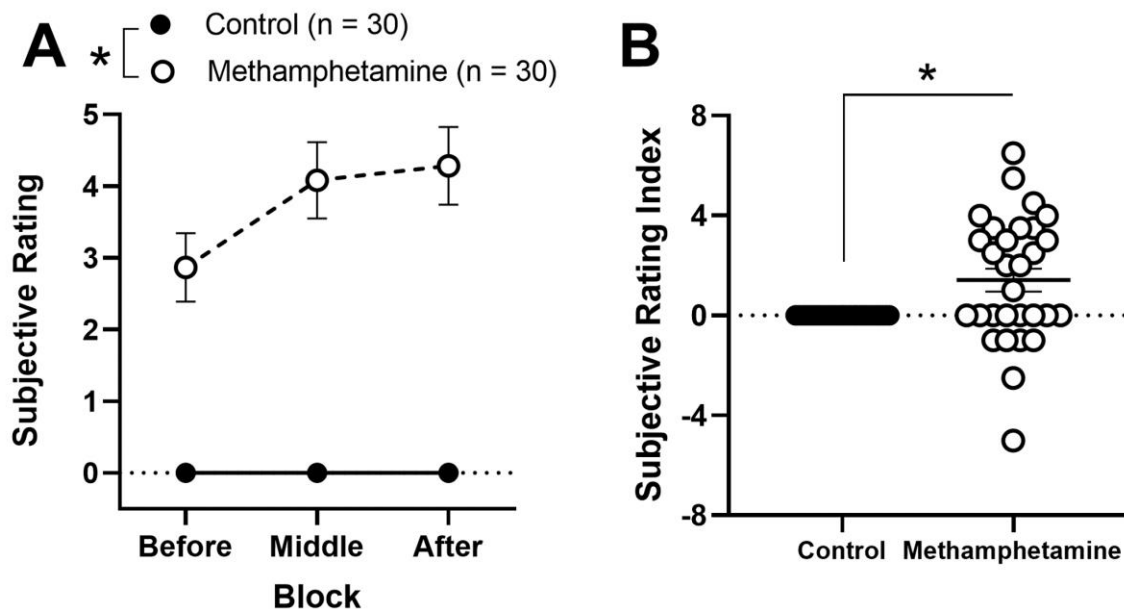


Figure 2. Self-reported subjective craving between people with methamphetamine use disorder and controls. **A. Change in rating over time.** Self-reported craving was significantly higher in people with methamphetamine use disorder on average across blocks (*p < 0.001). **B. Subjective rating index.** Overall, the index was significantly higher in people with methamphetamine use disorder compared to controls (*p = 0.003). Data either represented as mean $\pm$ SEM or individual values. Methamphetamine = people with methamphetamine use disorder; SEM = standard error of the mean.

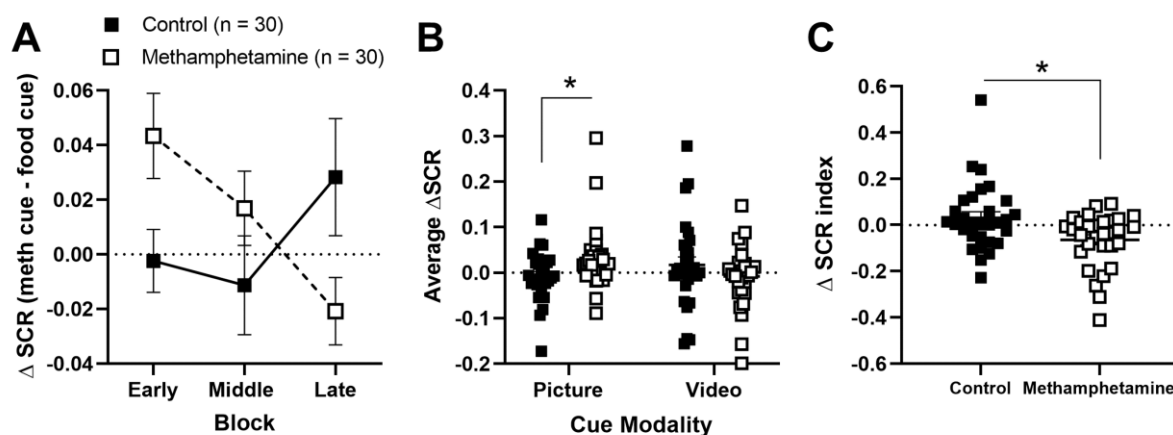


Figure 3. ΔSCR between people with methamphetamine use disorder and controls. A. ΔSCR across cue reactivity task. There were significant interactions between Group and Cue Modality, and Group and Block. **B. Average ΔSCR for each cue modality.** On average, people with methamphetamine use disorder showed higher ΔSCR to picture cues compared to controls (* $p = 0.026$). ΔSCR did not differ between groups for video cues. **C. ΔSCR index.** ΔSCR index was significantly lower in people with methamphetamine use disorder compared to controls (* $p = 0.008$). ΔSCR = differential skin conductance response calculated as response to methamphetamine cues minus response to food cues. Data either represented as mean $\pm$ SEM, or as individual values. Methamphetamine = people with methamphetamine use disorder; SEM = standard error of the mean.

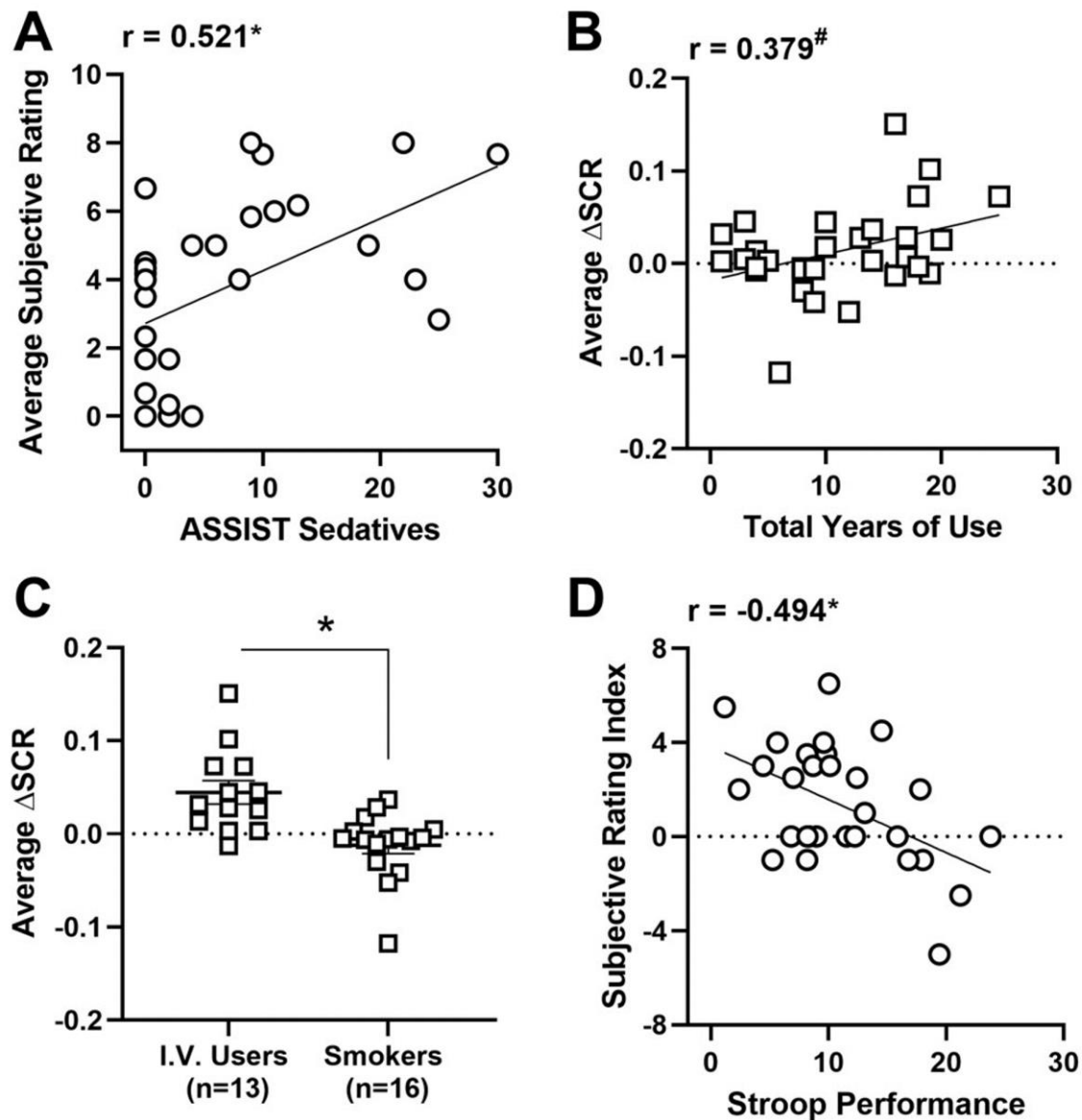


Figure 4. Exploratory analysis of factors modulating cue reactivity. A. Sedatives use. There was a strong positive association between severity of sedative use (as measured by the ASSIST) and subjective craving ($*p = 0.003$). **B. Total years of use.** There was a positive association between total years of methamphetamine use and Δ SCR ($\#p = 0.039$), which did not survive correction for multiple comparisons. **C. Mode of use.** People who predominantly injected methamphetamine displayed higher average Δ SCR than those who predominantly smoked methamphetamine ($*p < 0.001$). One participant reported using methamphetamine exclusively intranasally and was excluded from this analysis. **D. Stroop performance.** There was a strong negative association between performance in the Stroop Task (Inhibition) and subjective craving index ($*p = 0.006$). ASSIST = Alcohol, Smoking, and Substances Identification Test; Δ SCR = differential skin conductance response calculated as response to methamphetamine cues minus response to food cues. I.V. = intravenous. All data represented as individual values.

Chapter 3. Psychiatric and cognitive factors associated with age of onset of methamphetamine use in people with methamphetamine use disorder

3.1. Introduction

Methamphetamine use typically begins early in life, with 0.2% of young people aged 12-25 reporting using methamphetamine in the United States (SAMHSA, 2015). This is a major concern as substance use in adolescence may predict the severity of addiction later in life (Brown et al., 2009; Chen et al., 2009; Dawson et al., 2008; Grant and Dawson, 1998; King et al., 2007; Robins and Przybeck, 1985). In addition, people who have started using drugs in adolescence are typically more resistant to treatment interventions (Catalano et al., 1991; Perepletchikova et al., 2008). Psychiatric disorders and cognitive deficits are also associated with adolescent methamphetamine use (Kuitunen-Paul et al., 2020; Lyoo et al., 2015; Russell et al., 2008).

Similar to their adult counterparts, adolescents who use methamphetamine display a high rate of psychiatric symptoms (Kuitunen-Paul et al., 2020; Nutt et al., 2010; Russell et al., 2008). Notably, people who started using methamphetamine earlier in life are more likely to develop a formal psychotic disorder (Chen et al., 2003; Hides et al., 2015), and are at higher risk of mood and anxiety disorders (Akindipe et al., 2014; Rawson et al., 2005). In addition, antisocial behaviors and conduct disorders are significantly associated with methamphetamine use in young people (Embry et al., 2009; Russell et al., 2008). This is particularly important as such behaviors are precursors to antisocial personality disorder (ASPD) (American Psychiatric Association, 2013), which can be present long before the onset of methamphetamine use (Kramer et al., 2009).

People with methamphetamine use disorder also display deficits of medium effect size in cognitive abilities (Guerin et al., 2019; Potvin et al., 2018). Notably, executive

functions, such as inhibitory and impulse control are most consistently impaired in adolescents who use methamphetamine (Kim et al., 2016; King et al., 2010; Lyoo et al., 2015). This suggests that poor inhibitory control may be a predisposing factor to developing methamphetamine use disorder early in life. Further, poor impulse control is associated with treatment non-completion and relapse in this population (Pattij and De Vries, 2013; Winhusen et al., 2013).

Therefore, the aim of this chapter was to investigate the association between comorbid psychiatric symptoms, performance in cognitive tasks, and age of onset of methamphetamine use. Based on previous literature (Embry et al., 2009; Kramer et al., 2009), I hypothesized that a diagnosis of antisocial personality disorder would be associated with an earlier age of onset of methamphetamine use. While evidence suggests an association between methamphetamine use, depression and suicidality (McKetin et al., 2019), no studies have investigated their association with age of onset, making it challenging to hypothesize specific outcomes. In terms of cognitive factors, I hypothesized that inhibitory and impulse control deficits would be associated with age of onset of methamphetamine use based on the few studies to date in adolescents who use methamphetamine (Kim et al., 2016; King et al., 2010; Lyoo et al., 2015).

3.2. Methods

3.2.1. Participants

Forty people with methamphetamine use disorder were recruited between March 2018 and March 2020, through referrals at the Department of Addiction Medicine at St Vincent's Hospital Melbourne, and from local community groups in Melbourne metropolitan area. All participants provided written informed consent before any assessment. They were included in this study if they were 1) aged 18 to 50 years-old at the time of testing; and 2) met DSM-5 criteria for stimulant use disorder, methamphetamine-type (moderate or severe) in the past six months as assessed by the MINI International Neuropsychiatric Interview for the DSM-5

(M.I.N.I 7.0.2.). They were excluded if they 1) had a major neurological or medical illness; 2) a history of substance use disorder where methamphetamine was not the primary substance of abuse; or 3) if they were not able to provide informed consent (i.e. judged to be unable to provide informed consent due to intoxication, and if they had an IQ < 70). The IQ cut-off was determined based on the DSM-5 criteria for intellectual disability (i.e. IQ < 70), to ensure that participants were capable of understanding study instructions. To confirm recent drug use, participants were required to provide a urine sample for qualitative urinalysis (Pathtech, Melbourne, Australia), to test for amphetamines, cocaine, methamphetamine, opiates, benzodiazepines, and cannabis, as described in Chapter 2.

3.2.2. Study design and procedure

Participants were assessed individually in a single three-hour session at the Department of Addiction Medicine at St Vincent's Hospital Melbourne, Fitzroy, Australia. Participants were administered a clinical interview, a series of questionnaires, a cognitive task battery, and a cue reactivity task by a trained assessor (AAG). They were reimbursed with an \$AU75 gift card for their time and travel. Due to the length of the session, participants were provided with two five-minute breaks: one after the clinical interview, and one after the cognitive task battery. All testing was performed in accordance with the Declaration of Helsinki (World Medical Association, 2013) and received ethical approval from St Vincent's Hospital Melbourne Human Research Ethics Committee (HREC/17/SVHM/169).

3.2.3. Measures

3.2.3.1. Background measures

Demographic questionnaire: The demographics questionnaire was developed by the research team and collected information on key demographic and drug use characteristics (age, sex, ethnicity, education level, age of onset and total years of methamphetamine use).

Wechsler Test of Adult Reading (WTAR): as described in Chapter 2. In brief, the WTAR

assesses premorbid verbal IQ (Wechsler, 1997).

Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): as described in Chapter 2. In brief, the ASSIST assesses severity, frequency, and high-risk substance use (WHO ASSIST Working Group, 2010). It also collects information about mode of drug use.

Alcohol Use Disorder Identification Test (AUDIT): as described in Chapter 2. In brief, the AUDIT assesses excessive drinking (Saunders et al., 1993).

3.2.3.2. Psychiatric measures

MINI International Neuropsychiatric Interview (MINI): The M.I.N.I English Version 7.0.2 for the DSM-5 (Sheehan et al., 1998) was administered to all participants to 1) confirm stimulant use disorder, methamphetamine-type diagnosis, and 2) assess neuropsychiatric comorbidities. Neuropsychiatric disorders were grouped into nine clusters according to DSM-5 guidelines: Antisocial Personality Disorder, Anxiety Disorders, Bipolar and Related Disorders, Depressive Disorders, Eating Disorders, Obsessive-Compulsive Disorders, Post-traumatic Stress Disorder, Psychotic Disorders, and Suicidal Behavior Disorders.

Positive and Negative Syndrome Scale (PANSS): assesses symptomatology in psychiatric populations (Kay et al., 1987). The PANSS scale consists of three separate subscales; the Positive Scale, the Negative Scale, and the General Scale. The Positive Scale is used to measure productive symptoms / excess or distortion of normal functions (e.g., delusions, hallucinations and thought disorder), the Negative Scale is used to measure deficit features / a diminution or loss of normal functions (e.g., blunted affect, social withdrawal), and the General Scale is used to measure general psychopathology symptoms (e.g., depression, anxiety). The PANSS includes 30 items, each rated from 1 (absent) to 7 (extreme). PANSS Total score therefore ranges from a minimum of 30, to a maximum of 210.

Childhood Trauma Questionnaire (CTQ): assesses early life adversity. The CTQ consists of 28 items assessing five dimensions of childhood trauma: physical, sexual, and emotional abuse, as well as physical and emotional neglect. The different types of trauma were

analyzed separately, and as a Total CTQ score. Note that the CTQ was added to the study in January 2019, and therefore not all participants completed this assessment.

3.2.3.3. Cognitive measures

Cognitive task battery: All participants were administered a brief cognitive task battery, adapted from the MATRICS Consensus Cognitive Battery (Kern et al., 2008) and the Delis-Kaplan Executive Function System (Delis et al., 2001), as described in Chapter 2. In brief, the following tasks were administered: Trial Making Part A and Part B (TMT-A, TMT-B), the Symbol Coding task, the Spatial and Digit Span tasks, and the Word-Color Stroop Task. In addition, a Verbal Fluency task was administered, assessing semantic and phonological fluency (Lezak et al., 2012). The semantic fluency test requires participants to list as many animal names as possible, while the phonological test requires them to list as many words starting with the letters F, A, and S. The performance index was the number of correct words produced in one minute. A number of participants declined taking some of the tests, or were unable to complete the tasks (e.g., due to color-blindness), therefore total sample sizes vary from one test to the other.

Response inhibition: Methamphetamine-specific response inhibition was assessed using a cue reactivity paradigm. Details on paradigm development and validation are presented in Chapter 2. In brief, participants were presented with 40 picture cues (20 methamphetamine-related and 20 food-related control cues). Skin conductance responses (SCR) were recorded throughout the task, as previously described (Ganella, Drummond, Ganella, Whittle, & Kim, 2018; Guerin et al., under review). All analyses were performed on the differential SCR (Δ SCR) scores, which were calculated as the difference between methamphetamine-related cue response and food-related cue response. Therefore, a positive score indicates reactivity towards methamphetamine-related cues, whereas a negative score indicates reactivity towards food-related cues. While the cue reactivity

paradigm was used to assess Pavlovian conditioning in Chapter 2, this Chapter will focus on the Δ SCR index, which will be used as a measure of response inhibition. It was calculated as the response in the late block minus response in the early block of the task, with a positive score indicating an increase in response and a negative score indicating a decrease in response (i.e., response inhibition). Based on results in Chapter 2, only reactivity to picture cues was assessed in the present Chapter. Note that due to poor conductance and technical issues during SCR recording, not all participants contributed to SCR analyses.

Barratt Impulsiveness Scale (BIS): assesses impulsivity. It consists of 30 items assessing six different first order factors (attention, cognitive instability, motor, perseverance, self-control, and cognitive complexity impulsivity), and three second order factors (attentional, motor, and non-planning impulsivity) (Patton et al., 1995). The different factors were analyzed separately, and as a Total BIS score. Note that the BIS was added to the study in July 2019, and therefore not all participants completed this assessment.

3.2.4. Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics 26 (IBM Corp., NY, USA). Demographic variables were reported as mean, standard deviation (SD), and range for continuous variables (**Table 3.2**). For categorical variables, total number in each category and percentages were calculated. Whenever possible, demographic characteristics were compared against national data of people using methamphetamine published in the Australian Institute of Health Welfare (AIHW) 2019 National Drug Strategy Household Survey (AIHW, 2020).

For categorical variables (i.e., presence or absence of the nine psychiatric comorbidities; see **Table 3.3**), Mann-Whitney U tests were used, using age of onset as the dependent variable. For continuous variables (e.g., cognitive performance), Pearson's

correlations were used to examine the association between variables of interest and age of onset.

A hierarchical multiple regression was conducted on the significant associations to assess whether these variables could predict age of onset of methamphetamine use. Demographic variables significantly associated with age of onset were entered in Block 1 of the regression to control for their effect. Pearson's correlation and Chi-square analyses were conducted to identify such variables (**Table 3.1**). Only age at testing was significantly associated with age of onset, and therefore was included in Block 1. Significant psychiatric and cognitive predictors identified above were entered in Block 2 and 3, respectively. The statistics of interest were the change in F and p values associated with inclusion of predictors in the model. A significant change ($p < 0.05$) was interpreted as predictors contributing to age of onset of methamphetamine use over and above age at testing.

Table 3.1. Demographic characteristics associated with age of onset of methamphetamine use. Pearson's correlation analyses were used for continuous variables. Chi-square analyses were used for categorical variables. Values in **bold** are significant at $p < 0.05$.

	Age of Onset	
	r	p
Age	0.567	< 0.001
Premorbid IQ	0.03	0.856
Education Years	-0.029	0.86
	χ^2	p
Sex (Male/Female)	17.016	0.522
Ethnicity (Caucasian/Asian/Aboriginal/Pacific Islander)	68.084	0.094
Unemployed (Yes/No)	12.431	0.824

3.3. Results

3.3.1. Descriptive statistics

Key characteristics of the sample are presented in **Table 3.2**. The mean age of our sample was 33.68, which is slightly above the national average of 32 (AIHW, 2020). Consistent with national data, 57.5% of participants were males, and 60% were unemployed. Our sample

was predominantly of Caucasian descent. The mean age of onset of methamphetamine use was 22.76, which was slightly above the national median of 22 (AIHW, 2020). 95% of participants reported primarily smoking methamphetamine or using methamphetamine intravenously, with only two reporting using methamphetamine intranasally. Thirteen participants were currently seeking treatment in in-patient (DePaul House) or out-patient services (Department of Addiction Medicine), whereas the rest were recruited from the community. Over half of the participant met criteria for a polysubstance use disorder. The most common comorbid substance use disorders were nicotine and cannabis use disorders ($n = 8$; 20% of total sample). Other substance use disorders included alcohol, cocaine, inhalant, sedatives, and opioids use disorder. It should be noted that methamphetamine was their primary substance of use for all participants in this study.

On average, participants displayed more positive symptoms than negative symptoms on the PANSS, and scores were markedly lower than previous studies in similar populations (Medhus et al., 2013; Okada et al., 2016; Yamamuro et al., 2015). In contrast, Total BIS scores, and second-order subscale scores were much higher in our sample compared to previous studies in stimulant dependents (Stanford et al., 2009; Tziortzis et al., 2011). Lastly, CTQ scores ranged from “none” to “extreme” for all subscales, with emotional abuse and neglect the highest scores, which is in line with previous reports (Lopez-Patton et al., 2016; Pirnia et al., 2020). For descriptive statistics, refer to **Table 3.2**.

Table 3.2. Demographic, drug use, psychiatric and cognitive characteristics of the sample.

	Mean / n	SD / %	Range	N
Demographic:				
Age - years	33.68	6.69	22-51	40
Sex				
Females	17	42.5%		
Males	23	57.5%		
Premorbid IQ	104.15	10.00	74-122	40
Ethnicity				
Caucasian	29	72.5%		
Asian	6	15%		

Aboriginal/Torres Strait Islander	3	7.5%		
Pacific Islander	2	5%		
Education years	13.46	3.13	9-22	40
Unemployed	24	60%		
Methamphetamine Use:				
Age of onset of use	22.76	6.99	13-46	40
Total years of use	10.91	6.37	1-25	40
ASSIST Amphetamine score	31.38	5.61	13-39	40
Treatment seeking	13	32.5		
<i>Primary route of administration</i>				
Smoking	20	50%		
I.V	18	45%		
Intranasal	2	5%		
Other drug use:				
Polysubstance use disorder	21	53.8%		
Nicotine Use Disorder	8	20%		
Alcohol Use Disorder	3	7.5%		
Cannabis Use Disorder	8	20%		
Cocaine Use Disorder	1	2.5%		
Inhalant Use Disorder	1	2.5%		
Sedatives Use Disorder	1	2.5%		
Opioids Use Disorder	2	5%		
PANSS Scores:				
Total Score	56.85	11.31	36-82	40
Positive Symptoms	13.33	4.77	8-26	40
Negative Symptoms	11.73	5.23	7-27	40
General Symptoms	31.80	6.56	18-44	40
Cognitive Performance:				
TMT-A	3.48	1.45	1.29-8.03	37
TMT-B	1.53	0.46	0.74-2.61	37
Symbol Coding	50.51	9.80	33-71	37
Spatial Span	16.49	2.73	10-22	37
Digit Span	15.66	3.25	9-24	32
Verbal Fluency	16.34	4.16	11-25.5	35
Stroop (inhibition)	11.13	4.92	2.38-23.76	35
Stroop (switching)	2.57	1.28	0.7-7.91	34
BIS Scores:				
Total Score	85.53	11.91	68-114	17
<i>Second-order subscales</i>				
Attentional	22.82	5.28	13-32	17
Motor	32.59	5.15	19-40	17
Non-planning	30.12	4.50	23-42	17
<i>First-order subscales</i>				
Attention	13.82	3.49	5-20	17
Cognitive Instability	9.00	2.40	4-12	17
Motor	21.12	4.00	10-28	17
Perseverance	11.47	2.18	9-16	17
Self-Control	15.76	2.91	11-22	17
Cognitive Complexity	14.35	2.55	11-20	17
CTQ				

Total	65.29	22.67	28-118	28
Emotional Abuse	14.96	5.29	5-22	28
Physical Abuse	12.79	6.07	5-25	28
Sexual Abuse	12.11	7.33	5-25	28
Emotional Neglect	14.46	5.48	5-24	28
Physical Neglect	10.68	5.68	5-22	28

3.3.2. Psychiatric factors

3.3.2.1. Comorbid psychiatric disorders

Mann-Whitney U tests revealed that people with comorbid antisocial personality disorder (ASPD) started using methamphetamine earlier in life [$U = 47.50$, $z = -4.060$, $p < 0.001$] (**Figure 3.1**). There were no other statistically significant differences between participants with or without psychiatric co-morbidities (**Table 3.3**).

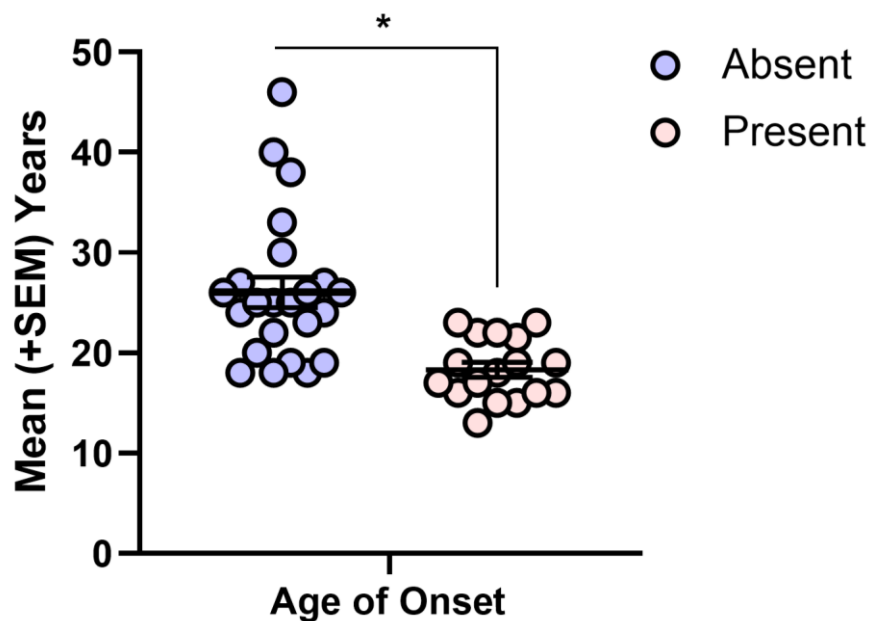


Figure 3.1. Comorbid antisocial personality disorder and age of onset of methamphetamine use. People with comorbid ASPD started using methamphetamine earlier in life ($p < 0.001$). Data presented as individual values, mean $\pm$ SEM.

Table 3.3. Comparison between presence or absence of comorbid psychiatric disorders and age of onset of methamphetamine use. Values in **bold** are significant.

		n	Age of Onset	
			Mean ($\pm$ SE)	Test; p-value
Antisocial Personality Disorder	Absent	23	26.04 ($\pm$ 1.52)	z = -4.060; p < 0.001
	Present	17	18.32 ($\pm$ 0.75)	
Anxiety Disorders	Absent	26	23.94 ($\pm$ 1.37)	z = 1.905; p = 0.057
	Present	14	20.57 ($\pm$ 1.78)	
Bipolar and Related Disorders	Absent	29	23.36 ($\pm$ 1.43)	z = -0.273; p = 0.785
	Present	11	21.18 ($\pm$ 1.39)	
Depressive Disorders	Absent	10	24.75 ($\pm$ 1.49)	z = -1.127; p = 0.260
	Present	30	22.10 ($\pm$ 1.22)	
Eating Disorders	Absent	36	22.74 ($\pm$ 1.09)	z = -0.678; p = 0.498
	Present	4	23.00 ($\pm$ 5.73)	
Obsessive-Compulsive Disorders	Absent	35	22.99 ($\pm$ 1.23)	z = -0.307; p = 0.758
	Present	5	21.20 ($\pm$ 2.20)	
Post-Traumatic Stress Disorder	Absent	29	21.78 ($\pm$ 1.03)	z = 1.108; p = 0.268
	Present	11	25.36 ($\pm$ 2.92)	
Psychotic Disorders	Absent	38	22.59 ($\pm$ 1.16)	z = 1.400; p = 0.162
	Present	2	26.00 ($\pm$ 1.00)	
Suicidal Behaviour Disorders	Absent	22	22.05 ($\pm$ 1.57)	z = -1.077; p = 0.282
	Present	18	23.64 ($\pm$ 1.56)	

3.3.2.2. Positive, negative and general symptoms

There were no significant correlations between any drug use parameters and PANSS scores (**Table 3.4**).

Table 3.4. Pearson's correlation analyses between PANSS scores and age of onset of methamphetamine.

	Age of Onset	
	r	p
Total PANSS Score	-0.049	0.766
Positive Symptoms	-0.037	0.82
Negative Symptoms	0.076	0.641
General Symptoms	-0.117	0.471

3.3.2.3. Childhood Trauma

There were no significant correlations between any drug use parameters and CTQ scores (**Table 3.5**).

Table 3.5. Pearson's correlation analyses between CTQ scores and age of onset of methamphetamine.

	Age of Onset	
	r	p
Total	0.087	0.661
Emotional Abuse	0.037	0.852
Physical Abuse	0.02	0.919
Sexual Abuse	0.085	0.666
Emotional Neglect	0.191	0.329
Physical Neglect	0.001	0.997

3.3.3. Cognitive factors

3.3.3.1. Cognitive performance

Pearson's correlation analyses revealed a positive correlation between age of onset of methamphetamine use and Stroop performance ($r = 0.432$, $p = 0.011$) (**Table 3.6; Figure 3.2**), suggesting that participants who started using methamphetamine earlier in life displayed poorer inhibitory control.

Table 3.6. Pearson's correlation analyses between cognitive performance and age of onset of methamphetamine use. Values in **bold** are significant.

	Age of Onset	
	r	p
TMTA	0.079	0.647
TMTB	0.096	0.578
Symbol Coding	0.087	0.615
Spatial Span	0.038	0.827
Digit Span	-0.188	0.303
Verbal Fluency	0.185	0.296
Stroop (Inhibition)	0.432	0.011
Stroop (Switching)	0.303	0.087

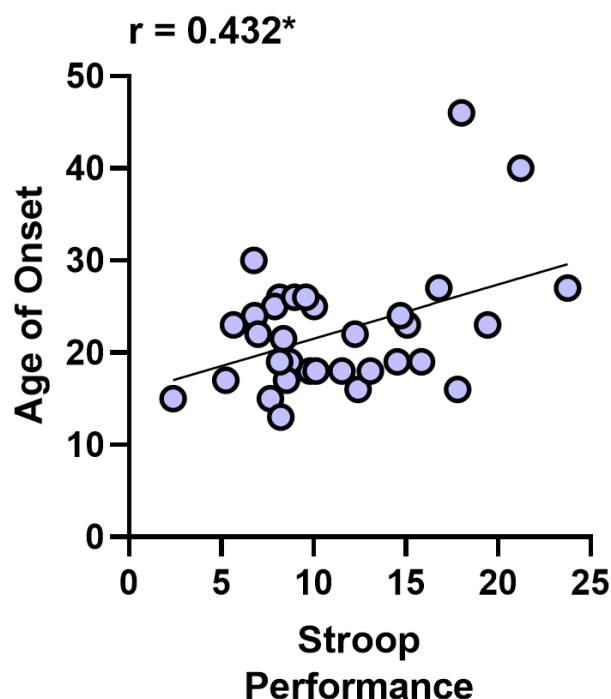


Figure 3.2. Pearson's correlation analyses between Stroop performance and age of onset of methamphetamine use. There was a positive association between performance on the Stroop Task (Inhibition) and Age of Onset of methamphetamine use (* $p = 0.011$; $n = 35$).

3.3.3.2. Impulsivity

Age of onset of methamphetamine use was negatively correlated with motor impulse ($r = -0.172$, $p = 0.025$) and cognitive complexity ($r = -0.487$, $p = 0.047$), indicating that people who started using methamphetamine earlier in life were more likely to be more impulsive (Table 3.7).

Table 3.7. Pearson's correlation analyses between Barratt Impulsiveness Scale and age of onset of methamphetamine use disorder. Values in **bold** are significant.

	Age of Onset	
	r	p
Total Score	-0.458	0.065
<i>Second-order subscales</i>		
Attentional	-0.172	0.508
Motor	-0.54	0.025
Non-planning	-0.391	0.121
<i>First-order subscales</i>		
Attention	-0.119	0.65
Cognitive Instability	-0.207	0.425
Motor	-0.435	0.081
Perseverance	-0.477	0.053
Self-Control	-0.178	0.493
Cognitive Complexity	-0.487	0.047

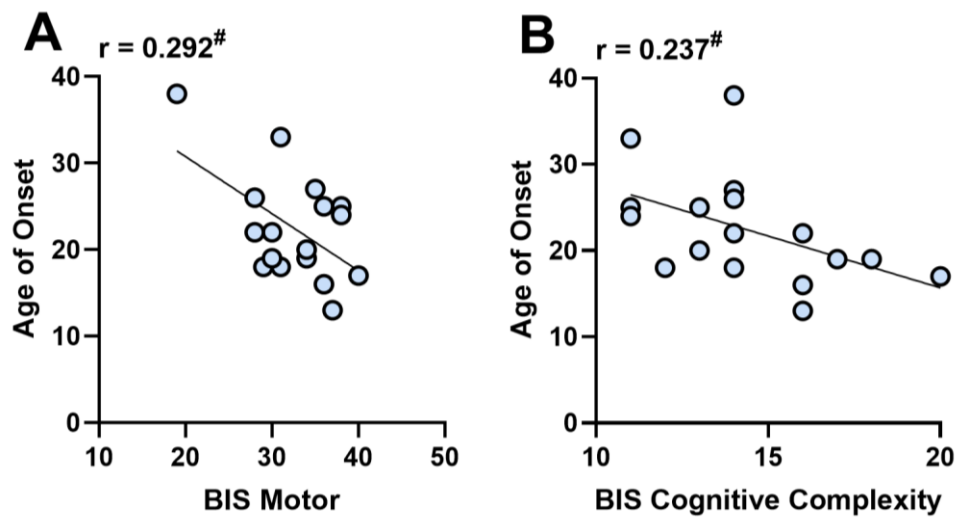


Figure 3.3. Pearson's correlation analyses between BIS and age of onset of methamphetamine use. A. Motor impulsivity. There was a negative association between performance on the BIS (motor impulsivity) and Age of Onset of methamphetamine use ($\#p = 0.025$; $n = 17$). **B. Cognitive complexity.** There was a negative association between performance on the BIS (cognitive complexity) and Age of Onset of methamphetamine use ($\#p = 0.047$; $n = 17$).

3.3.4. Hierarchical multiple regression analyses

A hierarchical regression was conducted to determine whether psychiatric and cognitive factors predicted age of onset of methamphetamine use after controlling for significant demographic variables (i.e., age at testing). Age at testing was entered in Block 1. In Block 2 and Block 3, significant psychiatric and cognitive factors were entered, respectively. Factors included in the model were based on significant factors described above. Note that BIS scores were not included as only a subgroup of participants were administered the test.

Therefore, a hierarchical multiple regression was run to determine if the addition of comorbid diagnosis of ASPD and performance in the Stroop task (Inhibition) improved the prediction of age of onset methamphetamine use over and above age of participants (**Table 3.8**). Age explained 28.8% of the variation in age of onset. The addition of ASPD diagnosis significantly improved the predictive value of the model [$F(1,31) = 17.914$, $p < 0.001$], and explained an additional 26.1% of the variance in age of onset. Addition of Stroop performance did not significantly improve the model further [$F(1,30) = 3.720$, $p = 0.063$],

and only explained an additional 5.0% of the variance. The overall model was significant [$F(4,29) = 14.904$, $p < 0.001$]. Diagnosis of ASPD was a significant predictor ($\beta = -0.477$, $p < 0.001$), whereas Stroop performance was not ($\beta = 0.235$, $p = 0.063$).

Table 3.8. Hierarchical multiple regressions examining the predictive value of psychiatric and cognitive factors on age of onset of methamphetamine use.

Dependent Variables	Predictor Variables	R ²	b	SE b	β	p-value
Age of Onset	<i>Block 1</i>	0.288				
	Age		0.541	0.15	0.537	0.001
	<i>Block 2</i>	0.549				
	Age		0.515	0.122	0.511	< 0.001
	ASPD		-6.802	1.607	-0.511	< 0.001
	<i>Block 3</i>	0.598				
	Age		0.453	0.121	0.449	0.001
	ASPD		-6.349	1.559	-0.477	< 0.001
	Stroop (Inhibition)		0.32	0.166	0.235	0.063

3.4. Discussion

The aim of the present chapter was to investigate the association between comorbid psychiatric symptoms, performance in cognitive tasks, and age of onset of methamphetamine use. I found strong associations between age of onset of methamphetamine use and comorbid diagnosis of ASPD and poor performance on the Stroop task, although the later was no longer significant in the final model. I identified ASPD as the strongest predictor of earlier age of onset of methamphetamine use, after controlling for age at test. There also were significant associations between BIS scores and age of onset of methamphetamine use, though these were not included in the final regression model as only a subgroup of people with methamphetamine use disorder were administered the questionnaire in this study.

3.4.1. Psychiatric factors

3.4.1.1. Antisocial personality disorder

In the present study, 42.5% of the total sample ($n = 17$) reported a comorbid diagnosis of antisocial personality disorder (ASPD). Frequency in our study was markedly lower than in a previous study reporting that over 75% of people with methamphetamine dependence also had ASPD (Zhang et al., 2018). It should be noted that all participants were male and undergoing compulsory rehabilitation in that study, whereas our sample was males and females recruited from both treatment facilities and the community. People with a comorbid diagnosis of ASPD were more likely to have started using methamphetamine earlier in life.

It is widely accepted that personality disorders predate onset of substance use disorders (Harro, 2015). A large cohort study ($n = 544$) found that over 30% of people who use methamphetamine had reported three or more conduct problems (which often predate development of ASPD) prior to initiating methamphetamine use (Kramer et al., 2009). In addition, antisocial behaviors were one of the strongest predictors of methamphetamine use in a cohort of American 8th graders (Embry et al., 2009), and Australian adolescents (Degenhardt et al., 2007). Another study reported that ASPD predicted persistent drug use (Fenton et al., 2012). Taken together, misconduct and antisocial behaviors in childhood and adolescence may contribute to the vulnerability towards early onset methamphetamine use that may lead to persistent methamphetamine taking.

3.4.1.2. Positive, negative, and general symptoms

There were no associations between any of the PANSS scales (positive, negative, and general) and age of onset of methamphetamine use in the present study. Evidence of an association between age of onset and psychotic symptoms is inconsistent. Studies investigating lifetime substance-induced psychotic disorders have found an association between age of onset of methamphetamine use and diagnosis (Chen et al., 2003; Hides et al., 2015). In contrast, studies investigating specific symptoms reported no differences in

age of onset of methamphetamine use between methamphetamine users with or without psychotic symptoms (McKetin et al., 2010, 2006; Salo et al., 2013b). In the present study, I only assessed current symptoms, as opposed to formal diagnosis of substance-induced psychosis. Therefore, my study provides additional evidence that while early onset of methamphetamine use may play a role in the development of formal psychotic disorders, development of discrete methamphetamine-induced symptoms is unlikely to be dependent on age of onset.

3.4.2. Cognitive factors

3.4.2.1. Inhibitory and impulse control

I found a strong correlation between age of onset of methamphetamine use and performance on the Stroop task. Results are consistent with previous studies reporting that adolescents with methamphetamine use disorder display poorer Stroop performance compared to controls of the same age (King et al., 2010; Lyoo et al., 2015). Positive correlation between age of onset of methamphetamine use and Stroop performance was also previously reported (Kim et al., 2016). Taken together, it is possible that reduced inhibitory control early in life is a risk factor that perpetuates methamphetamine use after experimentation in adolescence. Alternatively, early methamphetamine use may lead to greater deficits in inhibitory control.

Poor impulse control is associated with inhibitory control deficits (Logan et al., 1997). Consistent with these findings, I also found a negative correlation between age of onset and two dimensions of the BIS: cognitive and motor impulsivity, indicating the younger the age of onset, the higher the impulsivity. Cognitive impulsivity involves making quick decisions, while motor impulsivity involves acting without thinking (Patton et al., 1995). Notably, poor motor impulsivity predicts treatment non-completion in people with methamphetamine dependence (Winhusen et al., 2013), which may partly explain why young people are more resistant to treatment (Catalano et al., 1991; Perepletchikova et al., 2008). It should be noted

that only a subset of participants were administered the BIS ($n = 17$), and findings discussed should be treated with caution and replicated in a larger sample. Nonetheless, my results are consistent with previous studies reporting that participants who started using methamphetamine earlier in life have higher impulsivity scores (Cservenka and Ray, 2017; Kogachi et al., 2017; Semple et al., 2005). Notably, Kogachi and colleagues (2017) only observed the association between age of onset and BIS scores in female participants. When data collected in this Chapter were split by sex, motor impulsivity was only associated with age of onset in males ($r = -0.752$; $p = 0.031$), and cognitive impulsivity was only associated with onset in females ($r = -0.698$; $p = 0.036$). While my sample was relatively small (8 males and 9 females), findings from Kogachi et al. (2017) and herein suggest that different types of impulse control deficits may be sex-specific in people with methamphetamine use disorder. While the directionality of the association could not be assessed in this Chapter due to the cross-sectional nature of the study, extensive evidence from rodent literature suggests that impulsivity predicts susceptibility for substance use (Dalley et al., 2011; Dalley and Robbins, 2017; Jentsch et al., 2014).

3.4.3. Predicting age of onset of methamphetamine use

Using hierarchical multiple regressions, I found that comorbid diagnosis of ASPD significantly predicted age of onset of methamphetamine use, over and above age of participant at testing. In addition, while cognitive factors (i.e., Stroop performance) did not significantly improve the predictive model, they explained a further 5% of the variance between participants in age of onset. This suggests that ASPD is the single most important predictor of early onset and longer duration of methamphetamine use in this sample.

3.4.4. Limitations

This study is not without limitations. While the correlation analyses and hierarchical regression model revealed a role for psychiatric and cognitive factors in early-onset

methamphetamine use vulnerability, there is a critical need for more longitudinal studies in this population to ascertain the causal link between factors of interest and development of methamphetamine use disorder. To allow causal inference from cross-sectional data, a number of factors must be taken into consideration, including but not limited to temporality, directionality, and strength of the association (Hill, 1965). My study also exclusively recruited participants above the age of 18. This is because the vast majority of addiction treatment facilities in Australia are only available to those over 18 years of age (Department of the Prime Minister and Cabinet, 2015), and I could not directly recruit underage adolescents. It should also be noted that my sample size was rather small. This was due to suspension of the study from March 2020 onwards due the coronavirus pandemic. While this Chapter provides compelling preliminary data, future studies should aim to replicate findings in larger samples. Lastly, all participants were recruited in metropolitan Melbourne, Australia, and findings from this study may not reliably extrapolate to the general population.

3.4.5. Conclusions

In conclusion, this study adds to the growing body of literature showing that poor inhibitory and impulse control may be associated with age of onset of methamphetamine use. This study also provides further evidence that comorbid diagnosis of antisocial personality disorder is one of the strongest predictors of early onset methamphetamine use. Early interventions in youth at risk of developing methamphetamine use disorders should therefore target these factors.

Chapter 4. Genetics of methamphetamine use disorder: A systematic review and meta-analyses of gene association studies



Genetics of methamphetamine use disorder: A systematic review and meta-analyses of gene association studies

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ABSTRACT

Genetic susceptibility to methamphetamine use disorder is poorly understood. No twin or adequately powered genome-wide association studies (GWASs) have been conducted. However, there are a large number of hypothesis-driven candidate gene association studies, which were systematically reviewed herein. Seventy-six studies were identified, investigating markers of 75 different genes. Allele frequencies, odds ratios, 95 % confidence intervals and power were calculated. Risk of bias was also assessed as a quality measure. Meta-analyses were conducted for gene markers if three or more studies were available. Eleven markers from adequately powered studies were significantly associated with methamphetamine use disorder, with Fatty Acid Amide Hydrolase (FAAH) and Brain Derived Neurotrophic Factor (BDNF) representing promising targets. Limitations of these studies include unclear rationale for candidate gene selection, low power and high risk of bias. Future research should include replications to enable more meta-analyses, well-powered GWASs or whole exome or genome sequencing, as well as twin and family studies to further complement the findings of this review to uncover genetic contributions toward methamphetamine use disorder.

1. Introduction

Amphetamine-like stimulants are the second most used illicit drug in the world, behind cannabis (UNODC, 2017a). Among amphetamines, methamphetamine (meth) represents the greatest global health threat, with a rapidly increasing market and number of users worldwide (UNODC, 2017b). In their most recent report, the U.S. Substance Abuse and Mental Health Services Administration (SAMHSA) estimated that 13 million people in the U.S. used meth in their lifetime (~4% of the total population), with 569,000 people using meth in the past month (SAMHSA, 2015). Meth use appears to be increasing globally, most likely due to expanding markets (UNODC, 2017b), highlighting the need to better understand meth use disorder.

1.1. Heritability of meth use disorders

Evidence from family, twin and adoption studies suggests substantial heritability for substance use disorders (for comprehensive reviews see Ducci and Goldman, 2012; Goldman et al., 2005; Vanyukov and Tarter, 2000). Substance use disorders are regarded as highly polygenic, with many hundreds of genes likely contributing to their heritability, each of which contribute a miniscule fraction of the overall risk (Ducci and Goldman, 2012). While the heritability of stimulant use disorders is estimated to be close to 40 % (Goldman et al., 2005), no twin or family studies have been conducted in people with meth use disorder, making it challenging to uncover the heritability of risk for this particular drug. Notably, that 40 % figure seems consistent across numerous classes of abused drugs including stimulants, cannabinoids, opioids, nicotine and alcohol.

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Another approach to investigate the heritability of disorders are genome-wide association studies (GWASs). To date, only three GWASs have been conducted in people with meth use disorder (Ikeda et al., 2013; Sun et al., 2019; Uhl et al., 2008b). While Uhl et al. (2008b) and Ikeda et al. (2013) did not detect significant genome-wide single nucleotide polymorphism (SNP) associations, Sun et al. (2019) more recently reported an association between meth use disorder and three SNPs. It should be noted that the first two studies used overlapping samples, and all three studies were gravely under-powered for GWASs. Specifically, study with the largest sample size included 1750 people with meth use disorder, compared to the tens or even hundreds of thousands of participants required for reliable genetic findings for similarly polygenic complex psychiatric disorders. Of note, the three studies exclusively recruited people of Han Chinese or Japanese ethnicity. There is preliminary evidence of ethnic divergence of gene variants for meth use disorder (Bousman et al., 2010).

While twin studies have been lacking and GWAS results have been inconclusive, there has been an accumulation of candidate gene association studies in people with meth use disorder over the past two decades. Though not without important limitations, candidate gene studies can identify specific sequence variations in genes of interest and point towards potential molecular pathways affected to inform future research. While an association of genetic variants of a given candidate gene with some functional endpoint can potentially teach us about the biological effects of that gene and its naturally occurring sequence variations, this is very different from claiming that those gene variants contribute to heritable risk for a given psychiatric disorder. The latter claim can only be verified with rigorous genome-wide approaches or the careful study of large family pedigrees.

The first aim of the present study was therefore to systematically review gene association studies in people with meth use disorder to identify any promising targets and possible pathways underlying meth use disorder. Genotype, allele frequencies, and haplotypes were assessed. For specific markers with 3 or more studies available, meta-analyses were also conducted. Importantly, risk of bias assessment was conducted as a quality measure for each study using the National Heart, Lung, and Blood Institute Quality Assessment of Case-Control studies (NHLBI, 2020).

Candidate gene studies can be classified into two broad categories: those that examine possible influencing factors predisposing to the development of substance use disorders, (e.g., poor inhibition and impulse control), and those that specifically examine putative genetic variants in neurotransmitter and receptor systems involved in the substance use disorder of interest. In addition, Uhl et al. (2008a) suggested that the genetics of substance use vulnerability can either be due to individual differences in specific drug targets, or individual differences in receptor drug responses which may be shared across different class of substances (Uhl et al., 2008a). In that regard, the second aim of this review was to examine genetic contribution of broad substance use in meth use disorder to understand polysubstance use. We also systematically reviewed other secondary outcomes that the genetic association studies explored, including sex differences and age of first meth use.

2. Method

2.1. Literature search

This systematic review was conducted following the PRISMA guidelines (Moher et al., 2009). The protocol was registered with PROSPERO (CRD42020177826) after preliminary searches were conducted, but before literature search. The entire PubMed, EMBASE, PsycINFO and MEDLINE databases were searched for studies from their date of inception to April 10, 2020. The following search terms were used: “methamphetamine” OR “methylamphetamine”; and a combination of the following terms: “gene” OR “allele” OR “genotype” OR “polymorphism” OR “DNA” OR “haplotype” OR “twin” OR “linkage” OR

“expression” OR “protein” OR “RNA”. To limit results to studies in humans, terms “human” OR “participant” were also used. Duplicated papers were removed, then titles and abstracts were screened for inclusion by two authors (AAG and JHK) using Covidence (Veritas Health Innovation, Melbourne, Australia).

2.2. Inclusion and exclusion criteria

Studies were included if they: (1) included human participants with a formal diagnosis (DSM or ICD) of meth use disorder, dependence or abuse; (2) included a healthy comparison group; (3) collected genetic information using human tissues; (4) reported genotype/allele distribution; and (5) were published in English language in a peer-reviewed journal.

Candidate gene variant studies were included. Studies were excluded if they were: (1) other systematic reviews or meta-analyses; (2) conference proceedings published as abstract only; (3) case reports; (4) book chapters; or (5) animal studies.

2.3. Quality assessment

Each study was assessed by two authors (AAG and JHK) using the NHLBI Quality Assessment of Case-Control Studies, which consist of 12 items (NHLBI, 2020). Each item was given a score of 1 (low risk of bias) or 0 (high risk of bias) to result in the total score for each study ranging from 0–12, with 0 suggesting a very high risk of bias, and 12 representing little to no risk of bias. Each assessor scored each study included in the review, and any conflict between assessors was resolved through discussion. The percent agreement between the two assessors was calculated across all 12 items.

2.4. Data extraction

For each study, the following information were extracted: (1) sample size; (2) mean age, sex, and ethnicity; (3) diagnostic tool (DSM or ICD) used to confirm diagnosis of meth use disorder; (4) existing comorbidities (including polysubstance use and meth-induced psychosis); (5) drug use parameters for the meth use disorder group (age of onset, duration of use, and severity of dependence); (6) type of biospecimen collected; (7) genes studied, including their location, function, and variant type (e.g., SNP, deletion, repeat etc.) when relevant; and (8) genotype and allele frequency data.

2.5. Data synthesis and analysis

Chi-square analyses were used to calculate significance levels for genotype and allele distribution between controls and people with meth use disorder for each study. Odds ratios and 95 % confidence intervals were also calculated for minor alleles. Power for each association was calculated using the total sample size and effect size w , using the minor allele frequency and sample size reported in each study, as described in Bousman et al. (2009). When a study reported more than one association, frequencies and power were calculated for each association separately. When enough studies were available, a meta-analytic approach was used. The outcome measure used was the effect size (odds ratio) for association between minor allele and meth use disorder using a random-effects model. Weights for each study were calculated in RevMan5 (The Cochrane Collaboration, 2020) using sample size and number of minor alleles. Heterogeneity was estimated using the I^2 statistic, with $I^2 \leq 50$ % indicating low to moderate heterogeneity, and $I^2 > 50$ % indicating moderate to high heterogeneity. Sensitivity analyses were conducted based on quality of studies by systematically excluding the study with the highest risk of bias of each analysis. Publication bias assessment was not conducted as there were fewer than 10 studies included in each analysis (Higgins and Green, 2011).

Statistical analyses were performed using IBM SPSS Statistics 26

(IBM Corp., NY, USA) for genotype/allele analyses, G*Power 3.1 (Erdfeilder et al., 2009) for power analyses, and Review Manager 5.4 (The Cochrane Collaboration, 2020) for the meta-analyses. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Study selection and quality assessment

From the 2330 studies identified through database search, 1065 remained following removal of duplicates (Fig. 1). Following title and abstract screening by two independent assessors, 239 articles underwent full-text assessment for eligibility. In total, 79 studies met the inclusion criteria, for a total of 44,184 participants (17,682 cases and 26,502 controls). Three studies were GWASs, and 76 studies were hypothesis-driven candidate gene studies, investigating 75 unique genes (Supplementary Table S1).

Summary of eligible studies are found in Table 1 and Fig. 2. Eligible studies either used ICD-10, DSM-IV or DSM-5 criteria, with four studies using a combination of these (Fig. 2A). A large majority of studies reported co-morbidities, with the most common being meth-induced psychosis and polysubstance use disorder (Fig. 2B). The most studied populations were of Japanese and Han Chinese descent, with one study investigating both populations (Fig. 2C). Three studies recruited Malaysians participants of four different ethnicities: Malay, Chinese, Kadazan-Dusun and Bajau. Two studies reported predominantly Caucasian samples, which also included other ethnicities. Fourteen studies exclusively examined male participants, and three studies did not report sex distribution (Fig. 2D).

The quality of the studies based on the NHLBI Quality Assessment of Case-Control Studies (see Methods) ranged from 3 to 9, with the lower the score, the higher the risk of bias (Table 1). The percent agreement between the two assessors was 86.0 %. The main limitations were poorly defined and implemented exclusion and inclusion criteria (item 5; 93.4 % of all studies), poorly defined and differentiated cases and controls (item 6; 75.0 %) and unjustified sample size (item 3; 72.4 %).

3.2. Candidate gene association studies

Table 2 provides the genotype and allele frequencies, calculated odds

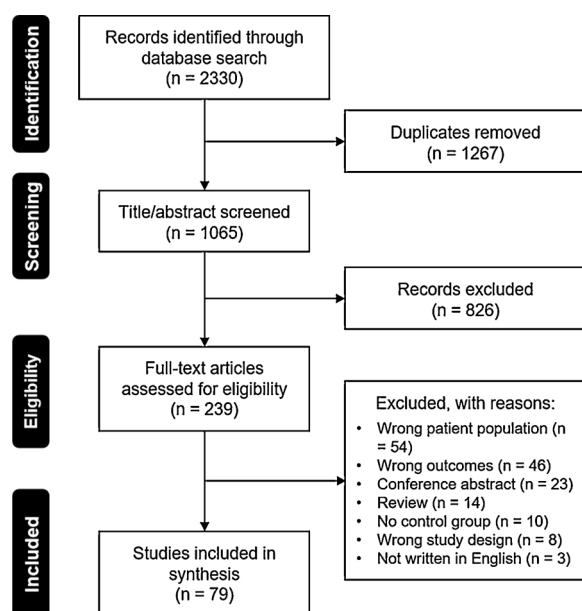


Fig. 1. PRISMA Diagram illustrating stages of article processing for the systematic review.

ratios (OR) and 95 % confidence intervals (95 % CI), p-values and power for each gene variant investigated. The most common type of variant studied were single nucleotide polymorphisms (SNPs). Deletions (Dels), variable number tandem repeats (VNTRs), restriction fragment length polymorphisms (RFLPs), and linked polymorphic regions (LPRs) were also examined. The characteristics of all genes investigated, including non-abbreviated full name, chromosomal location, and function are given in Supplementary Table S1. Note that the listed function of each gene was derived from each study's rationale, and that genes investigated may be involved in various other functions and pathways.

3.2.1. Genotype and allele frequencies

Forty markers in 29 different genes displayed significant genotypic or allelic association with meth use disorder. Full names of genes are available in Table S1. Characteristics of each significant marker (including location) are given in Table 3. Of these markers, 11 were located in coding regions of a gene. Five of them were missense mutation leading to a change in amino acid sequence (*BDNF* rs6265; *COMT* rs4680; *FAAH* rs324420; *GSTO1* rs4925; and *GSTP1* rs1695), whereas the other six were synonymous mutations (*ADORA2A* rs5751876; *ARRB2* rs1045280; *CHRNA4* rs2273506; *GRIA* rs502434; *PROKR2* rs3746682; and *SLC6A9* rs2248829). In addition, four markers were located in the 3' untranslated region (UTR; *CHRNA2* rs2072659 and rs3811450; *GCLM* rs3170633; and *PROKR2* rs3746682), three markers in the 5' UTR (*ADORA2A* rs13306114; *GABRG2* rs4480617; and *GAD2* rs2236418), and two in the promoter region of their respective gene (*PDYN* 68-bp repeat; and *SLC6A4* LPR). Ten markers were located within an intron (*AKT1* rs3730358; *ANRIL* rs1333045; *DTNBP1* rs3213207; *GABBR1* rs715044; *OPRM1* rs2075572; *PICK1* rs1713729; *PROKR2* rs6085086; *SLC22A3* rs3106164 and rs4709426; and *SLC6A9* rs2486001). One of the markers investigated was a deletion (*GSTT1*). The last nine markers were not directly located within a gene. Six of them were downstream (*ANRIL* rs1333048; *ARRB2* rs4790694 and rs2036657; *GRM2* rs4687771 and rs12487957; and *SIRT1* rs10997875) and three of them upstream (*CHRNA2* rs2072658; *DTNBP1* rs2619538; and *HTR6* rs6693503) of the targeted gene. The effect of most variants on their gene was unknown, and therefore any significant association with meth use disorder should be taken with caution.

Minor alleles for markers in 14 genes (*ADORA2A*, *AKT1*, *ANRIL*, *ARRB2*, *DTNBP1*, *FAAH*, *GABRG2*, *GAD2*, *GRIA3*, *GSTP1*, *GSTT1*, *HTR6*, *OPRM1*, *PDYN*) were found to confer a significant risk for meth use disorder (ORs > 1). Of these genes, three had more than one significant marker (*ANRIL* rs1333045 and rs1333048; *ARRB2* rs1045280, rs2036657 and rs4790694; and *DTNBP1* rs3213207 and rs2619538), and one was explored in more than one study (*FAAH* rs324420). Minor alleles for markers in six genes (*BDNF*, *COMT*, *CYP2D6*, *GABBR1*, *GRM2*, and *PICK1*) were found to be protective (ORs < 1). One of these genes had more than one significant marker (*GRM2* rs12487957 and rs4687771), and two genes were explored in more than one study (*COMT* rs4680; and *BDNF* rs6265). It should be noted that four genes (*CHRNA2*, *PROKR2*, *SLC6A9*, *SCL22A3*) had significant markers both conferring risk and protection. For *CHRNA2*, rs2072658 was protective (OR < 1), whereas rs2072659 and rs3811450 conferred a risk (ORs > 1). For *PROKR2*, rs3746682 was protective, whereas rs6085086 and rs4815787 were risk markers. For *SLC6A9*, rs2248829 was protective and rs2486001 conferred a risk. Lastly, for *SLC22A3*, rs3106164 was protective, while rs4709426 was a risk marker. The genotype of markers in five genes (*CHRNA4*, *GCLM*, *GSTO1*, *SLC6A4*, *SIRT1*) was significantly different between cases and controls, but failed to reach allelic statistical significance.

Power calculations revealed that a large majority of associations (~94 %) were underpowered ($1 - \beta < 0.8$) to detect appropriate genotypic or allelic associations. Power ranged from 0.05 to 0.99. For associations that were significant (*ADORA2A* rs5751876; *ANRIL* rs1333048; *ARRB2* rs1045280; *DTNBP1* rs3213207; *FAAH* rs324420; *GAD2* rs2236418; *GRM2* rs12487957 and rs4687771; *HTR6* rs6693503; *PDYN*

Table 1
Studies characteristics listed alphabetically based on first author.

Study	N	People with meth use disorder		Controls		Ethnicity	Diagnosis	NHLBI Score
		Age (mean $\pm$ SD)	Sex (% female)	Age (mean $\pm$ SD)	Sex (% female)			
Aoyama et al., 2006	655	37 $\pm$ 11.7	17.8	41.3 $\pm$ 14.4	59.8	Japanese	ICD-10 F15.2 and F15.5	5
Bousman et al., 2010	193	38.6 $\pm$ 8.2	0	39.6 $\pm$ 10.2	0	Caucasian	DSM-IV Dependence	8
Chanasong et al., 2013	203	29.5 $\pm$ 4.6	0	28.8 $\pm$ 6.4	0	Thai	DSM-IV Dependence	3
Chen et al., 2004	851	NR	36.8	NR	43	Han	DSM-IV Abuse	5
Cheng et al., 2005	225	27.8 $\pm$ 6.8	0	29.8 $\pm$ 8.5	0	Chinese	DSM-IV Dependence	7
Ezaki et al., 2008	363	37.13 $\pm$ 11.0	16.3	38.96 $\pm$ 13.0	20.8	Japanese	ICD-10 F15.2 and F15.5	7
Ghafouri-Fard et al., 2020	426	35.43 $\pm$ 11.77	0	36.76 $\pm$ 11.15	0	Iranian	DSM-5 Meth use disorder	6
Han et al., 2008	77	35.1 $\pm$ 6.8	13.6	34.4 $\pm$ 6.7	15	Korean	DSM-IV Dependence	6
Hashimoto et al., 2005	388	36.9 $\pm$ 11.9	21.1	37.2 $\pm$ 10.5	21.1	Japanese	ICD-10 F15.2 and F15.5	7
Hashimoto et al., 2008	451	36.9 $\pm$ 12.0	19.3	38.7 $\pm$ 12.6	19.7	Japanese	ICD-10 F15.2 and F15.5	5
Hong et al., 2003	215	28.7 $\pm$ 6.9	0	28.1 $\pm$ 6.4	0	Han	DSM-IV Dependence	5
Iamjan et al., 2015	202	29.5 $\pm$ 4.6	0	28.8 $\pm$ 6.4	0	Thai	DSM-IV Dependence	5
Iamjan et al., 2018	202	30.1 $\pm$ 5.1	0	28.6 $\pm$ 6.3	0	Thai	DSM-IV Dependence	5
Ide et al., 2004	351	35.7 $\pm$ 1.1	22.5	34.4 $\pm$ 1.6	25.8	Japanese	ICD-10 F15.2 and F15.5	6
Ide et al., 2006	360	35.9 $\pm$ 1.0	22.7	35.2 $\pm$ 1.8	22	Japanese	ICD-10 F15.2 and F15.5	7
Ikeda et al., 2006	619	36.7 $\pm$ 12.0	19.8	34.3 $\pm$ 13.6	52.2	Japanese	DSM-IV Dependence and Abuse	3
Ikeda et al., 2007	723	36.8 $\pm$ 12.0	18.1	37.0 $\pm$ 14.4	53.3	Japanese	DSM-IV Dependence/ ICD-10 F15.2 and F15.5	5
Ikeda et al., 2013	1100	NR	28.6	NR	52.5	Japanese	ICD-10 F15.2 and F15.5	6
Inada et al., 2004	326	36	21.7	37	21.9	Japanese	ICD-10 F15.2 and F15.5	6
Itoh et al., 2005	391	36.6 $\pm$ 11.9	20.6	37.2 $\pm$ 10.6	21.8	Japanese	ICD-10 F15.2 and F15.5	7
Iwata et al., 2004	473	NR	NR	NR	NR	Japanese	ICD-10 F15.2 and F15.5	5
Jugurnauth et al., 2011	983	28.15	39	34.4 $\pm$ 10.0	42.9	Han	DSM-IV Abuse	6
Kanahara et al., 2009	465	37.18 $\pm$ 11.73	20.7	39.26 $\pm$ 12.62	19.3	Chinese	ICD-10 F15.2 and F15.5	4
Kinoshita et al., 2008	393	NR	NR	NR	NR	Japanese	ICD-10 F15.2 and F15.5	3
Kishi et al., 2008a	1004	34.6	20	37.6 $\pm$ 14.3	55.6	Japanese	DSM-IV Dependence	4
Kishi et al., 2008b	944	36.2	19.9	37.3 $\pm$ 14.3	56.7	Japanese	DSM-IV Dependence	4
Kishi et al., 2010	536	36.3 $\pm$ 11.4	19.6	37.6 $\pm$ 14.3	19.6	Japanese	ICD-10 F15.2 and F15.5	5
Kishi et al., 2011a	515	37.1 $\pm$ 12.1	16.8	36.7 $\pm$ 12.8	18.6	Japanese	DSM-IV Dependence	6
Kishi et al., 2011b	533	37.1 $\pm$ 12.1	18.6	36.7 $\pm$ 12.8	18.6	Japanese	DSM-IV Dependence	6
Kishi et al., 2011c	534	37.6 $\pm$ 12.2	16.8	37.6 $\pm$ 14.3	19.6	Japanese	ICD-10 F15.2 and F15.5	4
Kishimoto et al., 2008	440	38.1 $\pm$ 12.6	17.8	37.2 $\pm$ 12.0	20.6	Japanese	ICD-10 F15.2 and F15.5	7
Kobayashi et al., 2004	331	37.6 $\pm$ 12.0	18.8	38.6 $\pm$ 12.0	48.4	Japanese	ICD-10 F15.2 and F15.5	6
Kobayashi et al., 2006	430	37.5 $\pm$ 12.0	19.4	35.0 $\pm$ 11.5	29.6	Japanese	ICD-10 F15.2 and F15.5	6
Kobayashi et al., 2010	400	37.5 $\pm$ 12.0	19.3	41.2 $\pm$ 12.3	48	Japanese	ICD-10 F15.2 and F15.5	6
Kobayashi et al., 2011a	405	37.4 $\pm$ 12.0	19.8	35.4 $\pm$ 11.5	30.9	Japanese	ICD-10 F15.2 and F15.5	6
Kobayashi et al., 2011b	400	37.5 $\pm$ 12.0	19.3	41.2 $\pm$ 12.3	48	Japanese	ICD-10 F15.2 and F15.5	5
Koizumi et al., 2004	357	Average 35.2; 37.0 $\pm$ 11.0 (males); 28.0 $\pm$ 5.0 (females)	20.4	Average 36.8; 37.0 $\pm$ 11.0 (males); 36.0 $\pm$ 10.0 (females)	21.5	Japanese	ICD-10 F15.2 and F15.5	6
Kotaka et al., 2008	489	37.5 $\pm$ 11.9	19.5	37.6 $\pm$ 13.1	20.8	Japanese	ICD-10 F15.2	6
Li et al., 2004	851	27.2 $\pm$ 7.0	36.8	34.4 $\pm$ 10.0	43	Han	DSM-IV Abuse	5
Lin et al., 2003	685	Average 27.3; 28.73 $\pm$ 7.14 (males); 25.29 $\pm$ 6.23 (females)	42.7	Average 34.2; 35.13 $\pm$ 9.93 (males); 32.93 $\pm$ 10.14 (females)	43.2	Chinese	DSM-IV Abuse	6
Liu et al., 2004	218	29.7	11	29.6 $\pm$ 6.8	8.33	Han	DSM-IV Dependence	7
Liu et al., 2006	788	27.2 $\pm$ 7.1	37.5	34.5 $\pm$ 9.5	42.4	Chinese	DSM-IV Abuse	5
Loftis et al., 2019	106	NR	NR	NR	NR	Multiple	DSM-IV Dependence	7
Matsuzawa et al., 2007	426	36.9 $\pm$ 11.3	18.8	39.0 $\pm$ 12.3	19.7	Japanese	ICD-10 F15.2 and F15.5	5
Morita et al., 2005a	353	37.8 $\pm$ 12.1	19	37.3 $\pm$ 12.1	19	Japanese	ICD-10 F15.2 and F15.5	9

(continued on next page)

Table 1 (continued)

Study	N	People with meth use disorder		Controls		Ethnicity	Diagnosis	NHLBI Score
		Age (mean $\pm$ SD)	Sex (% female)	Age (mean $\pm$ SD)	Sex (% female)			
Morita et al., 2005b	353	37.8 $\pm$ 12.1	19	37.3 $\pm$ 12.1	19	Japanese	ICD-10 F15.2 and F15.5	9
Morita et al., 2008	414	37.4 $\pm$ 11.9	18.1	36.5 $\pm$ 10.6	22.4	Japanese	ICD-10 F15.2 and F15.5	7
Nakamura et al., 2006	305	35.8 $\pm$ 11.0	18.1	37.3 $\pm$ 10.5	23.8	Japanese	ICD-10 F15.2 and F15.5	3
	339	27.3 $\pm$ 7.3	23.7	28.8 $\pm$ 7.4	47.1	Han Chinese		
Nakamura et al., 2009	317	35.8 $\pm$ 11.0	17.8	37.3 $\pm$ 10.5	22.6	Japanese	ICD-10 F15.2 and F15.5	6
Namvar et al., 2020	455	36.43 $\pm$ 9.79	0	37.76 $\pm$ 9.6	60	Iranian	DSM-5 Meth Use Disorder	4
Nishiyama et al., 2005	466	36.7 $\pm$ 12.0	19.1	33.6 $\pm$ 13.0	47.2	Japanese	DSM-IV Dependence and Abuse	6
Nomura et al., 2006a,	352	35.5 $\pm$ 11.3	20.3	36.5 $\pm$ 10.6	22.5	Japanese	ICD-10 F15.2	6
Nomura et al., 2006b,	406	36.8 $\pm$ 11.9	20	36.3 $\pm$ 10.5	22.6	Japanese	ICD-10 F15.2	5
Ohgake et al., 2005	398	36.7 $\pm$ 10.6	20.9	36.5 $\pm$ 11.9	22.2	Japanese	ICD-10 F15.2 and F15.5	7
Okahisa et al., 2009	510	36.9 $\pm$ 11.9	20.3	37.2 $\pm$ 13.2	22.2	Japanese	ICD-10 F15.2	5
Okochi et al., 2009	718	36.7 $\pm$ 11.6	17.4	37.5 $\pm$ 14.4	54.5	Japanese	ICD-10 F15.2 and F15.5	5
Okochi et al., 2011	450	36.7 $\pm$ 11.6	17.5	35.5 $\pm$ 14.4	18.7	Japanese	ICD-10 F15.2 and F15.5/ DSM-IV Dependence	8
Otani et al., 2008	539	36.9 $\pm$ 11.8	17.3	37.2 $\pm$ 13.1	19.6	Japanese	ICD-10 F15.2	7
Payer et al., 2012	100	34.4 $\pm$ 9.4	41.5	32.1 $\pm$ 9.5	46.8	Multiple	DSM-IV Dependence	7
Šerý et al., 2001	225	21.6 $\pm$ 4.5 (SEM)	40.9	21.3 $\pm$ 3.4 (SEM)	52.3	Caucasian	DSM-IV Dependence	3
Sim et al., 2010	340	31.0 $\pm$ 8.2	0	33.0 $\pm$ 12.2	0	Multiple (Asian)	DSM-IV Dependence	6
Sim et al., 2013	473	31.0 $\pm$ 8.4	0	32.0 $\pm$ 11.0	0	Multiple (Asian)	DSM-IV Dependence	8
Sim et al., 2014	555	30.0 $\pm$ 8.0	0	32.0 $\pm$ 11.0	0	Multiple (Asian)	DSM-IV Dependence	8
Su et al., 2014	419	30.79 $\pm$ 7.99	16.5	33.68 $\pm$ 9.87	21	Han Chinese	DSM-IV Abuse	8
Su et al., 2015a	419	30.79 $\pm$ 7.99	16.5	33.68 $\pm$ 9.87	21	Han Chinese	DSM-IV Abuse	7
Su et al., 2015b	572	31.46 $\pm$ 8.40	17.5	45.99 $\pm$ 12.96	60.6	Han Chinese	DSM-IV Dependence	6
Sun et al., 2019 (Discovery)	4608	30.51 $\pm$ 7.40	28.24	34.17 $\pm$ 6.41	35.3	Han Chinese	DSM-5 Meth Use Disorder	5
Sun et al., 2019 (Replication)	2667	36.36 $\pm$ 8.52	0	33.71 $\pm$ 11.80	0	Han Chinese	DSM-5 Meth Use Disorder	
Suzuki et al., 2006	343	35.8 $\pm$ 11.0	18.2	37.3 $\pm$ 10.5	22.5	Japanese	ICD-10 F15.2 and F15.5	8
Tsai et al., 2002	228	28.6 $\pm$ 6.6	0	28.1 $\pm$ 6.4	0	Han Chinese	DSM-IV Dependence	7
Tsunoka et al., 2010	998	37.0 $\pm$ 10.8	16.8	37.6 $\pm$ 14.3	56.2	Japanese	ICD-10 F15.2 and F15.5	5
Uhl et al., 2008b	380	32.5 $\pm$ 10.0	30	NR	NR	Han Chinese	DSM-IV Dependence	7
Ujike et al., 2003	200	39.9 $\pm$ 13.0	21	NR	NR	Japanese	ICD-10 F15.2 and F15.5	9
Ujike et al., 2009	284	35.8 $\pm$ 11.3	16.9	36.0 $\pm$ 10.2	21.9	Japanese	ICD-10 F15.2 and F15.5	
Veerasakul et al., 2016	445	38.1 $\pm$ 12.6	17.3	37.2 $\pm$ 12.0	20.6	Japanese	ICD-10 F15.2	9
Veerasakul et al., 2017	28	34.1 $\pm$ 6.2	0	32.9 $\pm$ 6.4	0	Thai	DSM-IV Dependence	4
Yoon et al., 2005	202	30.1 $\pm$ 5.1	0	28.6 $\pm$ 6.3	0	Thai	DSM-IV Dependence	8
Yoshimura et al., 2011	262	34.7 $\pm$ 7.4	12.7	47.1 $\pm$ 10.0	21.1	Korean	DSM-IV Dependence	8
Zhang et al., 2020	602	37.1 $\pm$ 11.8	18.7	40.0 $\pm$ 14.6	58.2	Japanese	ICD-10 F15.2	3
Zhao et al., 2018	1061	28.42 $\pm$ 7.35	15.3	36.08 $\pm$ 10.21	26.8	Han Chinese	ICD-10 F15.2/DSM-5 Meth Use Disorder	8
	1239	35.96 $\pm$ 0.32 (SEM)	24.8	35.25 $\pm$ 0.46 (SEM)	29.9	Han Chinese	DSM-IV Dependence and Abuse	4

Notes: N = total study sample size; NR = not reported; ICD-10 = International Classification of Diseases 10th edition; DSM-IV/5 = Diagnostic and Statistical Manual of Mental Disorders 4th and 5th Edition; F15.2 = Other stimulant dependence; F15.5 = Other stimulant-induced psychotic disorder; NHLBI Assessment = National Heart, Lung, and Blood Institute Study Quality and Risk of Bias Assessment, where with 0 = highest risk of bias and 12 = little to no risk of bias.

68-bp repeat; *PROKR2* rs4815787 and rs6085086; *SLC6A9* rs2486001), power ranged from 0.16 to 0.99. Out of the 40 significant markers, a total of 14 were adequately powered ($1 - \beta \geq 0.80$).

3.2.2. Haplotype analyses

Haplotype analyses revealed significant haplotypic associations between markers in 14 genes (*AKT1*, *ANRIL*, *ARRB2*, *BDNF*, *COMT*, *DTNBP1*, *GABBR1*, *GABRG2*, *GAD1*, *GRM2*, *HTR6*, *PICK1*, *PROKR2*,

SLC6A9, *SOD2*) and meth use disorder (Table 4). For *BDNF* and *GABBR1*, all markers included in haplotype analyses were located in introns, whereas markers for the other genes were a combination of intronic, synonymous and up/downstream variants, with the exception of *COMT* which included the functional variant rs4680 (Table 3). None of the markers assigned to *GRM2* were directly located within the gene, but rather at the 5' and 3' ends. *GAD1* and *SOD2* were the only two genes that did not show genotypic or allelic significance when markers were

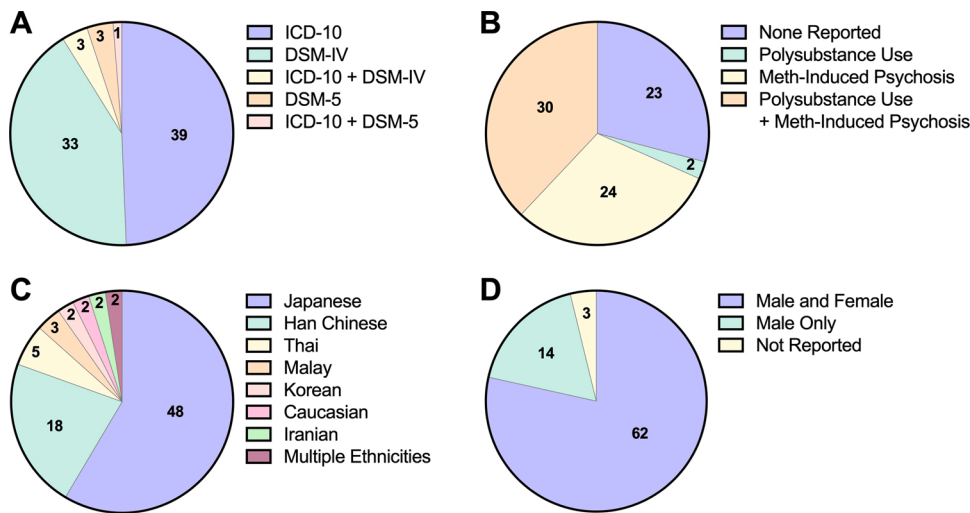


Fig. 2. Key characteristics of included studies. A. Diagnostic criteria. Studies predominantly used the ICD-10 and DSM-IV criteria. B. Comorbidities. Comorbidities were reported in 71 % of studies, with polysubstance use disorder and meth-induced psychosis the most common. C. Ethnicities. Participants of Japanese and Han Chinese descent were the most studied. Some studies belonged to more than 1 category. D. Sex. Most studies included both male and female participants, but 14 studies only included males. Three studies did not report participant sex. Numbers on each wedge represent the number of studies with the characteristic of interest.

investigated individually.

3.3. Meta-analyses

Specific markers of seven genes (*BDNF*, *COMT*, *DRD2*, *DRD4*, *FAAH*, *SLC6A3*, *SLC6A4*) were studied in three or more studies, and a meta-analytic approach was used to determine any potential association between markers in these genes and meth use disorder. Summary of the key meta-analytic findings are presented in Table 5.

3.3.1. *BDNF*

There were seven studies examining the rs6265 marker of *BDNF* (Bousman et al., 2010; Cheng et al., 2005; Jamjan et al., 2015; Itoh et al., 2005; Sim et al., 2010; Su et al., 2015b, 2014). rs6265 is located within the coding region of *BDNF*, in exon 9 (Fig. 3A). It is a missense mutation, substituting a valine (Val) for a methionine (Met) residue. The meta-analysis revealed a significant protective effect of the minor allele on meth use disorder ($p < 0.0001$), with an overall effect size of 0.78 [95% CI, 0.69, 0.88] (Fig. 3B). Heterogeneity analyses revealed low heterogeneity ($I^2 = 0\%$). Sensitivity analyses revealed that removing the study with the highest risk of bias (NHLBI score = 5) had no significant impact on the results or heterogeneity.

3.3.2. Dopaminergic signaling

There were five studies examining the rs1800497 marker of *DRD2* (Chen et al., 2004; Han et al., 2008; Šerý et al., 2001; Tsai et al., 2002; Ujike et al., 2009), four studies examining the exon 3 VNTR of *DRD4* (Chen et al., 2004; Li et al., 2004; Tsai et al., 2002; Ujike et al., 2009), four studies examining the rs4680 marker of *COMT* (Bousman et al., 2010; Jugurnauth et al., 2011; Li et al., 2004; Suzuki et al., 2006), and three studies examining the 3' VNTR of *SLC6A3* (Hong et al., 2003; Liu et al., 2004; Ujike et al., 2003). rs1800497 is located downstream of *DRD2* (Fig. 4A), and is associated with reduced dopamine D2 receptor (*DRD2*) binding (Tunbridge et al., 2019). The exon 3 VNTR is located within a coding region of *DRD4* (Fig. 4C) and consists of 2–11 48-bp repeats. rs4680 is located within the coding region of *COMT*, in exon 5 (Fig. 4E). It is a missense mutation, substituting a Val for a Met residue, with the Val resulting in greater protein abundance and stability (Tunbridge et al., 2019). The 3' VNTR is located within the 3' UTR of *SLC6A3* (Fig. 4G) and consists 9–10 repeat alleles.

The meta-analysis showed no effect of any markers on meth use disorder (smallest $p = 0.09$), with effect sizes of 0.95 [95% CI, 0.83, 1.09] for *DRD2* (Figure 4B), 1.10 [95% CI, 0.92, 1.32] for *DRD4* (Figure 4D), 0.90 [95% CI, 0.79, 1.02] for *COMT* (Fig. 4F), and 1.17 [95% CI, 0.81, 1.69] for *SLC6A3* (Fig. 4H). Heterogeneity analyses revealed low

heterogeneity for *DRD2*, *DRD4* and *SLC6A3* ($I^2 = 0$ –20%), whereas *COMT* had considerable heterogeneity ($I^2 = 70\%$). Sensitivity analyses revealed that removing the study with the highest risk of bias for each gene (NHLBI score = 3 (*DRD2*) and 5 (*DRD4*, *COMT* and *SLC6A3*)) had no significant impact on the results or heterogeneity.

3.3.3. Serotonergic signaling

There were three studies examining the LPR marker of *SLC6A4* (Ezaki et al., 2008; Hong et al., 2003; Payer et al., 2012). The LPR is located in the promoter region of *SLC6A4* (Fig. 5A), with the short variant associated with reduced gene expression (Greenberg et al., 1999; Lesch et al., 1996). The meta-analysis revealed a moderate but significant protective effect of the long variant on meth use disorder ($p = 0.04$), with an overall effect size of 0.78 [95% CI, 0.61, 0.99] (Fig. 5B). Sensitivity analyses revealed that when the study with the highest risk of bias (NHLBI score = 5) was removed, the significant overall effect did not survive ($p = 0.07$). Heterogeneity analyses revealed low heterogeneity ($I^2 = 0\%$).

3.3.4. Endocannabinoid signaling

There were three studies examining the rs324420 marker of *FAAH* (Morita et al., 2005a; Sim et al., 2013; Zhang et al., 2020). rs324420 is located within the coding region of *FAAH*, in exon 4 (Fig. 6A). It is a missense mutation, substituting a proline (Pro) for a threonine (Thr) residue. The meta-analysis revealed a significant risk of the minor allele on meth use disorder ($p < 0.00001$), with an overall effect size of 1.64 [95% CI, 1.40, 1.92] (Fig. 6B). Heterogeneity analyses revealed considerable heterogeneity ($I^2 = 66\%$). Sensitivity analyses revealed that removing the study with the highest risk of bias (NHLBI score = 8) had no significant impact on the results or heterogeneity.

3.4. Secondary outcomes

3.4.1. Polysubstance use disorder

To investigate whether markers were specific to meth use disorder or common to multiple substances, the difference in genotype and allele distribution between people with polysubstance use disorder and people who exclusively used meth was examined (Table 6). Of the 34 studies reporting a co-morbid diagnosis of polysubstance use disorder, only 14 provided data on polysubstance use for a total of 38 markers in 17 different genes. Two markers (*GSTP1* rs1695; and *PICK1* rs713729) displayed significant genotypic or allelic association with meth use disorder only, three markers (*NPY1R* rs7687423; *SLC22A3* rs509707, rs7745775 and rs3106164; and *XBPI* −116C > G) with polysubstance use disorder only, and three markers (*ADORA2A* rs5751876; *GDNF*

Table 2
Genotype and allele frequencies of candidate gene association studies in meth use disorder.

Gene	Code	Variant	Reference	Meth				Control				p-value	Meth		Control		OR	95 % CI	p-value	Power (1 – β)
				M/ M	M/ m	m/ m	n	M/ M	M/ m	m/ m	n		M	m	M	m				
ACE	rs4646994	Del	Sery 2001	17	51	19	87	33	61	32	126	0.323	85	89	127	125	1.06	0.72–1.57	0.754	0.06
	rs56298433	SNP	Kobayashi 2011b	166	2	0	168	222	2	0	224	0.772	334	2	446	2	1.34	0.19–9.53	0.772	0.06
	rs10920568	SNP	Kobayashi 2011b	132	36	3	171	162	63	4	229	0.332	300	42	387	71	0.76	0.51–1.15	0.196	0.26
	rs5780149	SNP	Kobayashi 2011b	108	55	8	171	150	69	10	229	0.718	271	71	369	89	1.09	0.77–1.54	0.642	0.07
	rs6427994	SNP	Kobayashi 2011b	128	38	5	171	181	46	2	229	0.248	294	48	408	50	1.33	0.87–2.04	0.183	0.26
ADORA1	rs41264025	SNP	Kobayashi 2011b	162	9	0	171	215	14	0	229	0.937	333	9	444	14	0.86	0.37–2.00	0.721	0.07
	rs16851030	SNP	Kobayashi 2011b	80	67	24	171	89	116	24	229	0.071	227	115	294	164	0.91	0.68–1.22	0.522	0.10
	rs13306114	SNP	Kobayashi 2010	168	3	0	171	229	0	0	229	0.044	339	3	458	0	N/A	N/A	0.045	0.52
	Exon1 + 219	SNP	Kobayashi 2010	169	2	0	171	228	1	0	229	0.401	340	2	457	1	2.69	0.24–29.77	0.402	0.14
	rs13306116	SNP	Kobayashi 2010	162	8	1	171	219	10	0	229	0.504	332	10	448	10	1.35	0.56–3.28	0.507	0.10
ADORA2A	rs5751876	SNP	Kobayashi 2010	35	85	51	171	70	114	45	229	0.018	155	187	254	204	1.50	1.13–1.99	0.005	0.81
	rs34923252	SNP	Kobayashi 2010	144	26	1	171	181	43	5	229	0.257	314	28	405	53	0.68	0.42–1.10	0.116	0.35
AGT	rs699	SNP	Sery 2001	32	46	11	89	41	58	26	125	0.274	110	68	140	110	0.79	0.53–1.17	0.23	0.22
	rs3730358	SNP	Bousman 2010	86	26	5	117	50	24	2	76	0.32	198	36	124	28	0.81	0.47–1.39	0.433	0.12
AKT1	rs3803300	SNP	Ikeda 2006	136	43	3	182	364	68	5	437	0.048	315	49	796	78	1.59	1.09–2.32	0.017	0.67
	rs1130214	SNP	Ikeda 2006	63	91	28	182	124	234	79	437	0.286	217	147	482	392	0.83	0.65–1.07	0.149	0.30
	rs2498799	SNP	Ikeda 2006	40	98	44	182	121	211	105	437	0.3	178	186	453	421	1.12	0.88–1.44	0.348	0.16
	rs2494732	SNP	Ikeda 2006	86	79	17	182	212	192	33	437	0.756	251	113	616	258	1.07	0.82–1.40	0.594	0.08
	rs2498804	SNP	Ikeda 2006	63	92	27	182	142	206	89	437	0.275	218	146	490	384	0.85	0.67–1.10	0.215	0.23
ANRIL	rs2494743	SNP	Ghafouri-Fard 2020	120	82	18	220	109	85	12	206	0.516	322	118	303	109	1.02	0.75–1.38	0.905	0.05
	rs2498794	SNP	Ghafouri-Fard 2020	41	120	59	220	50	112	44	206	0.235	202	238	212	200	1.25	0.95–1.64	0.105	0.36
	rs1333045	SNP	Namvar 2020	48	94	63	205	73	129	48	250	0.016	190	220	275	225	1.42	1.09–1.84	0.009	0.74
	rs1333048	SNP	Namvar 2020	41	89	75	205	98	117	35	250	<0.001	171	239	313	187	2.34	1.79–3.06	<0.0001	0.99
	rs4790694	SNP	Bousman 2010	84	31	2	117	48	25	3	76	0.36	199	35	121	31	0.69	0.40–1.17	0.166	0.29
ARRB2	rs1045280	SNP	Ikeda 2007	138	39	0	177	470	74	2	546	0.02	315	39	1014	78	1.61	1.07–2.41	0.02	0.64
	rs2036657	SNP	Ikeda 2007	117	54	6	177	417	122	7	546	0.012	288	66	956	136	1.61	1.17–2.22	0.004	0.83
			Bousman 2010	129	44	4	177	436	103	7	546	0.133	302	52	975	117	1.43	1.01–2.04	0.043	0.52
			Cheng 2005	86	29	2	117	45	29	2	76	0.12	201	33	119	33	0.59	0.35–1.01	0.052	0.49
			Iamjan 2015	31	57	15	103	23	68	31	122	0.046	119	87	114	130	0.64	0.44–0.93	0.019	0.65
BDNF	rs6265	SNP	Itoh 2005	38	42	20	100	23	54	25	102	0.057	118	82	100	104	0.67	0.45–0.99	0.044	0.52
			Sim 2010	70	96	23	189	70	107	25	202	0.884	236	142	247	157	0.95	0.71–1.26	0.71	0.07
			Su 2014	56	99	31	186	36	84	34	154	0.256	211	161	156	152	0.78	0.58–1.06	0.114	0.36
			Su 2015b	64	96	40	200	51	118	50	219	0.136	224	176	220	218	0.79	0.60–1.04	0.095	0.39
			Su 2015a	62	93	39	194	94	189	90	373	0.2	217	171	377	369	0.81	0.63–1.03	0.085	0.40
CHRNA4	132C > T	SNP	Itoh 2005	175	14	0	189	183	19	0	202	0.477	364	14	385	19	0.78	0.39–1.58	0.487	0.11
	rs2030324	SNP	Su 2015a	52	97	51	200	67	102	48	217	0.492	201	199	236	198	1.18	0.90–1.55	0.233	0.22
	rs16917204	SNP	Su 2015a	64	97	39	200	53	119	47	219	0.206	225	175	225	213	0.82	0.63–1.08	0.157	0.29
	rs16917234	SNP	Su 2015a	132	59	8	199	144	66	8	218	0.976	323	75	354	82	1.00	0.71–1.42	0.989	0.05
	rs755203	SNP	Kishi 2008b	74	77	34	185	259	358	130	747	0.284	225	145	876	618	0.91	0.72–1.53	0.446	0.12
CHRNA4	rs2273506	SNP	Kishi 2008b	138	40	7	185	564	176	7	747	0.016	316	54	1304	190	1.17	0.85–1.63	0.338	0.16
	rs2273504	SNP	Kishi 2008b	56	84	45	185	232	351	164	747	0.786	196	174	815	679	1.07	0.85–1.34	0.585	0.09
	rs1044396	SNP	Kishi 2008b	107	61	17	185	393	297	57	747	0.225	275	95	1083	411	0.91	0.70–1.18	0.478	0.11
	rs1044397	SNP	Kishi 2008b	79	78	28	185	273	349	125	747	0.303	236	134	895	599	0.85	0.67–1.07	0.172	0.28
	rs2236196	SNP	Kishi 2008b	137	44	4	185	563	177	7	747	0.383	318	52	1303	191	1.12	0.80–1.55	0.516	0.10
CHRNA4	rs4522666	SNP	Kishi 2008b	59	94	32	185	247	357	143	747	0.737	212	158	851	643	0.99	0.78–1.24	0.907	0.05
	rs4845652	SNP	Kishi 2008b	141	48	2	191	607	132	10	749	0.062	330	52	1346	152	1.40	1.00–1.96	0.052	0.50
	rs2072658	SNP	Kishi 2008b	134	53	4	191	466	256	25	747	0.124	321	61	1188	306	0.74	0.55–1.00	0.047	0.51
	rs2072659	SNP	Kishi 2008b	128	55	8	191	559	179	13	751	0.034	311	71	1297	205	1.44	1.07–1.94	0.015	0.68
	rs2072660	SNP	Kishi 2008b	97	79	15	191	440	268	45	753	0.15	273	109	1148	358	1.28	1.00–1.65	0.054	0.48
CHRNA4	rs3811450	SNP	Kishi 2008b	130	59	2	191	580	163	8	751	0.028	319	63	1323	179	1.46	1.07–1.99	0.017	0.67

(continued on next page)

Table 2 (continued)

Gene	Code	Variant	Reference	Meth				Control				p-value	Meth		Control		OR	95 % CI	p-value	Power (1 – β)
				M/ M	M/ m	m/ m	n	M/ M	M/ m	m/ m	n		M	m	M	m				
COMT	rs4680	SNP	Bousman 2010	23	65	29	117	18	45	13	76	0.43	111	123	81	71	1.26	0.84–1.90	0.261	0.20
			Jugurnauth 2011	262	166	46	474	179	170	37	386	0.021	690	258	528	244	0.81	0.66–0.99	0.046	0.51
			Li 2004	228	150	32	410	181	172	37	390	0.034	606	214	534	246	0.77	0.62–0.95	0.016	0.69
			Suzuki 2006	57	65	21	143	94	85	21	200	0.313	179	107	273	127	1.28	0.93–1.77	0.123	0.34
CSNK1E	rs165599	SNP	Jugurnauth 2011	93	194	92	379	109	210	109	428	0.833	380	378	428	428	0.99	0.82–1.21	0.958	0.05
			Kotaka 2008	126	78	11	215	151	100	23	274	0.346	330	100	402	146	0.83	0.62–1.12	0.226	0.18
			Otani 2008				202				336		151	51	218	118	0.62	0.42–0.92	0.017	0.67
			Liu 2006	252	106	5	363	302	110	13	425	0.194	610	116	714	136	1.00	0.76–1.31	0.991	0.05
CYP2D6	N/A	SNP	Chen 2004	141	209	66	416	137	228	70	435	0.749	491	341	502	368	0.95	0.78–1.15	0.583	0.08
			Han 2008	8	26	3	37	19	18	3	40	0.054	42	32	56	24	1.78	0.92–3.45	0.088	0.40
			Sery 2001	57	34	2	93	75	53	4	132	0.769	148	38	203	61	0.85	0.54–1.35	0.5	0.10
			Tsai 2002	48	52	16	116	39	52	21	112	0.464	148	84	130	94	0.78	0.54–1.14	0.208	0.24
DRD1	rs1800497	RFLP	Ujike 2009	83	84	35	202	92	114	37	243	0.523	250	154	298	188	0.98	0.74–1.28	0.863	0.05
			Ujike 2009	143	51	8	202	181	60	2	243	0.08	337	67	422	64	1.31	0.90–1.90	0.152	0.30
			Ujike 2009	185	17	0	202	231	12	0	243	0.139	387	17	474	12	1.74	0.82–3.68	0.146	0.31
			Chen 2004	206	172	34	412	217	206	44	467	0.552	584	240	605	259	0.96	0.78–1.18	0.702	0.07
DRD2	rs1801028	SNP	Ujike 2009	100	87	15	202	126	95	22	243	0.639	287	117	347	139	1.02	0.76–1.36	0.906	0.05
			Chen 2004				393				414		751	35	780	48	0.76	0.48–1.18	0.222	0.23
			Li 2004	196	179	40	415	220	161	34	415	0.244	571	259	601	229	1.19	0.96–1.47	0.106	0.37
			Tsai 2002				116				112		215	17	212	12	1.40	0.65–3.00	0.389	0.14
DRD3	rs6280	SNP	Ujike 2009				202				243		191	11	229	14	0.94	0.42–2.12	0.885	0.05
			Ujike 2009	61	106	35	202	66	134	43	243	0.775	228	176	266	220	0.93	0.72–1.22	0.611	0.08
			Kishimoto 2008	175	22	0	197	239	4	0	243	<0.001	372	22	482	4	7.13	2.44–20.86	0.0001	0.98
			Sim 2014	237	14	3	254	283	17	1	301	0.499	488	20	583	19	1.26	0.66–2.38	0.481	0.11
DTNBP1	rs2619539	SNP	Kishimoto 2008	78	94	18	190	118	107	15	240	0.173	250	130	343	137	1.30	0.97–1.74	0.074	0.43
			Kishimoto 2008	182	15	0	197	225	7	0	232	0.031	379	15	457	7	2.58	1.04–6.40	0.034	0.56
			Morita 2005a	105	43	5	153	139	58	3	200	0.541	253	53	336	64	1.10	0.74–1.64	0.64	0.08
			Sim 2013	100	101	31	232	149	82	10	241	<0.001	301	163	380	102	2.02	1.51–2.70	<0.0001	0.99
FAAH	rs324420	SNP	Zhang 2020	227	186	17	430	426	192	13	631	<0.001	640	220	1044	218	1.65	1.33–2.03	<0.001	0.99
			Zhao 2018	492	260	39	791	258	171	18	447	0.147	1244	338	687	207	0.90	0.74–1.10	0.302	0.18
			Zhao 2018	373	347	71	791	212	189	44	445	0.82	1093	489	613	277	0.99	0.83–1.18	0.912	0.05
			Zhao 2018	668	118	5	791	353	90	5	448	0.039	1454	128	796	100	0.70	0.53–0.92	0.011	0.72
GABRA1	rs2279020	SNP	Lin 2003	83	117	46	246	131	201	100	432	0.358	283	209	463	401	0.85	0.68–1.07	0.162	0.29
GABRA6	rs4480562	SNP	Lin 2003	130	87	30	247	205	177	53	435	0.335	347	147	587	283	0.88	0.69–1.12	0.29	0.19
GABRB2	rs2229944	SNP	Lin 2003	230	17	1	248	407	29	1	437	0.915	477	19	843	31	1.08	0.61–1.94	0.788	0.06
GABRG2	rs4480617	SNP	Lin 2003	222	25	1	248	410	26	1	437	0.128	469	27	846	28	1.74	1.01–2.99	0.042	0.53
			Lin 2003	73	119	54	246	119	200	117	436	0.364	265	227	438	434	0.86	0.69–1.08	0.197	0.25
			Lin 2003	110	118	20	248	217	175	46	438	0.133	338	158	609	267	1.07	0.84–1.35	0.597	0.08
			Nishiyama 2005	87	75	16	178	157	113	18	288	0.363	249	107	427	149	1.23	0.92–1.65	0.164	0.29
GAD1	1128 + 99 > 4A	SNP	Nishiyama 2005	56	79	43	178	80	128	80	288	0.589	191	165	288	288	0.86	0.66–1.13	0.278	0.19
			Veerasakul 2017	25	53	22	100	27	49	26	102	0.761	103	97	103	101	0.96	0.65–1.42	0.839	0.05
			Veerasakul 2017	54	38	8	100	51	43	8	102	0.829	146	54	145	59	0.91	0.59–1.40	0.667	0.07
			Veerasakul 2017	22	50	28	100	44	40	18	102	0.005	94	106	128	76	1.90	1.28–2.83	0.001	0.89
GAD2	rs2236418	SNP	Hashimoto 2008	126	76	16	218	125	90	18	233	0.669	328	108	340	126	0.89	0.66–1.20	0.437	0.12
			Kishi 2008a	108	64	13	185	450	311	56	817	0.675	280	90	1211	423	0.92	0.71–1.20	0.534	0.10
			Hashimoto 2008	150	62	6	218	172	59	2	233	0.214	362	74	403	63	1.31	0.91–1.88	0.149	0.30
			Kishi 2008a	117	62	6	185	572	228	19	819	0.206	296	74	1372	266	1.29	0.97–1.72	0.081	0.42
GCLM	rs718875	SNP	Kishi 2008a	116	63	6	185	571	221	27	819	0.153	295	75	1363	275	1.26	0.95–1.68	0.111	0.36
			Kishi 2008a	107	73	5	185	532	250	37	819	0.047	287	83	1314	324	1.17	0.89–1.54	0.252	0.21
			Yoshimura 2011	112	72	21	205	202	155	24	381	0.147	296	114	559	203	1.06	0.81–1.39	0.669	0.07
			Yoshimura 2011	78	94	32	204	145	183	53	381	0.82	250	158	473	289	1.03	0.81–1.32	0.789	0.06
GDNF	rs10941370	SNP	Yoshimura 2011	85	91	29	205	154	183	44	381	0.567	261	149	491	271	1.03	0.81–1.32	0.789	0.06
			Yoshimura 2011	85	101	20	206	164	172	46	382	0.549	271	141	500	264	0.99	0.77–1.27	0.909	0.05

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Table 2 (continued)

Gene	Code	Variant	Reference	Meth				Control				p-value	Meth		Control		OR	95 % CI	p-value	Power (1 – β)
				M/ M	M/ m	m/ m	n	M/ M	M/ m	m/ m	n		M	m	M	m				
GHRL	rs2910704	SNP	Yoshimura 2011	88	94	24	206	143	190	48	381	0.471	270	142	476	286	0.88	0.68–1.13	0.297	0.18
	rs884344	SNP	Yoshimura 2011	128	69	8	205	219	140	22	381	0.402	325	85	578	184	0.82	0.61–1.10	0.185	0.27
	rs1549250	SNP	Yoshimura 2011	95	90	20	205	198	158	25	381	0.246	280	130	554	208	1.24	0.95–1.61	0.112	0.35
	rs2973049	SNP	Yoshimura 2011	51	111	43	205	109	178	93	380	0.241	213	197	396	364	1.01	0.79–1.28	0.96	0.05
	rs35683	SNP	Yoon 2005	89	28	1	118	103	33	8	144	0.114	206	30	239	49	0.71	0.44–1.16	0.171	0.28
	rs1050450	SNP	Hashimoto 2008	189	29	0	218	207	23	3	233	0.134	407	29	437	29	1.07	0.63–1.83	0.793	0.06
	rs1428920	SNP	Iamjan 2018	40	51	9	100	48	46	8	102	0.599	131	69	142	62	1.21	0.80–1.83	0.378	0.14
	rs3813296	SNP	Iamjan 2018	23	37	40	100	15	50	37	102	0.155	83	117	80	124	0.91	0.61–1.35	0.64	0.07
	rs989638	SNP	Iamjan 2018				100				102		91	9	93	9	1.02	0.39–2.69	0.965	0.05
	rs3761554	SNP	Iamjan 2018				100				102		64	36	72	30	1.35	0.75–2.44	0.318	0.17
GRIN1	rs502434	SNP	Iamjan 2018				100				102		45	55	60	42	1.75	1.00–3.05	0.049	0.50
	rs11146020	SNP	Chanasong 2013	56	35	9	100	65	33	5	103	0.4	147	53	163	43	1.37	0.86–2.17	0.182	0.26
	rs1126442	SNP	Chanasong 2013	62	36	2	100	54	46	3	103	0.381	160	40	154	52	0.74	0.46–1.18	0.207	0.25
	rs3821829	SNP	Tsunoka 2010	181	14	1	196	731	67	4	802	0.856	376	16	1529	75	0.87	0.50–1.51	0.613	0.08
GRM2	rs12487957	SNP	Tsunoka 2010	106	79	11	196	346	378	78	802	0.013	291	101	1070	534	0.70	0.54–0.89	0.004	0.82
	rs4687771	SNP	Tsunoka 2010	100	82	14	196	300	401	101	802	0.001	282	110	1001	603	0.65	0.51–0.83	<0.001	0.94
GSTA1	–69C > T	SNP	Hashimoto 2008	158	56	4	218	180	50	3	233	0.492	372	64	410	56	1.26	0.86–1.85	0.239	0.22
GSTM1	Del	Del	Koizumi 2004	82	63	12	157	88	97	15	200	0.269	227	87	273	127	0.82	0.60–1.14	0.242	0.22
GSTO1	rs4925	SNP	Hashimoto 2008	163	53	2	218	166	55	12	233	0.035	379	57	387	79	0.74	0.51–1.07	0.104	0.37
GSTP1	rs1695	SNP	Bousman 2010	15	58	44	117	9	29	38	76	0.22	88	146	47	105	0.74	0.48–1.15	0.178	0.27
		SNP	Hashimoto 2005	144	41	4	189	167	32	0	199	0.038	329	49	366	32	1.70	1.07–2.73	0.025	0.61
GSTT1	Deletion	Del	Hashimoto 2008				218				233		109	109	140	93	1.51	1.04–2.19	0.031	0.57
GSTT2	rs1622002	SNP	Hashimoto 2008	149	63	6	218	157	73	3	233	0.485	361	75	387	79	1.02	0.72–1.44	0.921	0.05
HTR2A	rs6311	SNP	Tsunoka 2010	58	96	42	196	262	374	166	802	0.708	212	180	898	706	1.08	0.87–1.35	0.497	0.10
	rs6313	SNP	Tsunoka 2010	52	95	49	196	220	386	196	802	0.965	199	193	826	778	1.03	0.83–1.28	0.795	0.06
	rs6693503	SNP	Kishi 2011c	124	59	14	197	257	70	10	337	0.002	307	87	584	90	1.84	1.33–2.55	0.0002	0.96
HTR6	rs1805054	SNP	Kishi 2011c	90	92	15	197	162	153	22	337	0.816	272	122	477	197	1.09	0.83–1.42	0.55	0.09
	rs4912138	SNP	Kishi 2011c	55	93	49	197	87	170	80	337	0.764	203	191	344	330	0.98	0.77–1.26	0.879	0.05
	rs3790757	SNP	Kishi 2011c	115	70	12	197	220	105	12	337	0.179	300	94	545	129	1.32	0.98–1.79	0.067	0.45
	rs9659997	SNP	Kishi 2011c	146	45	6	197	243	82	12	337	0.868	337	57	568	106	0.91	0.64–1.29	0.581	0.09
MAOA	T941G	SNP	Nakamura 2009				117				198		64	74	123	120	1.19	0.78–1.80	0.426	0.13
	30bp	VNTR	Nakamura 2009				115				197		82	52	146	95	0.97	0.63–1.50	0.907	0.05
	rs1800779	SNP	Okochi 2011	140	43	0	183	219	46	2	267	0.139	323	43	484	50	1.29	0.84–1.98	0.248	0.21
NOS3	rs2070744	SNP	Okochi 2011	140	42	1	183	219	47	1	267	0.358	322	44	485	49	1.35	0.88–2.08	0.168	0.28
	rs1799983	SNP	Okochi 2011	152	31	0	183	221	44	2	267	0.5	335	31	486	48	0.94	0.58–1.50	0.797	0.06
	rs3918188	SNP	Okochi 2011	107	62	14	183	140	110	17	267	0.286	276	90	390	144	0.88	0.65–1.20	0.425	0.13
	rs743507	SNP	Okochi 2011	130	49	4	183	183	78	6	267	0.849	309	57	444	90	0.91	0.63–1.31	0.61	0.08
	rs7830	SNP	Okochi 2011	49	91	43	183	85	121	61	267	0.496	189	177	291	243	1.12	0.86–1.46	0.399	0.13
NPY	rs16147	SNP	Okahisa 2009	96	99	24	219	119	128	41	288	0.537	291	147	366	210	0.88	0.68–1.14	0.339	0.16
NPY1R	rs7687423	SNP	Okahisa 2009	55	126	41	222	90	129	65	284	0.04	236	208	309	259	1.05	0.82–1.35	0.693	0.07
NQO1	rs1800566	SNP	Ohgake 2005	69	97	25	191	85	95	27	207	0.572	235	147	265	149	1.11	0.83–1.48	0.467	0.11
NQO2	N/A	Del	Ohgake 2005	117	59	15	191	123	74	10	207	0.333	293	89	320	94	1.03	0.74–1.44	0.843	0.05
NRG1	rs35753505	SNP	Okochi 2009	50	104	30	184	144	262	118	524	0.162	204	164	550	498	0.89	0.70–1.13	0.329	0.16
	rs241930	SNP	Okochi 2009	139	43	1	183	405	100	5	510	0.476	321	45	910	110	1.16	0.80–1.68	0.431	0.12
	rs6994992	SNP	Okochi 2009	48	102	30	180	146	280	107	533	0.53	198	162	572	494	0.95	0.75–1.20	0.659	0.07
	rs3924999	SNP	Okochi 2009	103	58	10	171	339	171	24	534	0.653	264	78	849	219	1.15	0.85–1.54	0.364	0.15
	IVS1 + 18	SNP	Kobayashi 2006	140	27	3	170	220	40	0	260	0.097	307	33	480	40	1.29	0.80–2.09	0.3	0.18
OPRD1	rs2234918	SNP	Kobayashi 2006	101	63	6	170	181	71	8	260	0.089	265	75	433	87	1.41	1.00–1.99	0.051	0.50
	rs4654327	SNP	Kobayashi 2006	109	54	7	170	190	62	8	260	0.143	272	68	442	78	1.42	0.99–2.03	0.056	0.48
			Bousman 2010	20	57	40	117	23	26	27	76	0.05	97	137	72	80	1.27	0.84–1.92	0.252	0.21
OPRM1	rs2075572	SNP	Ide 2006	73	43	12	128	154	72	6	232	0.012	189	67	380	84	1.60	1.11–2.31	0.011	0.72
			Ide 2004	50	56	25	131	67	99	47	213	0.434	156	106	233	193	0.82	0.60–1.12	0.213	0.23
	rs1799971	SNP	Ide 2006	49	54	25	128	67	99	47	213	0.435	152	104	233	193	0.83	0.60–1.13	0.233	0.22

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Table 2 (continued)

Gene	Code	Variant	Reference	Meth				Control				p-value	Meth		Control		OR	95 % CI	p-value	Power (1 – β)
				M/ M	M/ m	m/ m	n	M/ M	M/ m	m/ m	n		M	m	M	m				
PAI-1	IVS1–4980G	SNP	Ide 2004	130	8	0	138	177	10	0	187	0.99	268	8	364	10	1.09	0.42–2.79	0.863	0.05
	rs599548	SNP	Ide 2006	96	28	4	128	135	41	3	179	0.696	220	36	311	47	1.08	0.68–1.73	0.739	0.06
	rs598682	SNP	Ide 2006	101	26	1	128	154	23	2	179	0.207	228	28	331	27	1.51	0.86–262	0.146	0.31
	rs1799889	Del	Iwata 2004	87	80	17	184	115	138	35	288	0.251	254	114	368	208	0.79	0.60–1.05	0.105	0.37
	68-bp	VNTR	Nomura 2006a	85	48	10	143	149	59	1	209	0.001	218	68	357	61	1.83	1.24–2.68	0.002	0.87
PDYN	rs737622	SNP	Matsuzawa 2007	85	93	30	208	89	107	22	218	0.355	263	153	285	151	1.10	0.83–1.45	0.513	0.10
	rs3026682	SNP	Matsuzawa 2007	85	93	30	208	89	107	22	218	0.355	263	153	285	151	1.10	0.83–1.45	0.513	0.10
	rs11089858	SNP	Matsuzawa 2007	167	39	2	208	180	37	1	218	0.727	373	43	397	39	1.17	0.74–1.85	0.491	0.11
	rs713729	SNP	Matsuzawa 2007	166	37	5	208	150	63	5	218	0.025	369	47	363	73	0.63	0.43–0.94	0.022	0.62
	rs3952	SNP	Matsuzawa 2007	85	93	30	208	89	107	22	218	0.355	263	153	285	151	1.10	0.83–1.45	0.513	0.10
PICK1	rs2076369	SNP	Matsuzawa 2007	73	99	36	208	82	111	25	218	0.228	245	171	275	161	1.19	0.91–1.57	0.211	0.24
	rs2072513	SNP	Kanahara 2009	54	130	48	232	60	120	53	233	0.618	238	226	240	226	1.01	0.78–1.30	0.949	0.05
	rs165862	SNP	Kanahara 2009	54	126	52	232	59	117	57	233	0.676	234	230	235	231	1.00	0.77–1.29	1	0.05
	rs165793	SNP	Kanahara 2009	86	117	29	232	90	107	36	233	0.525	289	175	287	179	0.97	0.75–1.27	0.827	0.06
	rs165789	SNP	Kanahara 2009	55	127	50	232	60	118	55	233	0.676	237	227	238	228	1.00	0.77–1.29	1	0.05
PLAT	Alu	Del	Iwata 2004	48	96	41	185	77	146	65	288	0.967	192	178	300	276	1.01	0.78–1.31	0.954	0.05
	rs10108011	SNP	Kinoshita 2008	58	60	10	128	124	119	22	265	0.933	176	80	367	163	1.02	0.74–1.41	0.888	0.05
PPP3CC	rs2461491	SNP	Kinoshita 2008	34	68	26	128	82	130	53	265	0.654	136	120	294	236	1.10	0.82–1.48	0.536	0.09
	rs2461490	SNP	Kinoshita 2008	42	72	14	128	99	131	35	265	0.443	156	100	329	201	1.05	0.77–1.43	0.758	0.06
	rs2449340	SNP	Kinoshita 2008	44	69	15	128	112	122	31	265	0.293	157	99	346	184	1.19	0.87–1.61	0.279	0.19
	rs1116085	SNP	Kinoshita 2008	35	67	26	128	72	137	56	265	0.982	137	119	281	249	0.98	0.73–1.32	0.896	0.05
	rs17721321	SNP	Kishi 2010	170	29	0	199	292	43	2	337	0.47	369	29	627	47	1.05	0.65–1.70	0.847	0.05
PROKR2	rs6085086	SNP	Kishi 2010	77	97	25	199	185	121	31	337	0.001	251	147	491	183	1.57	1.21–2.05	0.001	0.92
	rs3746684	SNP	Kishi 2010	59	96	44	199	110	153	74	337	0.75	214	184	373	301	1.07	0.83–1.37	0.617	0.08
	rs3746682	SNP	Kishi 2010	91	84	24	199	121	155	61	337	0.042	266	132	397	277	0.71	0.55–0.92	0.01	0.73
	rs4815787	SNP	Kishi 2010	61	109	29	199	158	142	37	337	0.001	231	167	458	216	1.53	1.19–1.98	0.001	0.91
	rs1799729	SNP	Inada 2004	70	61	12	143	90	78	13	181	0.921	201	85	258	104	1.05	0.75–1.48	0.783	0.06
SIGMAR1	rs1800866	SNP	Inada 2004	61	65	17	143	86	83	14	183	0.396	187	99	255	111	1.22	0.87–1.69	0.245	0.22
	rs12778366	SNP	Kishi 2011a	146	47	4	197	249	64	5	318	0.546	339	55	562	74	1.23	0.85–1.79	0.273	0.19
	rs2273773	SNP	Kishi 2011a	98	83	16	197	155	131	32	318	0.762	279	115	441	195	0.93	0.71–1.23	0.617	0.08
	rs4746720	SNP	Kishi 2011a	66	94	37	197	113	155	50	318	0.656	226	168	381	255	1.11	0.86–1.43	0.42	0.13
	rs10997875	SNP	Kishi 2011a	144	46	7	197	199	111	8	318	0.02	334	60	509	127	0.72	0.51–1.01	0.055	0.49
SLC22A3	rs509707	SNP	Aoyama 2006	92	93	25	210	226	175	41	442	0.192	277	143	627	257	1.26	0.98–1.66	0.069	0.44
	rs4709426	SNP	Aoyama 2006	59	108	44	211	165	199	78	442	0.061	226	196	529	355	1.29	1.02–1.63	0.031	0.58
	rs7745775	SNP	Aoyama 2006	116	84	7	207	246	146	33	425	0.055	316	98	638	212	0.93	0.71–1.23	0.622	0.08
	rs3106164	SNP	Aoyama 2006	118	74	15	207	205	198	37	440	0.046	310	104	608	272	0.75	0.58–0.98	0.032	0.56
	rs3088442	SNP	Aoyama 2006	50	92	68	210	119	208	111	438	0.167	192	228	446	430	1.23	0.98–1.56	0.08	0.42
SLC6A3			Hong 2003	84	16	1	101	92	17	0	109	0.58	184	18	201	17	1.16	0.58–2.31	0.68	0.07
	3' VNTR	VNTR	Liu 2004	107	38	1	146	56	16	0	72	0.636	252	40	128	16	1.27	0.69–2.36	0.448	0.12
			Ujike 2003	105	18	1	124	138	20	2	160	0.834	228	20	296	24	1.08	0.58–2.01	0.803	0.05
	242C > T	SNP	Ujike 2003	101	2	0	103	104	2	0	106	0.977	204	2	210	2	1.03	0.14–7.38	0.977	0.07
	1342A > G	SNP	Ujike 2003	101	22	1	124	129	26	4	159	0.54	224	24	284	34	0.89	0.52–1.55	0.693	0.08
SLC6A4	2319 G > A	SNP	Ujike 2003	69	48	7	124	92	57	8	157	0.882	186	62	241	73	1.10	0.75–1.62	0.629	0.08
			Ezaki 2008	107	56	3	166	116	65	16	197	0.026	270	62	297	97	0.70	0.49–1.01	0.054	0.49
	5-HTTLPR	LPR	Hong 2003	59	33	10	102	61	35	16	112	0.603	151	53	157	67	0.82	0.54–1.26	0.366	0.15
			Payer 2012	14	22	17	53	11	20	16	47	0.939	50	56	42	52	0.90	0.52–1.58	0.724	0.06
	intron 2	VNTR	Hong 2003	86	16	1	103	100	12	0	112	0.324	188	18	212	12	1.69	0.79–3.60	0.169	0.28
SLC6A9	5-HTTVNTR	VNTR	Payer 2012	23	20	10	53	23	18	6	47	0.688	66	40	64	30	1.29	0.72–2.32	0.389	0.14
	rs2486001	SNP	Morita 2008	106	82	16	204	139	63	8	210	0.009	294	114	341	79	1.67	1.21–2.32	0.002	0.87
	rs2248829	SNP	Morita 2008	119	75	10	204	110	76	24	210	0.049	313	95	296	124	0.72	0.53–0.99	0.042	0.53
	rs2248632	SNP	Morita 2008	122	70	12	204	113	81	16	210	0.442	314	94	307	113	0.81	0.59–1.12	0.199	0.25
	rs1372520	SNP	Kobayashi 2004	142	27	1	170	141	18	2	161	0.388	311	29	300	22	1.27	0.72–2.26	0.413	0.13
SNCA	rs3756063	SNP	Kobayashi 2004	140	29	1	170	141	18	2	161	0.263	309	31	300	22	1.37	0.77–2.42	0.279	0.19

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Table 2 (continued)

Gene	Code	Variant	Reference	Meth				Control				p-value	Meth		Control		OR	95 % CI	p-value	Power (1 – β)
				M/ M	M/ m	m/ m	n	M/ M	M/ m	m/ m	n		M	m	M	m				
SOD2	rs2870027	SNP	Kobayashi 2004	60	88	22	170	55	75	31	161	0.281	208	132	185	137	0.86	0.63–1.17	0.33	0.16
	rs3756059	SNP	Kobayashi 2004	141	28	1	170	141	18	2	161	0.322	310	30	300	22	1.32	0.74–2.34	0.341	0.16
	T10A	Var	Kobayashi 2004				170				161	0.677								
	rs2758357	SNP	Nakamura 2006 (Japanese)	75	36	2	113	130	47	9	186	0.22	186	40	307	65	1.02	0.66–1.57	0.944	0.05
			Nakamura 2006 (Han)	79	53	2	134	126	70	4	200	0.677	211	57	322	78	1.12	0.76–1.64	0.577	0.09
			Nakamura 2006 (Japanese)	84	30	2	116	152	33	4	189	0.211	198	34	337	41	1.41	0.87–2.30	0.164	0.28
	rs4880	SNP	Nakamura 2006 (Han)	86	43	2	131	150	47	5	202	0.145	215	47	347	57	1.33	0.87–2.03	0.183	0.27
	rs2855116 & rs2758332	SNP	Nakamura 2006 (Japanese)	91	24	1	116	153	33	3	189	0.689	206	26	339	39	1.10	0.65–1.86	0.73	0.06
			Nakamura 2006 (Han)	90	41	2	133	151	48	4	203	0.338	221	45	350	56	1.27	0.83–1.95	0.268	0.20
	rs2758330	SNP	Nakamura 2006 (Japanese)	27	53	35	115	54	83	48	185	0.497	107	123	191	179	1.23	0.88–1.71	0.224	0.23
			Nakamura 2006 (Han)	42	63	29	134	54	105	41	200	0.59	147	121	213	187	0.94	0.69–1.28	0.684	0.07
	rs2842980	SNP	Nakamura 2006 (Japanese)	27	53	35	115	53	84	50	187	0.606	107	123	190	184	1.19	0.85–1.65	0.307	0.18
			Nakamura 2006 (Han)	41	62	31	134	55	104	41	200	0.59	144	124	214	186	0.99	0.73–1.35	0.953	0.05
	TAAR1	rs8192620	SNP	Loftis 2019			75				31		37	38	17	14	1.25	0.54–2.89	0.606	0.08
	TNFA	308G > A	SNP	Nomura 2006b	175	9	0	184	207	7	0	0.412	359	9	421	7	1.51	0.56–4.09	0.417	0.13
		857C > T	SNP	Nomura 2006b	123	52	8	183	134	68	17	0.267	298	68	336	102	0.75	0.53–1.06	0.103	0.37
	TNFARI	36A > G	SNP	Nomura 2006b	131	44	4	179	151	59	6	0.763	306	52	361	71	0.86	0.59–1.28	0.461	0.11
TPH2	rs11178998	SNP	Kobayashi 2011a	130	29	3	162	197	46	0	243	0.102	289	35	440	46	1.16	0.73–1.84	0.534	0.10
	Exon2+C18A	SNP	Kobayashi 2011a	146	16	0	162	222	21	0	243	0.673	308	16	465	21	1.15	0.59–2.24	0.68	0.07
	rs10879348	SNP	Kobayashi 2011a	136	26	0	162	206	37	0	243	0.823	298	26	449	37	1.06	0.63–1.79	0.83	0.06
	rs17110566	SNP	Kobayashi 2011a	123	35	4	162	173	64	6	243	0.552	281	43	410	76	0.83	0.55–1.24	0.351	0.16
	rs4760816	SNP	Kobayashi 2011a	49	85	28	162	65	121	57	243	0.313	183	141	251	235	0.82	0.62–1.09	0.176	0.27
	rs4290270	SNP	Kobayashi 2011a	53	80	29	162	79	115	49	243	0.841	186	138	273	213	0.95	0.72–1.26	0.728	0.06
	Exon11+(C3)500 (C2)	SNP	Kobayashi 2011a	159	3	0	162	242	1	0	243	0.151	321	3	485	1	4.53	0.47–43.77	0.152	0.30
	rs17110747	SNP	Kobayashi 2011a	92	63	7	162	136	95	12	243	0.955	247	77	367	119	0.96	0.69–1.37	0.815	0.06
	rs41317114	SNP	Kobayashi 2011a	119	38	5	162	187	50	6	243	0.717	276	48	424	62	1.19	0.79–1.79	0.402	0.13
	rs1630250	SNP	Kishi 2011b	70	100	45	215	84	165	69	318	0.293	240	190	333	303	0.87	0.68–1.11	0.267	0.20
TSNAX	rs766288	SNP	Kishi 2011b	93	91	31	215	128	141	49	318	0.786	277	153	397	239	0.92	0.71–1.18	0.507	0.10
	rs6662926	SNP	Kishi 2011b	58	105	52	215	90	164	64	318	0.537	221	209	344	292	1.11	0.87–1.42	0.388	0.14
TSPO/ PBR	rs6971	SNP	Rathitharan 2020				11				26		6	5	18	8	1.88	0.44–7.99	0.392	0.14
XBPI	–116C > G	SNP	Morita 2005b	72	64	17	153	80	88	32	200	0.274	208	98	248	152	0.77	0.56–1.05	0.1	0.38

CI = confidence interval; Del = deletion; LPR = linked polymorphic region; M = major allele; m = minor allele; OR = odds ratio; RFLP = restriction fragment length polymorphism; SNP = single nucleotide polymorphism; Var = sequence variation; VNTR = variable number tandem repeat. Values in **bold** are statistically significant ($p < 0.05$).

Table 3

Characteristics of markers significantly associated with meth use disorder.

Gene	Marker	Location	Alleles (M > m)	Variant effect
ADORA2A	rs5751876	Exon 2	C > T	Synonymous variant (Tyr > Tyr).
	rs13306114	5' UTR	C > T	No known effect.
	rs3730358	Intron variant	C > A	No known effect.
AKT1	rs3803300	8kb upstream of <i>AKT1</i>	C > T	No known effect.
	rs1130214	Intron variant	C > A	No known effect.
	rs2498799	Exon 8	C > T	Synonymous variant (Glu > Glu).
ANRIL	rs1333048	5 kb downstream of <i>ANRIL</i>	A > C	No known effect.
	rs1333045	Intron variant	T > C	No known effect.
	rs1045280	Exon 11	G > A	Synonymous variant (Ser > Ser).
ARRB2	rs4790694	1.5 kb downstream of <i>ARRB2</i>	A > C	No known effect.
	rs2036657	500 bases downstream of <i>ARRB2</i>	G > A	No known effect.
	rs6265	Exon 9	G > A	Missense variant (Val > Met). Met is associated with reduced secretion of BDNF protein (Egan et al., 2003).
BDNF	rs16917204	Intron variant	G > C	No known effect.
	rs16917234	Intron variant	T > C	No known effect.
	rs2030324	Intron variant	A > G	No known effect.
CHRNA4	rs2273506	Exon 2	G > A	Synonymous variant (Leu > Leu).
	rs1044397	Exon 5	G > C	Synonymous variant (Ala > Ala).
	rs2072659	3' UTR	C > G	No known effect.
CHRN2	rs3811450	3' UTR	C > G	No known effect.
	rs2072658	2kb upstream of <i>CHRN2</i>	G > A	A-allele associated with reduced gene expression in vitro (Hoft et al., 2011).
				Missense variant (Val > Met). Val associated with greater protein abundance and stability, and greater enzyme activity (Tunbridge et al., 2019).
COMT	rs4680	Exon 4	G > A	No consistent effect reported across studies (Tunbridge et al., 2019).
	rs165599	3' UTR	A > G	A range of allele combinations are association with increased, reduced, or no enzymatic activity (Bertilsson et al., 2002).
CYP2D6	N/A	Combination of alleles	N/A	A2 allele is associated with increased D2R binding (Tunbridge et al., 2019).
DRD2	rs1800497	downstream of <i>DRD2</i>	A2 > A1	No consistent effect reported across studies (Tunbridge et al., 2019).
DRD4	48bp-repeat	Exon 3	2–11 repeats	No known effect.
DTNBP1	rs3213207	Intron variant	A > G	No known effect.
	rs2619538	2kb upstream of <i>DTNBP1</i>	A > T	No known effect.
	rs2619539	Intron variant	C > A	No known effect.
FAAH	rs324420	Exon 3	C > A	Missense variant (Pro > Thr). Thr associated with reduced enzymatic catalytic activity and reduced protein stability (Chiang et al., 2004; Dincheva et al., 2015).
GABRA1	rs2279020	Intron variant	G > A	No known effect.
GABBR1	rs715044	Intron variant	G > T	No known effect.
	rs2076483	Intron variant	A > G	No known effect.
	rs29221	Intron variant	C > G	No known effect.
GABRG2	rs4480617	5' UTR	C > T	No known effect.
	315C > T	Exon 3	C > T	Synonymous variant (Asn > Asn).
	1128 + 99C > A	Intron variant	C > A	No known effect.
GAD1	rs769404	Exon 2	T > C	Synonymous variant (His>His).
	rs701492	Intron variant	C > T	No known effect.
	rs2236418	5' UTR	A > G	G-allele associated with 6-fold increase in transcription levels (Boutin et al., 2003).
GCLM	rs3170633	3' UTR	C > T	No known effect.
GDNF	rs2910702	Intron variant	T > C	No known effect.
	rs2910704	Intron variant	C > G	No known effect.
	rs884344	Intron variant	A > C	No known effect.
GRIA3	rs502434	Exon 10	T > C	Synonymous variant (Asn > Asn).
	rs3821829	4kb upstream of <i>GRM2</i>	C > T	No known effect.
	rs4687771	2.5 kb downstream of <i>GRM2</i>	T > A	No known effect.
GRM2	rs12487957	3.5 kb downstream of <i>GRM2</i>	T > C	No known effect.
				Missense variant (Ala > Asp). Asp associated with reduced catalytic enzymatic activity (Menon and Board, 2013).
GSTO1	rs4925	Exon 4	C > A	Missense variant (Ile > Val). Val is associated with reduced catalytic enzymatic activity and reduced protein stability (Johansson et al., 1998).
GSTP1	rs1695	Exon 5	A > G	Deletion of the <i>GSTT1</i> .
GSTT1	Deletion	N/A	N/A	No known effect.
HTR6	rs6693503	4kb upstream of <i>HTR6</i>	A > G	Synonymous variant (Tyr > Tyr).
	rs1805054	Exon 1	C > T	No known effect.
	rs4912138	Intron variant	G > A	No known effect.
NPY1R	rs7687423	Intron variant	A > G	No known effect.
OPRM1	rs2075572	Intron variant	G > C	No known effect.
PDYN	68-bp repeat	5' UTR	1–5 repeats	Three or four repeats associated with increased <i>PDYN</i> expression compared to one or two copies (Zimprich et al., 2000).
	rs713729	Intron variant	T > A	No known effect.
	rs737622	5' UTR	C > G	G-allele associated with increased gene expression (Matsuzawa et al., 2007).
PICK1	rs3026682	5' UTR	G > A	A-allele associated with increased gene expression (Matsuzawa et al., 2007).

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Table 3 (continued)

Gene	Marker	Location	Alleles (M > m)	Variant effect
PROKR2	rs11089858	Intron variant	G > A	No known effect.
	rs3952	Intron variant	A > G	No known effect.
	rs2076369	Intron variant	T > G	No known effect.
	rs6085086	Intron variant	G > A	No known effect.
	rs4815787	3' UTR	G > A	No known effect.
	rs3746682	Exon 2	C > A	Synonymous variant (Thr > Thr).
	rs3746684	Exon 2	G > A	Synonymous variant (Leu > Leu).
SIRT1	rs17721321	Intron variant	G > A	No known effect.
	rs10997875	1.5 kb downstream of SIRT1	C > T	No known effect.
SLC22A3	rs3106164	Intron variant	C > T	No known effect.
	rs4709426	Intron variant	C > G	No known effect.
	rs509707	Intron variant	C > T	No known effect.
SLC6A3	rs7745775	Intron variant	T > G	No known effect.
	3' VNTR	3' UTR	9–10 repeats	Some evidence of association of 10 repeats with dopamine transporter binding (Tunbridge et al., 2019).
SLC6A4	LPR	Promoter region	Long > Short	Short variant is associated with reduced gene expression, transporter levels and serotonin uptake in vitro (Greenberg et al., 1999; Lesch et al., 1996).
SLC6A9	rs2486001	Intron variant	T > C	No known effect.
	rs2248829	Exon 7	G > A	Synonymous variant (Asp > Asp).
	rs1372520	Intron variant	G > A	No known effect.
SNCA	rs3756063	Intron variant	C > G	No known effect.
	rs3756059	Intron variant	G > A	No known effect.
	rs2758357	Intron variant	C > T	No known effect.
	rs4880	Exon 2	A > G	Missense variant (Val > Ala). Val is associated with reduced enzymatic activity and increased oxidative stress (Sutton et al., 2005).
SOD2	rs2855116	Intron variant	A > C	No known effect.
	rs2758332	Intron variant	C > A	No known effect.
	rs2758330	Intron variant	G > T	No known effect.
	rs2842980	3' UTR	A > T	No known effect.
TSNAX	rs1630250	2kb upstream of TSNAX	G > C	No known effect.
XBPI	–116C > G	Promoter region	C > G	G-allele associated with reduced transcriptional activity (Kakiuchi et al., 2003).

Ala = alanine; Asn = asparagine; Asp = aspartic acid; DRD2 = dopamine D2 receptor; Glu = glutamic acid; His = histidine; Ile = isoleucine; kb = kilobase; Leu = leucine; LPR = linked polymorphic region; M = major allele; m = minor allele; Met = methionine; Pro = proline; Ser = serine; Thr = threonine; Try = tyrosine; UTR = untranslated region; Val = valine; VNTR = variable number tandem repeat.

rs2910704; and PDYN 68-bp repeat) with both diagnoses. Of these, the genotype distribution of two markers (*GSTP1* rs1695; and *NPY1R* rs7687423) was significantly different between cases and controls but failed to reach allelic statistical significance. Characteristics for each significant marker are given in Table 3.

The minor allele of an intronic variant in *PICK1* (rs713729) was found to be protective ($OR < 1$) in people who exclusively use meth, suggesting a specific role for this gene in meth use disorder susceptibility. An intronic variant of *SLC22A3* (rs3106164) and a marker in the promoter region of *XBPI* (–116C > G) were found to be protective in polysubstance use only, while a different intronic marker in *SLC22A3* (rs509707) was found to confer a risk ($OR > 1$) for polysubstance use disorder. This suggests that both genes may be involved in the development of substance use disorders other than meth. A marker in the 5' UTR of *ADORA2A* (rs5751876) and the promoter region of *PDYN* (68-bp repeat) were found to confer a risk for both meth use and polysubstance use disorders, highlighting a potential shared genetic vulnerability for more than one substance use disorder. Subgroup analyses for *ADORA2A* and *PDYN* were adequately powered ($1 - \beta = 0.80–0.86$).

One marker in *GDNF* (rs2910702) was found to be protective in meth only ($OR = 0.59$), while a different marker (rs884344) was protective in polysubstance use only ($OR = 0.69$). In addition, a third marker (rs2910704) conferred a risk in people who exclusively used meth ($OR = 1.5$), whereas it was protective in polysubstance users ($OR = 0.7$). This suggests a complex involvement of *GDNF* in substance use disorder vulnerability. It should be noted that all three *GDNF* markers were located within an intron. In addition, none of the associations were adequately powered ($1 - \beta = 0.52–0.67$).

3.4.2. Sex differences

A total of 12 studies examined genotype and allele distribution between male and female participants separately for 45 markers across 18 different genes (Table S2). Notably, three markers (*ADORA2A* rs5751876; *ARRB2* rs1045280; and *GABRG2* rs4480617) previously shown to confer a significant risk for meth use disorder (Table 2) were only significant in females and not in males (Table S2). In addition, six markers (*CHRNA4* rs1044397; *GABRA1* rs2279020; *SNCA* rs1372520, rs3756063 and rs3756059; and *TSNAX* rs1630250) displayed significantly different allele distribution between people with meth use disorder and controls when split by sex, although significance was not reached when combined. The intronic variant of *GABRA1* (rs2279020) was only protective in females, whereas the synonymous variant located in exon 5 of *CHRNA4* (rs1044397) was only protective in males. In contrast, three intronic markers of *SNCA* (rs1372520, rs3756063, rs3756059) were shown to confer a risk for meth use disorder in females only, whereas a marker ~2 kilobases upstream of *TSNAX* (rs1630250) was a risk marker in males only. It should be noted that nearly all associations were underpowered in this subgroup analyses ($1 - \beta = 0.05–0.81$), with the association between *GABRG2* rs4480617 and meth use disorder in female participants the only study adequately powered ($1 - \beta = 0.81$). Characteristics for each significant marker are given in Table 3.

3.4.3. Age of onset of meth use

Nine studies investigated the difference in genotype and allele distribution between adolescent-onset (< 20 years of age) and adult-onset of meth use (≥ 20 years of age) in markers in 9 different genes

Table 4
Summary of haplotype studies.

Gene	Ref	Haplotype markers				Frequency		p-value
						Meth	Control	
AKT1	Ghafouri-Fard 2020	rs2494743	rs2498794					
		T	T			0.4	0.43	0.93
		T	C			0.33	0.3	0.94
		C	C			0.21	0.18	1
		T	T			0.06	0.08	1
ANRIL	Ikeda 2006	rs3803300	rs1130214	rs3730358	rs2498799			
		A	G	C	G	0.28	0.2	0.023
		A	G	T	A	0.074	0.048	0.049
		G	G	C	G	0.12	0.21	0.0032
	Namvar 2020	rs1333045	rs1333048					
ARRB2	Ikeda 2007	C	A			0.391	0.322	0.918
		T	A			0.026	0.303	<0.0001
		C	C			0.072	0.227	<0.0001
		T	C			0.51	0.146	<0.0001
		rs1045280	rs4790694	rs2036657		Global		0.0175
BDNF	Su 2015a	T	C	A		0.802	0.854	0.0194
		C	A	G		0.086	0.052	0.021
		rs16917204	rs16917234	rs2030324				
		C	C	T		0.028	0.068	0.008
		C	T	T		0.381	0.393	0.772
COMT	Jugurnauth 2011	G	C	C		0.148	0.104	0.049
		G	T	C		0.316	0.325	0.826
		G	T	T		0.087	0.076	0.539
		rs4680	rs165599					
		A	A			0.24	0.23	0.46
DTNBP1	Kishimoto 2008	A	G			0.03	0.08	0.0006
		G	A			0.26	0.27	0.59
		G	G			0.47	0.42	0.15
		rs2619539	rs3213207	rs2619538		Global		0.0005
		C	A	A		0.6046	0.7101	0.0013
GABBR1	Zhao 2018	G	A	A		0.3315	0.2741	0.076
		C	G	T		0.0318	0.0022	0.0012
		C	G	A		0.0178	0.0023	0.11
		C	A	T		0.0055	0.0073	0.83
		G	G	A		0.0089	0	0.15
GABRG2	Nishiyama 2005	G	A	T		0	0.0039	0.18
		rs2076483	rs29221	rs715044				
		C	G	A		0.081	0.108	0.024
		C	G	C		0.133	0.123	0.477
		T	C	C		0.309	0.312	0.884
GAD1	Veerasakul 2017	T	G	C		0.477	0.457	0.339
		315C4T	1128+99C4A			Global		0.044
		C	C			0.275	0.314	
		C	A			0.425	0.428	
		T	C			0.262	0.186	
GRM2	Tsunoka 2010	T	A			0.039	0.072	
		rs769404	rs701492			Global		0.038
		C	C			0.46	0.385	0.125
		C	T			0.055	0.12	0.021
		T	C			0.27	0.326	0.218
HTR2A	Tsunoka 2010	T	T			0.215	0.169	0.241
		rs3821829	rs12487957	rs4687771		Global		0.00746
		C	C	A		0.746	0.673	0.00822
		C	T	T		0.254	0.327	0.00822
		rs6311	rs6313			Global		0.589
HTR6	Kishi 2011c	A	T			0.083	0.0778	0.327
		G	T			0.465	0.467	0.468
		G	C			0.452	0.455	0.922
		rs6693503	rs1805054					
		A	C			0.519	0.574	0.0824
OPRM1	Ide 2006	A	T			0.287	0.292	0.863
		G	C			0.194	0.134	0.00931
		rs6693503	rs1805054	rs4912138				
		A	C	A		0.444	0.488	0.227
		A	C	G		0.0744	0.0862	0.351
OPRM1	Ide 2006	A	T	G		0.284	0.292	0.0772
		G	C	A		0.0798	0.00161	0.0001
		G	C	G		0.118	0.132	0.646
		rs1799971	rs2075572	rs599548	rs598682	Global		0.3
		A	C	A	A	0	0.002	
OPRM1	Ide 2006	A	C	A	G	0.005	0.024	
		A	C	G	A	0	0.014	

(continued on next page)

Table 4 (continued)

Gene	Ref	Haplotype markers						Frequency		p-value
								Meth	Control	
		A	C	G	G			0.336	0.329	
		A	G	A	A			0	0.005	
		A	G	A	G			0.115	0.08	
		A	G	G	A			0.109	0.051	
		A	G	G	G			0.028	0.029	
		G	C	A	G			0.015	0.006	
		G	C	G	G			0.382	0.441	
		G	G	A	G			0.005	0.015	
		G	G	G	A			0	0.004	
		G	G	G	G			0.004	0	
<i>PICK1</i>	Matsuzawa 2007	rs737622	rs3026682	rs11089858	rs713729	rs3952	rs2076369			
		C	G	G	T	A	T	0.337	0.352	0.63
		G	A	G	T	G	G	0.323	0.323	0.85
		C	G	G	A	A	G	0.092	0.145	<0.02
		C	G	A	T	A	G	0.074	0.083	0.66
		C	G	G	T	A	G	0.089	0.055	<0.09
		G	A	G	T	G	T	0.035	0.007	0.01
		C	G	G	A	A	T	0.017	0.012	0.66
		G	A	G	A	G	G	0.004	0.01	0.4
<i>PIK4CA</i>	Kanahara 2009	rs2072513	rs165862	rs165793	rs165789					
		G	C	T	A			0.457	0.451	1
		A	A	C	G			0.352	0.357	1
		A	A	T	G			0.112	0.105	0.996
		A	C	T	A			0.047	0.053	1
<i>PLG</i>	Iwata 2004	rs3757017	rs14224					Global	Global	0.75
		D	D					0.47	0.48	
		D	I					0.11	0.09	
		I	I					0.01	0.01	
		D						0.41	0.42	
<i>PROKR2</i>	Kishi 2010	rs17721321	rs6085086	rs3746684	rs3746682	rs4815787		Global		0.000959
		G	G	G	C	G		0.351	0.488	0.00019
		G	A	A	G	A		0.403	0.281	0.000471
		G	G	A	G	G		0.171	0.164	0.791
		G	G	G	G	G		0.0745	0.0673	0.704
<i>SLC6A9</i>	Morita 2008	rs2486001	rs2248829							
		C	G					0.48	0.54	0.08
		C	A					0.24	0.27	0.3
		T	G					0.28	0.16	0.000039
		T	A					0	0.027	N/A
<i>SLC22A3</i>	Aoyama 2006	rs509707	rs4709426					Global		0.178
		C	G					0.5104	0.566	
		T	C					0.3158	0.2607	
		C	C					0.1487	0.1432	
		T	G					0.02513	0.02998	
<i>SOD2</i>	Nakamura 2006 (Japanese)	rs2758357	rs4880	rs2855116 & rs2758332	rs2758330	rs2842980				
		C	V	A	G	A		0.4663	0.4819	0.7244
		C	V	A	T	T		0.3872	0.3755	0.7887
		T	A	B	T	T		0.1202	0.1024	0.5212
		T	V	A	T	T		0.0262	0.0311	0.7495
		C	V	A	T	A		0	0.0091	0.0861
	Nakamura 2006 (Han)	rs2758357	rs4880	rs2855116 & rs2758332	rs2758330	rs2842980				
		C	V	A	G	A		0.437	0.4374	0.9841
		C	V	A	T	T		0.3583	0.3852	0.4944
		T	A	B	T	T		0.1575	0.1038	0.0493
		T	V	A	G	A		0.0236	0.0489	0.0997
		T	A	A	T	T		0.0236	0.0246	0.9384
<i>TNFA</i>	Nomura 2006b	308G>A	857C>T							
		G	C					0.79	0.75	0.22
		G	T					0.19	0.23	0.12
		A	C					0.025	0.017	0.42

(Supplementary Table S3). *BDNF* (rs2910704)—discussed above—was the only significantly different marker at the genotypic level but failed to reach allelic statistical significance. It was also greatly underpowered ($1 - \beta = 0.07$).

4. Discussion

The present study systematically reviewed gene association studies in people with meth use disorder. A previous systematic review

uncovered 38 studies and provided a thorough examination of the field from 2000 to 2009 (Bousman et al., 2009). In the present review, we provide a significant update of this expanding field and reviewed a total of 79 studies. While the number of candidate gene studies has almost doubled in the past decade, there is still a clear lack of twin and family studies, as well as adequately powered GWASs. Our meta-analyses of gene markers investigated in three or more studies show that candidate gene association studies can be informative. However, it is critical that the choice of candidate variant is well rationalized. For example, a focus

Table 5

Summary of meta-analytic investigation of minor allele frequency in people with meth use disorder compared to controls.

SNP	OR	95 % CI	z-score	p-value	I ²
<i>BDNF</i> (rs6265)	0.78	0.69–0.88	4.13	<0.0001	0%
<i>DRD2</i> (rs1800497)	0.95	0.83–1.09	0.77	0.44	14%
<i>DRD4</i> (Exon 3 VNTR)	1.10	0.92–1.32	1.05	0.29	20%
<i>COMT</i> (rs4680)	0.90	0.79–1.02	1.68	0.09	72%
<i>SLC6A3</i> (3' UTR)	1.17	0.81–1.69	0.83	0.41	0%
<i>SLC6A4</i> (LPR)	0.78	0.61–0.99	2.01	0.04	0%
<i>FAAH</i> (rs324420)	1.64	1.40–1.92	6.21	<0.00001	66%

Notes: CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism.

on a functional variant with known biological significance would be important in understanding the role of a particular gene in meth use disorder. In the present study, we found that only ~25 % of the markers significantly associated with meth use disorder were located within a coding region, with only five markers leading to amino acid changes (Table 3). In addition, investigating several candidates from the same neurochemical pathway could reveal how these genes interact and may lead to susceptibility of the disorder of interest. Rationale for a candidate gene study can also include significant findings from preclinical studies involving rodents. There were many studies without a clear rationale.

Of the 75 genes examined in the literature to date, 29 had at least one marker displaying significant genotypic or allelic association with meth use disorder. Utilizing our present findings showing the power of each association experiment, we will focus our discussion on associations with adequate power across the entire sample ($1 - \beta \geq 0.80$), and any related secondary outcomes. We will also discuss key limitations in the current literature and highlight future research directions to further understand the potential genetic contributions toward meth use disorder.

4.1. Genes associated with meth use disorder

Fourteen markers in 11 genes were adequately powered, with NHLBI risk bias scores ranging from 4 (high risk of bias) to 9 (relatively low risk of bias). The large majority of genes examined were involved in neurotransmission, highlighting the need for future studies to examine other emerging areas identified to be involved in meth use such as inflammation and oxidative stress (Hacimusalar et al., 2019; Kohno et al., 2019; Luikinga et al., 2018; Shin et al., 2018; Zhang et al., 2018).

4.1.1. Endocannabinoid system

The most robust evidence with the biggest effect size for a genetic susceptibility to meth use disorder was the rs324420 variant of *FAAH*. Two reports found that the minor allele (A) was a risk for meth use disorder in Malaysian and Han Chinese samples, respectively (Sim et al., 2013; Zhang et al., 2020). A third report found no association in a Japanese sample (Morita et al., 2005a), though the study was highly underpowered ($1 - \beta = 0.08$). Meta-analysis revealed a strong overall effect ($p < 0.00001$), with the variant conferring ~1.64 greater odds for risk of meth use disorder (Fig. 6B). All three studies had a relatively low risk of bias, and sensitivity analyses revealed that removing the study with highest risk of bias had no significant impact on the results.

rs324420 is a variant in exon 3 of *FAAH* (Fig. 6A). It is a missense mutation which converts a proline (Pro/C-allele) to a threonine (Thr/A-allele) residue (Pro129Thr) in the Fatty Acid Amide Hydrolase (FAAH) protein. The main function of *FAAH* is to break down anandamide, an endocannabinoid (Deutsch and Chin, 1993). The Thr variant leads to reduced enzymatic catalytic activity and reduced protein stability in cell lines and rodents (Chiang et al., 2004; Dincheva et al., 2015). This leads to increased levels of anandamide in the brain. A study reported that participants carrying the minor allele displayed heightened reward-related ventral striatal activity and impulsivity (Hariri et al.,

2009). In addition, the variant may modulate the subjective effect of stimulants. In people with cocaine use disorder, minor allele carriers reported a stronger drug effect, and a higher “high” compared to homozygous major allele carriers when receiving a cocaine injection (Patel et al., 2018). The minor allele variant has previously been reported to confer a risk for comorbid illicit drug or alcohol use (OR = 4.88) (Sipe et al., 2002). This suggests that the association between the variant and substance use disorders is not specific to meth use. Consistent with this idea, we found no difference in allele distribution between people who exclusively use meth and polysubstance users (Table 6). Therefore, the rs324420 variant of *FAAH* may, in concert with other genetic and environmental factors, promote substance use disorders to a limited extent by increasing levels of anandamide in the brain, leading to heightened subjective response to drugs, higher impulsivity, and increased in reward-related activity in the ventral striatum. It would be informative to validate the effect of the minor allele on levels of anandamide in the brain of people with meth use disorder. New pharmacotherapies, such as the newly discovered inhibitor LEI-401 (Mock et al., 2020), could then be trialed to reduce levels of anandamide in minor allele carriers, and may be a promising approach to treat substance use disorders.

4.1.2. *BDNF*

While none of the seven studies exploring the *BDNF* rs6265 variant were adequately powered (range 0.07 – 0.52), the meta-analysis suggested an overall protective effect of the minor allele (A) on meth use disorder (OR = 0.78; $p < 0.0001$) (Fig. 3B). Sensitivity analyses revealed that removing the study with the highest risk of bias had no significant impact on the results. *BDNF* codes for brain-derived neurotrophic factor (BDNF), which plays a critical role in growth, differentiation and survival of neurons and synapses (Huang and Reichardt, 2001). Importantly, BDNF acting in the mesocorticolimbic dopamine system is a positive modulator of the rewarding effect of psychostimulants (evidence reviewed in Ghitza et al., 2010).

The rs6265 variant has been widely studied across a range of neurological and psychiatric disorders (Brueinig et al., 2016; Chen et al., 2017; Lin et al., 2014). It is a missense mutation substituting a valine (Val/G-allele) for a methionine (Met/A-allele) residue, with the Met variant leading to impaired intracellular trafficking and secretion of BDNF (Egan et al., 2003). The Met/A-allele may confer protection against meth use disorder by reducing levels of BDNF, and therefore the rewarding effect of meth. In addition, the Met/A-allele appears to be protective in people with heroin and nicotine use disorders (Haerian, 2013; Zhao et al., 2020), suggesting a shared genetic risk across substances.

Su et al. (2014, 2015b) investigated the effect of this variant on cognition and impulsivity in people with meth use disorder. While they found that meth users displayed poorer performance in memory, language, and visuospatial tasks, there were no genotype effects (Su et al., 2015b). In a second study, they reported an association between Val66Met and aspects of impulse control in people with meth use disorder, with homozygous Met/A-allele carriers reporting higher attentional impulsivity (Su et al., 2014). It should be noted that both studies were underpowered ($1 - \beta = 0.39–0.40$), and further research is needed to better understand the potential impact of rs6265 on cognitive function in people with meth use disorder.

4.1.3. Dopaminergic system

Considering the critical involvement of dopamine in the development of meth and other substance use disorders, it is not surprising that genes involved in mediation and modulation of dopaminergic signaling were among the most studied. Three markers in dopamine-related genes were adequately powered (*ADORA2A* rs5751876; *ARRB2* rs1045280; and *DTNBP1* rs3213207)

The gene with the largest effect size was *DTNBP1*, with the minor allele (G) of the rs3213207 variant conferring ~7.13 greater odds of

Table 6
Genotype and allele frequencies of candidate gene association studies between non-polydrug users and polydrug users.

Gene	Code	Reference	Drug Use Status	Meth				Control				p-value	Meth		Control		OR	95 % CI	p-value	Power (1 – β)
				M/M	M/m	m/m	n	M/M	M/m	m/m	n		M	m	M	m				
ADORA2A	rs5751876	Kobayashi 2010	Meth Only	6	29	17	52	70	114	45	229	0.01	41	63	254	204	1.91	1.24–2.95	0.003	0.84
			Polydrug Use	26	54	32	112	70	114	45	229	0.127	106	118	254	204	1.39	1.01–1.91	0.046	0.52
BDNF	rs6265	Itoh 2005	Meth Only	21	27	8	56	70	107	25	202	0.812	69	43	247	157	0.98	0.64–1.51	0.928	0.05
			Polydrug Use	47	60	15	122	70	107	25	202	0.768	154	90	247	157	0.92	0.66–1.28	0.616	0.08
	132C > T	Itoh 2005	Meth Only	51	5	0	56	183	19	0	202	0.913	107	5	385	19	0.95	0.35–2.60	0.915	0.05
			Polydrug Use	114	8	0	122	183	19	0	202	0.369	236	8	385	19	0.69	0.30–1.59	0.379	0.14
FAAH	rs324420	Morita 2005a	Meth Only	30	17	1	48	139	58	3	200	0.642	77	19	336	64	1.30	0.73–2.29	0.371	0.14
			Polydrug Use	75	26	4	105	139	58	3	200	0.352	176	34	336	64	1.01	0.64–1.60	0.951	0.05
	rs2973033	Yoshimura 2011	Meth Only	26	21	10	57	202	155	24	381	0.012	73	41	559	203	1.55	1.02–2.34	0.38	0.54
			Polydrug Use	82	48	11	141	202	155	24	381	0.366	212	70	559	203	0.91	0.66–1.25	0.553	0.09
	rs10941370	Yoshimura 2011	Meth Only	22	23	12	57	145	183	53	381	0.312	67	47	473	289	1.15	0.77–1.71	0.499	0.10
			Polydrug Use	54	66	20	140	145	183	53	381	0.983	174	106	473	289	1.00	0.75–1.32	0.984	0.05
	rs12518844	Yoshimura 2011	Meth Only	24	23	10	57	154	183	44	381	0.35	71	43	491	271	1.10	0.73–1.65	0.655	0.07
			Polydrug Use	57	65	19	141	154	183	44	381	0.82	179	103	491	271	1.04	0.79–1.39	0.774	0.06
GDNF	rs2910702	Yoshimura 2011	Meth Only	34	19	4	57	164	172	46	382	0.057	87	27	500	264	0.59	0.37–0.93	0.021	0.64
			Polydrug Use	50	77	15	142	164	172	46	382	0.17	177	107	500	264	1.14	0.86–1.52	0.348	0.16
	rs2910704	Yoshimura 2011	Meth Only	15	30	12	57	143	190	48	381	0.111	60	54	476	286	1.50	1.01–2.23	0.44	0.52
			Polydrug Use	70	60	12	142	143	190	48	381	0.042	200	84	476	286	0.70	0.52–0.94	0.017	0.67
	rs884344	Yoshimura 2011	Meth Only	30	23	4	57	219	140	22	381	0.776	83	31	578	184	1.17	0.75–1.83	0.481	0.11
			Polydrug Use	94	43	4	141	219	140	22	381	0.112	231	51	578	184	0.69	0.49–0.98	0.037	0.54
	rs1549250	Yoshimura 2011	Meth Only	26	28	3	57	198	158	25	381	0.547	80	34	554	208	1.13	0.74–1.74	0.573	0.09
			Polydrug Use	65	60	16	141	198	158	25	381	0.155	190	92	554	208	1.29	0.96–1.73	0.091	0.39
	rs2973049	Yoshimura 2011	Meth Only	18	28	11	57	109	178	93	380	0.685	64	50	396	364	0.85	0.57–1.26	0.421	0.13
			Polydrug Use	33	77	31	141	109	178	93	380	0.274	143	139	396	364	1.06	0.81–1.39	0.689	0.07
GSTP1	rs1695	Hashimoto 2005	Meth Only	44	9	2	55	167	32	0	199	0.026	97	13	366	32	1.53	0.77–3.03	0.217	0.24
			Polydrug Use	87	28	1	116	167	32	0	199	0.085	202	30	366	32	1.70	1.00–2.88	0.053	0.51
NPY	rs16147	Okahisa 2009	Meth Only	29	27	3	59	119	128	41	288	0.138	85	33	366	210	0.68	0.44–1.05	0.078	0.42
			Polydrug Use	64	69	20	153	119	128	41	288	0.945	197	109	366	210	0.96	0.72–1.29	0.805	0.06
NPY1R	rs7687423	Okahisa 2009	Meth Only	12	32	15	59	90	129	65	284	0.217	56	62	309	259	1.32	0.89–1.97	0.169	0.28
			Polydrug Use	40	91	25	156	90	129	65	284	0.031	171	141	309	259	0.98	0.75–1.30	0.908	0.05
NQO1	rs1800566	Ohgake 2005	Meth Only	17	31	4	52	85	95	27	207	0.188	65	39	265	149	1.07	0.68–1.67	0.775	0.06
			Polydrug Use	41	53	19	113	85	95	27	207	0.559	135	91	265	149	1.20	0.86–1.67	0.286	0.19
NQO2	N/A	Ohgake 2005	Meth Only	35	12	5	52	123	74	10	207	0.13	82	22	320	94	0.91	0.54–1.54	0.734	0.06
			Polydrug Use	68	36	9	113	123	74	10	207	0.464	172	54	320	94	1.07	0.73–1.57	0.733	0.06
PDYN	68-bp	Nomura 2006a	Meth Only	21	16	2	39	149	59	1	209	0.01	58	20	357	61	2.02	1.13–3.59	0.015	0.68
			Polydrug Use	56	31	8	95	149	59	1	209	<0.001	143	47	357	61	1.92	1.26–2.95	0.002	0.86
	rs737622	Matsuzawa 2007	Meth Only	23	23	9	55	89	107	22	218	0.364	69	41	285	151	1.12	0.73–1.73	0.604	0.08
			Polydrug Use	58	63	19	140	89	107	22	218	0.547	179	101	285	151	1.06	0.78–1.46	0.694	0.07
	rs3026682	Matsuzawa 2007	Meth Only	23	23	9	55	89	107	22	218	0.364	69	41	285	151	1.12	0.73–1.73	0.604	0.08
			Polydrug Use	58	63	19	140	89	107	22	218	0.547	179	101	285	151	1.06	0.78–1.46	0.694	0.07
	rs11089858	Matsuzawa 2007	Meth Only	44	11	0	55	180	37	1	218	0.772	99	11	397	39	1.13	0.56–2.29	0.732	0.06
			Polydrug Use	112	26	2	140	180	37	1	218	0.563	250	30	397	39	1.22	0.74–2.02	0.434	0.12
PICK1	rs713729	Matsuzawa 2007	Meth Only	47	7	1	55	150	63	5	218	0.045	101	9	363	73	0.44	0.21–0.92	0.025	0.61
			Polydrug Use	109	28	3	140	150	63	5	218	0.163	246	34	363	73	0.69	0.44–1.07	0.092	0.39
	rs3952	Matsuzawa 2007	Meth Only	23	23	9	55	89	107	22	218	0.364	69	41	285	151	1.12	0.73–1.73	0.604	0.08
			Polydrug Use	58	63	19	140	89	107	22	218	0.547	179	101	285	151	1.06	0.78–1.46	0.694	0.07
	rs2076369	Matsuzawa 2007	Meth Only	15	30	10	55	82	111	25	218	0.226	60	50	275	161	1.42	0.93–2.17	0.101	0.37
			Polydrug Use	53	62	25	140	82	111	25	218	0.195	168	112	275	161	1.14	0.84–1.55	0.409	0.13
	rs509707	Aoyama 2006	Meth Only	32	25	2	59	226	175	41	442	0.317	89	29	627	257	0.79	0.51–1.24	0.31	0.17
			Polydrug Use	57	65	22	144	226	175	41	442	0.024	179	109	627	257	1.49	1.12–1.96	0.005	0.80
SLC22A3	rs4709426	Aoyama 2006	Meth Only	21	32	6	59	165	199	78	442	0.255	74	44	529	355	0.89	0.60–1.32	0.55	0.09
			Polydrug Use	40	73	20	133	165	199	78	442	0.134	153	113	529	355	1.10	0.83–1.45	0.499	0.10
	rs7745775	Aoyama 2006	Meth Only	30	24	4	58	246	146	33	425	0.576	84	32	638	212	1.15	0.74–1.77	0.54	0.10
			Polydrug Use	81	59	2	142	246	146	33	425	0.014	221	63	638	212	0.86	0.62–1.18	0.348	0.16

(continued on next page)

Table 6 (continued)

Gene	Code	Reference	Drug Use Status	Meth				Control				Meth				p-value	Control		OR	95 % CI	p-value	Power (1 - β)
				M/M	M/m	m/m	n	M/M	M/m	m/m	n	M	m	M	m		M	m				
SLC6A3	rs3106164	Aoyama 2006	Meth Only	30	24	4	58	205	198	37	440	84	32	608	272	0.749	608	272	0.85	0.55–1.31	0.47	0.11
			Polydrug Use	84	48	11	143	205	198	37	440	216	70	608	272	0.036	608	272	0.72	0.53–0.98	0.038	0.55
	rs3088442	Aoyama 2006	Meth Only	13	25	21	59	119	208	111	438	51	67	446	430	0.239	446	430	1.36	0.93–2.01	0.117	0.34
			Polydrug Use	34	65	45	144	119	208	111	438	133	155	446	430	0.357	446	430	1.21	0.93–1.58	0.163	0.29
	exon 15	Ujike 2003	Meth Only	30	1	1	32	138	20	2	160	61	3	296	24	0.233	296	24	0.61	0.18–2.08	0.422	0.12
			Polydrug Use	74	15	0	89	138	20	2	160	163	15	296	24	0.377	296	24	1.13	0.58–2.22	0.712	0.07
	242C > T	Ujike 2003	Meth Only	33	0	0	33	104	2	0	106	66	0	210	2	0.427	210	2	NA	NA	0.428	0.12
			Polydrug Use	67	2	0	69	104	2	0	106	136	2	210	2	0.662	210	2	1.54	0.22–11.09	0.663	0.07
	1342A > G	Ujike 2003	Meth Only	28	7	1	36	129	26	4	159	63	9	284	34	0.898	284	34	1.19	0.55–2.61	0.658	0.07
			Polydrug Use	72	17	0	89	129	26	4	159	161	17	284	34	0.288	284	34	0.88	0.48–1.63	0.688	0.07
TAAR1	2319 G > A	Ujike 2003	Meth Only	21	13	2	36	92	57	8	157	55	17	241	73	0.994	241	73	1.02	0.56–1.87	0.948	0.05
			Polydrug Use	47	37	4	88	92	57	8	157	131	45	241	73	0.675	241	73	1.13	0.74–1.74	0.564	0.09
	rs8192620	Loffis 2019	Meth Only				32				31	19	13	17	14		17	14	0.83	0.31–2.26	0.716	0.07
			Polydrug Use				43				31	18	25	17	14		17	14	1.69	0.67–4.28	0.27	0.20
TNFA	308G > A	Nomura 2006b	Meth Only	55	3	0	58	207	7	0	214	113	3	421	7	0.495	421	7	1.60	0.41–6.27	0.499	0.10
			Polydrug Use	125	7	0	132	207	7	0	214	257	7	421	7	0.351	421	7	1.64	0.57–4.72	0.356	0.15
	857C > T	Nomura 2006b	Meth Only	35	18	5	58	134	68	17	219	88	28	336	102	0.976	336	102	1.05	0.65–1.69	0.848	0.05
TNFAR1			Polydrug Use	87	37	6	130	134	68	17	219	211	49	336	102	0.4	336	102	0.76	0.52–1.21	0.168	0.28
	36A > G	Nomura 2006b	Meth Only	41	14	1	56	151	59	6	216	96	16	361	71	0.849	361	71	0.85	0.47–1.53	0.58	0.09
XBPI			Polydrug Use	91	34	3	128	151	59	6	216	216	40	361	71	0.956	361	71	0.94	0.62–1.44	0.78	0.06
	–116C > G	Morita 2005b	Meth Only	19	22	7	48	80	88	32	200	60	36	248	152	0.961	248	152	0.98	0.62–1.55	0.928	0.05
			Polydrug Use	53	42	10	105	80	88	32	200	148	62	248	152	0.129	248	152	0.68	0.48–0.98	0.037	0.55

CI = confidence interval; M = major allele; m = minor allele; OR = odds ratio. Values in **bold** are statistically significant ($p < 0.05$).

developing meth use disorder in a sample of Japanese descent (Kishimoto et al., 2008). rs3213207 is an intronic variant. *DTNBP1* codes for Dystrobrevin-binding protein 1, or simply Dysbindin, a protein found in certain axon terminals throughout the brain (Benson et al., 2001). Dysbindin deficiency leads to increased extracellular dopamine receptor D2 (D2R), and increase strength of D2R signaling (Iizuka et al., 2007). Given that meth leads to an increase of dopamine concentration in the synaptic cleft (Cruickshank and Dyer, 2009; Riddle et al., 2008; Sulzer et al., 2005), it is possible that higher levels of extracellular dopamine in combination with increased concentration of D2R may contribute to the pathophysiology of meth use disorder. The rs3213207 variant has previously been shown to be associated with bipolar disorder, first episode psychosis and schizophrenia (Gaysina et al., 2009; Varela-Gomez et al., 2015; Wirgenes et al., 2009). In addition, there is evidence that haplotypes including rs3213207 are involved in the susceptibility for bipolar disorder and major depressive disorder (Duan et al., 2007; Kim et al., 2008; Pae et al., 2009, 2007). This is particularly notable because every participant with meth use disorder in Kishimoto et al. (2008) also presented with a co-morbid diagnosis of meth-induced psychosis. It is possible that this particular variant may be more broadly associated with psychotic disorders rather than meth use specifically.

Kobayashi et al. (2010) reported an association between the rs5751876 variant of *ADORA2A* and meth use disorder, with the C allele conferring a risk in a Japanese sample (OR = 1.50) (Kobayashi et al., 2010). *ADORA2A* codes for the adenosine A2A receptor, and plays an important role in striatal function (Fredholm et al., 2005). It is reported to form heterodimers with D2R, which may contribute towards the reinforcing effect of psychostimulants (Ballesteros-Yáñez et al., 2018; Fuxe et al., 2005). While the rs5751876 variant is a synonymous mutation (Table 3), it has been associated with the anxiogenic effect of amphetamines, with C-allele carriers reporting lower levels of anxiety (Hohoff et al., 2005). This has led to the suggestion that carrying this allele might lessen the adverse effect of amphetamine use, therefore promoting sustained use. Subgroup analyses revealed that the C allele was a risk allele in people who exclusively use meth as well as poly-substance users. Sex analyses revealed that the significant distribution was only found in female participants, although this secondary outcome analyses were underpowered ($1 - \beta = 0.19$ for males and 0.73 for females). There is other research to have illustrated sex-specific associations between the rs5751876 variant and anxiety and emotion processing (Domschke et al., 2012; Gajewska et al., 2013). However, at present there is no insight into how a change in DNA sequence that does not change protein sequence leads to a functional effect. Further work is needed to understand whether the C allele might alter the transcription, stability or translatability of the encoded RNA and hence receptor levels or turnover. Until such insight is available, weak associations between synonymous mutations and function must be viewed with extreme caution.

The minor allele (G) of *ARRB2* rs1045280 variant was found to confer a risk for meth use disorder (OR = 1.61) in Japanese people (Ikeda et al., 2007). Subgroup analyses revealed that the variant was only associated with meth use in female participants, although underpowered ($1 - \beta = 0.72$). While located in an exon, rs1045280 is a synonymous variant. *ARRB2* codes for the beta-arrestin-2 protein, which plays a critical role in modulating dopamine and many other G protein-coupled receptors and downstream behaviors (Beaulieu et al., 2005). Along with rs1045280, Ikeda et al. (2007) found that two other SNPs located downstream of *ARRB2* (rs2036657 and rs4790694) were also significantly associated with meth use disorder, though the associations were underpowered ($1 - \beta = 0.52$ – 0.64). In addition, they reported an association with two haplotypes comprising all three variants (Ikeda et al., 2007). In contrast, another report found a divergent trend in a Caucasian sample, with the rs4790694 variant seemingly protective (OR = 0.69; Bousman et al., 2010). As the role of these variants is still unknown, further work is needed to elucidate the potential influence of *ARRB2* polymorphisms on meth use disorder, including examination of

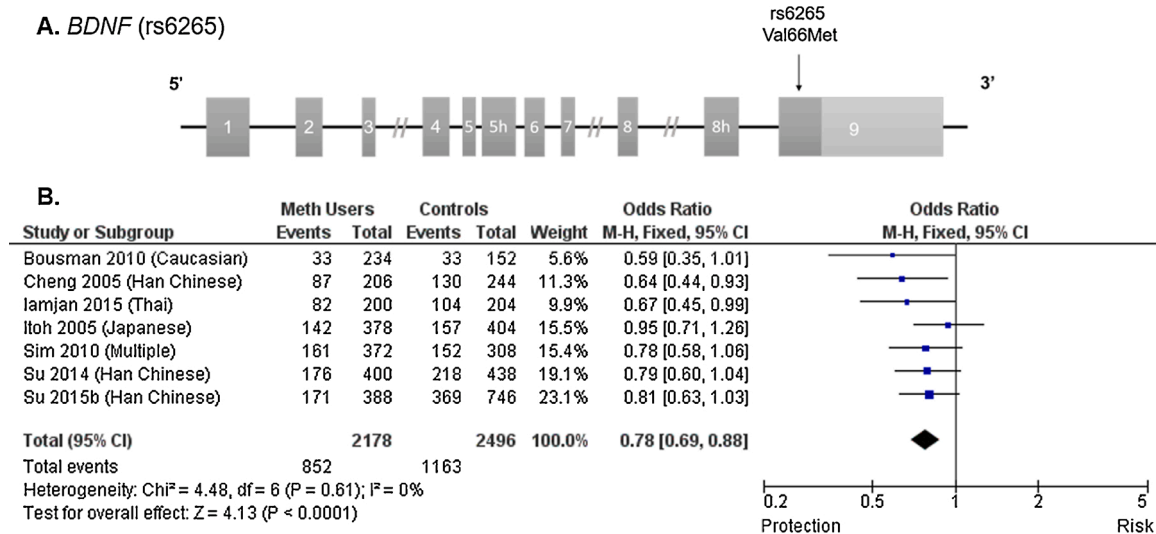


Fig. 3. *BDNF* rs6265. A. Schematic representation of *BDNF* and the location of rs6265. Numbers represent exons. Lighter shaded boxes represent non-coding regions. B. Forrest plot of the association between the rs6265 variant of *BDNF* and meth use disorder. There was a significant overall effect ($p < 0.0001$) of the variant, with the minor allele associated with protection against meth use disorder ($OR = 0.78$). Event = number of minor alleles; OR = odds ratio.

sex and ethnicity.

A meta-analytic approach was conducted on four markers in genes directly involved in dopamine signaling (*DRD2* rs1800497; *DRD4* Exon 3 VNTR; *COMT* rs4680; and *SLC6A3* 3' VNTR). None of the variants were associated with meth use disorder (smallest $p = 0.09$; [Fig. 4](#); [Table 5](#)), even after removing the study with the highest risk of bias for each analysis.

4.1.4. Serotonergic system

Three studies investigated the association between the linked polymorphic region (LPR) repeat in the promoter region of *SLC6A4* and meth use disorder (Ezaki et al., 2008; Hong et al., 2003; Payer et al., 2012). *SLC6A4* codes for the serotonin transporter (SERT), which primarily regulates serotonergic signaling by serotonin reuptake from the synaptic cleft back into the pre-synaptic neuron (Coleman et al., 2016). The short variant of the LPR is associated with reduced gene expression, transporter levels and serotonin uptake in vitro (Greenberg et al., 1999; Lesch et al., 1996). While individually none of the studies reported a significant allelic association, meta-analysis revealed a moderate overall protective effect of the LPR on meth use disorder (OR = 0.78; $p = 0.04$, Fig. 5B; Table 5). However, the significance did not survive sensitivity analyses, with exclusion of the study with the highest risk of bias resulting in a non-significant result.

Despite the lack of evidence for a role of genetic variations in *SLC6A4* in contributing to heritable risk for meth use disorder, the gene is implicated in the pathophysiology of meth addiction. There is evidence for a brain-region specific decrease in serotonergic activity and SERT expression in people with meth use disorder (Kish et al., 2009; Sekine

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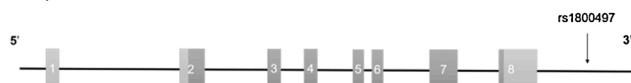
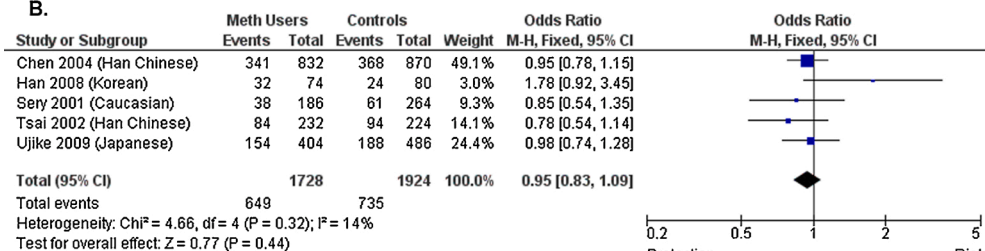
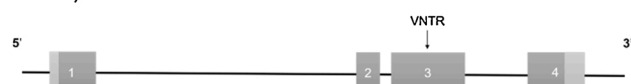
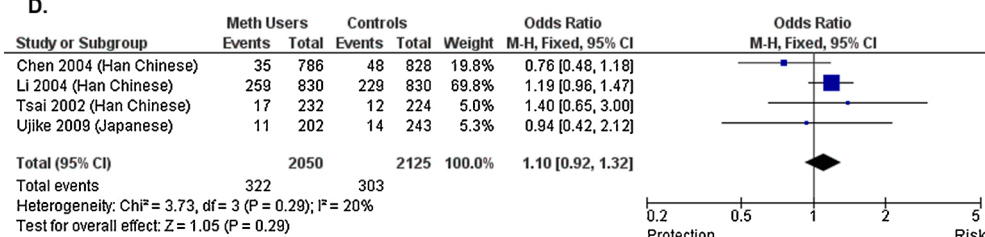
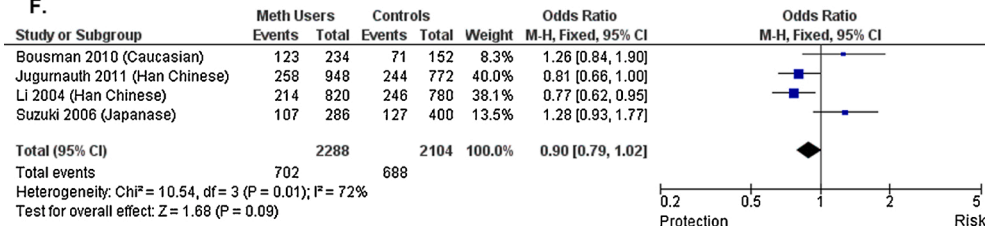
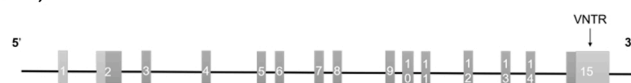
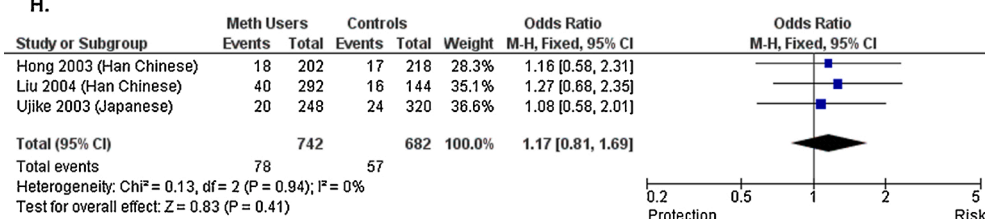
A. DRD2 (rs1800497)**B.****C. DRD4 (Exon 3 VNTR)****D.****E. COMT (rs4680)****F.****G. SLC6A3 (3' UTR)****H.**

Fig. 4. Makers in dopamine-related genes. A. Schematic representation of *DRD2* and the location of rs1800497. Numbers represent exons. Lighter shaded boxes represent non-coding regions. B. Forrest plot of the association between the rs1800497 variant of *DRD2* and meth use disorder. C. Schematic representation of *DRD4* and the location of the exon 3 VNTR. D. Forrest plot of the association between the exon 3 VNTR of *DRD4* and meth use disorder. E. Schematic representation of *COMT* and the location of rs4680. F. Forrest plot of the association between the rs4680 variant of *COMT* and meth use disorder. G. Schematic representation of *SLC6A3* and the location of the 3' VNTR. H. Forrest plot of the association between the 3' VNTR of *SLC6A3* and meth use disorder. There was no significant overall effect ($p > 0.05$) of any of the variants on meth use disorder. Event = number of minor alleles; VNTR = variable number tandem repeat.

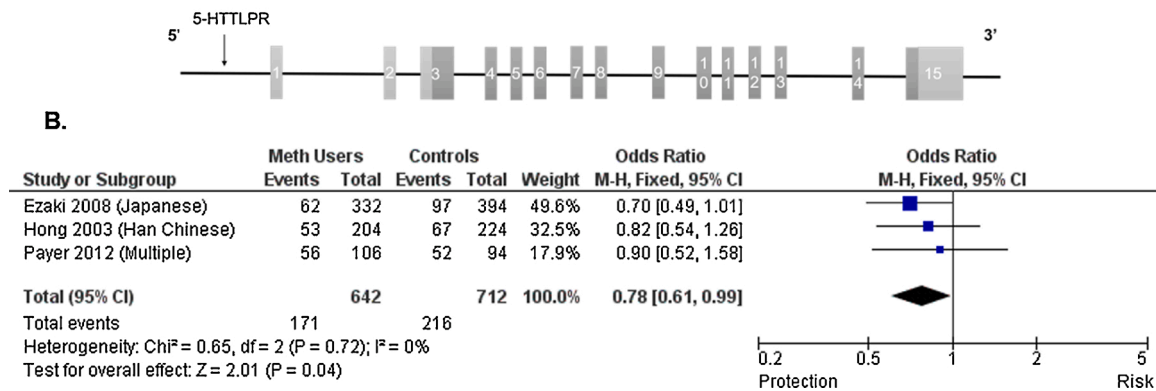
A. *SLC6A4* (LPR)

Fig. 5. A. *SLC6A4* LPR. Schematic representation of *SLC6A4* and the location of the LPR. Numbers represent exons. Lighter shaded boxes represent non-coding regions. B. Forrest plot of the association between the LPR variant of *SLC6A4* and meth use disorder. There was a moderate overall effect ($p = 0.04$) of the variant, with the minor allele associated with protection against meth use disorder (OR = 0.78). Event = number of minor alleles; LPR = linked polymorphic region; OR = odds ratio.

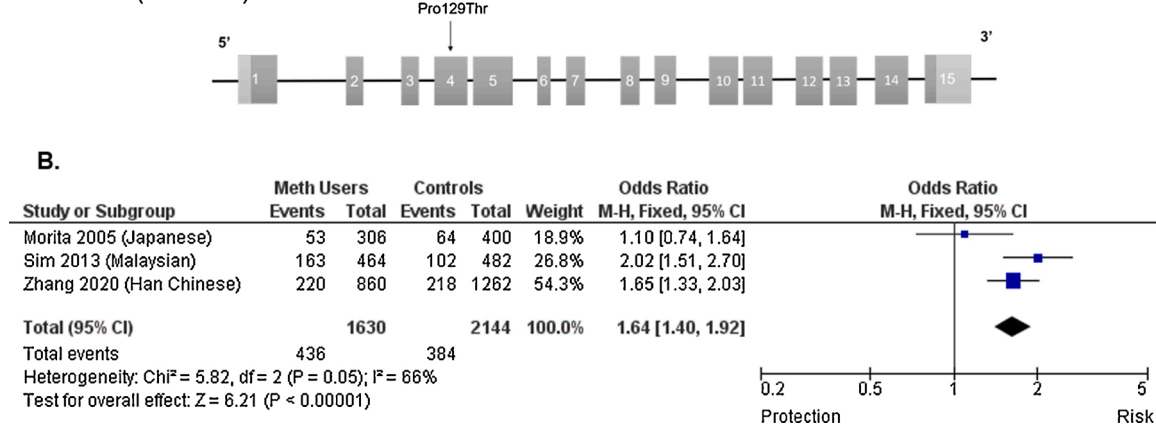
A. *FAAH* (rs324420)

Fig. 6. A. *FAAH* rs324420. Schematic representation of *FAAH* and the location of rs324420. Numbers represent exons. Lighter shaded boxes represent non-coding regions. B. Forrest plot of the association between the rs324420 variant of *FAAH* and meth use disorder. There was a significant overall effect ($p < 0.00001$) of the variant, with the minor allele associated with a risk for meth use disorder (OR = 1.64). Event = number of minor alleles; OR = odds ratio.

for meth use disorder in a Japanese sample (Morita et al., 2008). *SLC6A9* codes for the glycine transporter 1 (GlyT-1), which controls glycine neurotransmission and is implicated in modulating the N-methyl-D-aspartate (NMDA) receptor in the brain (Smith et al., 1992). The rs2486001 variant is an intronic variant without known function, whereas rs2248829 is located within exon 7 of *SLC6A9*. However, rs2248829 is a synonymous variant and has no effect on the structure of the GlyT-1 (Table 3). While silent mutations may have an effect on gene expression and rate of translation (Shabalina et al., 2013), this was never demonstrated for this variant of *GLYT1*. In addition, it is possible, though not yet demonstrated, that rs2486001 and rs2248829 are in linkage disequilibrium with other functional variants.

Another study reported that two variants (rs12487957 and rs4687771) of *GRM2* were protective (ORs = 0.70 and 0.65, respectively), with people with meth use disorder less likely to carry the minor alleles (C and A) compared to controls (Tsunoka et al., 2010). The haplotypes rs3821829(C)-rs12487957(C)-rs4687771(A) were associated with a risk for meth use disorder. This suggests that, while carrying only one of the minor alleles is protective, carrying both rs12487957 and rs4687771 may confer a risk. *GRM2* codes for the metabotropic

glutamate receptor 2 (mGlu2), and is expressed in neurons throughout the brain (Flor et al., 1995). Activation of mGlu2 generally inhibits glutamate release at synapses (Niswender and Conn, 2010). Neither variants are directly located in *GRM2*, with rs4687771 and rs12487957 located ~ 2.5 kilobases and ~3.5 kilobases downstream of the gene, respectively. While these variants may modulate gene expression, this has yet to be determined. Preclinical evidence suggests that activation of mGlu2 can reduce reinstatement of meth seeking after extinction and abstinence (Caprioli et al., 2015; Kufahl et al., 2013). It is therefore possible that the minor alleles of rs12487957 and rs4687771 lead to increased activity of mGlu2, and thereby less sensitivity to the behavioral effects of meth.

4.1.7. Endogenous opioid system

The 68-bp repeat in the promoter region of *PDYN* has been shown to be associated with a 1.83 increase in the risk of developing meth use disorder in a Japanese sample (Nomura et al., 2006a, 2006b). *PDYN* codes for prodynorphin, a precursor protein that gives rise to dynorphins when cleaved (Day et al., 1998). Dynorphins are endogenous ligands for the kappa opioid receptor, and form part of the endogenous opioid

system (Chavkin et al., 1982). Meth can increase expression of *PDYN* in the rodent brain (Adams et al., 2003; Cadet et al., 2016). The *PDYN* promoter region can contain one to five 68-bp tandem repeats (Rouault et al., 2011). Three or four copies of the 68-bp repeat increase *PDYN* expression compared to one or two copies (Zimprich et al., 2000). Another report suggests that changes in expression may be cell specific, with more repeats leading to increased gene expression in certain cell lines, and the reverse in others (Rouault et al., 2011). This highlights a complex relationship between the tandem repeat and *PDYN* expression. Subgroup analyses revealed that a greater number of tandem repeats were more common in both people who exclusively use meth and people with polysubstance use disorder compared to controls (Table 6). This suggests a potential shared genetic vulnerability for more than one substance use disorder. This is consistent with studies reporting an association between the number of repeats and heroin use disorder, as well as comorbid alcohol and cocaine use disorders (Williams et al., 2007; Yuanyuan et al., 2018). It is also consistent with a large literature implicating dynorphinergic signaling as a homeostatic, negative-feedback adaptation to chronic stimulant exposure (Carlezon et al., 2005).

4.1.8. Other notable genes

A recent study by Namvar et al. (2020) reported a strong association between the rs1333048 variant (C allele) of *ANRIL* and meth use disorder (OR = 2.34) (Namvar et al., 2020). rs1333048 is located ~5 kilobases downstream of *ANRIL*, and its effect on *ANRIL* expression is still unknown. *ANRIL*, or antisense non-coding RNA in the *INK4* locus, is a non-coding RNA involved in transcriptional silencing of other genes (Yap et al., 2010). Evidence suggests that *ANRIL* is only functional in higher primates (He et al., 2013), like many other long non-coding RNAs. It is associated with a wide range of disorders, including coronary heart disease, aneurysms, diabetes and several cancers (Pasmant et al., 2011). In their study, Namvar et al. (2020) also found an association between rs1333048 and a risk for bipolar disorders type 1 (OR = 2.33), type 2 (OR = 2.00), and major depressive disorder (OR = 2.42), suggesting a shared risk across neuropsychiatric disorders. The exact mechanism of action of *ANRIL* is still unknown. Results from a pre-clinical study suggest that *ANRIL* decreases apoptosis of hippocampal neurons and promotes memory recovery in a diabetic rat model (Wen et al., 2018). Memory deficits have been well documented in people with meth use disorder (Guerin et al., 2019; Potvin et al., 2018), and polymorphisms in *ANRIL* may be a contributing factor.

Kishi et al. (2010) reported an association between two variants (rs6085086(A) and rs4815787(A)) and two haplotypes of *PROKR2* and a risk for meth use disorder (Kishi et al., 2010). rs6085086 is located within an intron, whereas rs4815787 is within the 3' UTR of *PROKR2* and may modulate gene expression. *PROKR2* codes for the prokineticin receptor 2 (PKR₂), which primary function in the nervous system is regulation of circadian rhythm (Cheng et al., 2002). While the authors previously reported an association between these variants and mood disorders (Kishi et al., 2009), the biological effect of these SNPs and the role of PKR₂ in meth use disorder remains obscure.

4.2. Limitations

While the evidence reviewed in this study provides some insight into the genetic basis of meth use disorder, prominent limitations should be noted, and any results discussed in the present review should be taken with great caution. Major limitations for most gene markers include lack of independent replication, unclear rationale for candidate gene selection, study population selection, low power, and high risk of bias, especially in the absence of genome-wide significance. It should also be emphasized that the contribution of any of the candidate gene variants studied to date to the heritable risk for meth use disorder is likely to be extremely small. In fact, with the absence of genome-wide significance or robust association in a large family pedigree, demonstrating a

functional correlate of a genome sequence variation in one of these candidate genes is better interpreted as a contribution of that gene on the pathophysiology of meth use disorder and not specifically related to its genetic risk.

Of the 75 genes explored, 58 were only explored once. Replication in independent samples is critical to establish the validity of findings in individual studies. Also, in many instances there was no clear rationale for the selection of variants investigated, with ~65 % having no known or poorly understood biological function (Table 3). Further, none of the variants with a significant association have come up on GWASs. This may be because only three meth use disorder GWASs have been conducted to date, with small sample sizes and overlapping samples (Ikeda et al., 2013; Sun et al., 2019; Uhl et al., 2008b), highlighting the need for further research.

The present review also highlights that most studies were conducted in Japanese and Han Chinese samples. While historically China was one of the largest markets for meth production and use, recent evidence suggests that South-East Asia is the fastest growing meth market (UNODC, 2019). In addition, a recent analysis of metabolites in wastewaters revealed high use of meth in East and North-Central Europe, the United States, and Australia (González-Mariño et al., 2020). Preliminary evidence from Bousman et al. (2010) suggests that risk and protection conferred by putative gene variants may be ethnically divergent. It is therefore crucial to investigate the associations between gene and meth use disorder in a wider variety of populations.

Lastly, only a handful of studies were adequately powered to detect differences in genotype and allele distribution (Table 2). This was not surprising, as over 70 % of all studies failed to conduct appropriate power analyses and justify their sample size. In addition to low power, most studies reviewed had a high risk of bias. Over 90 % of all studies did not have clear inclusion and exclusion criteria, and the cases were not clearly differentiated from control in 75 % of studies. These bias risks undermine potential interpretation of the findings, as it is possible that genotype differences may arise from the heterogeneity between cases and controls. In addition, the lack of clear reporting of study design makes it challenging for future replication studies.

4.3. Implications and future directions

The findings summarized in the present review provide some evidence of the involvement of certain genes in meth use disorder. The most promising marker associated with meth use disorder is the functional variant rs324420 (Pro129Thr) of *FAAH* in people of East Asian descent, with a large overall effect size and studies with the highest quality and lowest risk of bias reviewed. While the exact role of Pro129Thr in susceptibility for meth use is unknown, it is possible that it plays a role in increasing the subjective effect of the drug, reducing impulse control and enhancing reward-related brain activity. Future studies should aim to replicate these findings in other populations. In contrast, while a meta-analysis of seven studies revealed that the rs6265 variant (Val66Met) of *BDNF* may confer protection against meth use disorder, the effect size was weak. It should be noted that both *FAAH* Pro128Thr and *BDNF* Val66Met have been implicated in other substance use and comorbid disorders.

To date, literature on the genetics of meth use disorder almost exclusively focuses on hypothesis-driven candidate gene association studies, and specific heritability scores are yet to be calculated. To uncover the heritability of the disorder, family and twin studies should be conducted, preferably across different populations. In addition, very few GWASs have been conducted in people with meth use disorder (see Limitations), and it appears clear that large-scale GWASs involving international collaborations are necessary to advance the field. Many studies reviewed reported significant associations with silent variants with no clear biological function. It is possible that genes involved in genetic susceptibility may be in linkage disequilibrium with the variants studied. In addition, due to the highly polygenic nature of substance use

disorders (Ducci and Goldman, 2012), a well-powered GWAS would not only complement the candidate gene studies, but also identify alleles in linkage disequilibrium and allow for calculation of polygenic scores.

Of the 76 candidate gene association studies reviewed, 70.9 % of them reported a co-morbid diagnosis, with participants also diagnosed with either meth-induced psychosis, polysubstance use disorder, or both (Figure 2B). While substance-induced psychosis occurs in 36.5 % of people who use meth (Lecomte et al., 2018), and polysubstance use is common in this population (Connor et al., 2014), future studies should include a separately analysis of people exclusively diagnosed with meth use disorder to uncover genes specific to meth use.

5. Conclusion

In this study we systematically reviewed gene association studies in people with meth use disorder to identify any promising targets and pathways underlying the disorder. We assessed the quality and risk of bias of each study and calculated the power of each association. In addition, a meta-analytic approach was conducted when three or more studies were available for any given marker. We found that most significant associations were identified in neurotransmitter-related genes, with functional variants in *FAAH* and *BDNF* being the most robust targets. These findings highlight the utility of candidate gene studies conducted by different laboratories. We also identified key limitations in current literature. A large number of associations were underpowered to detect appropriate effect sizes, and quality assessment revealed that most studies had a high risk of bias, with poorly defined and differentiated cases and controls in nearly three quarters of them. Rationale for candidate gene selection was also poor, with only a small number of variants located in coding region of genes of interest. In addition, very few studies explored association in samples of non-Asian descent.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.neubiorev.2020.11.001>.

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Chapter 5. Genetic factors associated with methamphetamine use disorder

5.1. Introduction

The heritability of stimulant use disorders is estimated to be close to 40% (Goldman et al., 2005). In Chapter 4, I systematically reviewed candidate-gene association studies. Though not without limitations, candidate gene studies can identify specific sequence variations in genes of interest (e.g., single nucleotide polymorphisms (SNP) and deletions), and highlight potential molecular pathways to inform future research. One of the main limitations of the current candidate-gene literature is the lack of rationale for gene and variant selection (Guerin et al., 2021a). Therefore, this Chapter aimed to conduct candidate gene analyses on well-rationalized potential variants.

Chapter 4 revealed that variants in *FAAH* (rs324420) and *BDNF* (rs6265) were most robustly associated with methamphetamine use disorder, conferring a risk for and protection against the disorder, respectively (Guerin et al., 2021a). Notably, *FAAH* and *BDNF* variants are associated with cognitive performance and impulsivity in healthy participants and people with methamphetamine use disorder (Hariri et al., 2009a; Su et al., 2015, 2014). It is therefore possible that mutations in these genes contribute to the inhibitory control deficits observed in people with methamphetamine use disorder in Chapter 3, thus playing a role in vulnerability to early onset methamphetamine use. Importantly, every study which has found an association between these *FAAH* and *BDNF* variants and methamphetamine use disorder have been conducted in East Asian populations. Evidence suggests that risk and protection conferred by putative gene variants may be ethnically divergent (Bousman et al., 2010b). It is therefore critical to replicate such findings in other populations.

In addition to focusing on the genes identified in **Chapter 4**, I also considered other relevant genes as an exploratory angle. Genes were selected based on strong preclinical

evidence and/or their integral role in neurochemical systems implicated in the development of substance use disorders. In fact, we have compelling preclinical evidence of the role of *SLC18A1* in methamphetamine use disorder (**Chapter 1**). Our transcriptome study in rats is the first to observe a link between *SLC18A1* expression and early-onset methamphetamine use (Luikinga et al., unpublished observations). *SLC18A1* codes for the VMAT1 protein, which regulates monoamine transport from cytosol to synaptic vesicles (Schuldiner et al., 1995). While *SLC18A1* SNPs have been shown to be associated with schizophrenia and alcohol use disorder (**Chapter 1, Table 1.3**), no candidate gene-association study has been conducted in methamphetamine use disorder. Due to the similarities shared between these disorders (Gordon, 2008; Guerin et al., 2019; Hingson and Zha, 2009; Lecomte et al., 2010), it is clear that distribution of *SLC18A1* variants should be investigated in methamphetamine use disorder. Historically, most of the research on the role of vesicular monoamine transporters and methamphetamine use has been conducted in VMAT2 (Eiden and Weihe, 2011; Fleckenstein et al., 2007; Fumagalli et al., 1999; McFadden et al., 2012; Nickell et al., 2014; Uhl et al., 2000), which is encoded by *SLC18A2*. A study found a 10% reduction of VMAT2 binding availability in the striatal subregions in methamphetamine users (Johanson et al., 2006), suggesting that methamphetamine use downregulates *SLC18A2* expression. Several *SLC18A2* polymorphisms have been shown to be associated with schizophrenia, opioid and cocaine use (**Chapter 1, Table 1.4**), although *SLC18A2* polymorphisms have not yet been assessed in methamphetamine use disorder. More research on *SLC18A2* variants in methamphetamine use disorder is therefore warranted.

In addition to the variants mentioned thus far, this Chapter assessed functional variants in two other dopamine-related genes: the enzyme catechol-O-methyltransferase (*COMT*), and the dopamine receptor D2 (*DRD2*) genes. While meta-analysis revealed no association between the rs4680 (Val158Met) polymorphism of *COMT* and methamphetamine use disorder (Guerin et al., 2021a), it is associated with executive

dysfunction in other neuropsychiatric disorders (Bilder et al., 2002; Carrà et al., 2017; Egan et al., 2001). Likewise, the rs1800497 (TaqA1) variant of *DRD2* was not significantly associated with methamphetamine use disorder in my meta-analysis. Nonetheless, its association with reduced striatal D2R density and availability makes it a worthwhile target (Jönsson et al., 1999; Pohjalainen et al., 1998). I also investigated a deletion of *DRD2*, known as the -141C Ins/Del (rs1799732) variant, due to its effect on reduced *DRD2* expression (Arinami et al., 1997).

At the time of designing this chapter, only two genome-wide association studies (GWASs) had been conducted in people with methamphetamine use disorder, using overlapping samples of Japanese and Taiwanese descent (Ikeda et al., 2013; Uhl et al., 2008). While there were no genome-wide significant single SNP associations in these studies, several genes had clusters of nominally associated SNPs. The top hits were neuregulin-1 (*NRG1*), sarcoglycan zeta (*SGCZ*), and neuronal PAS domain protein 3 (*NPAS3*). I therefore added them to the candidate gene list to investigate whether they were associated with methamphetamine use disorder in my sample.

Therefore, the primary aim of this Chapter was to investigate differences in SNP distribution in these candidate genes between controls and people with methamphetamine use disorder. **Table 5.1** describes all candidate genes and their variants examined in this Chapter. It should be noted that I could not genotype *FAAH* variants due to restricted access to resources during the COVID-19 pandemic. Based on findings from our preclinical model, Chapter 4, and literature in schizophrenia and other substance use disorders, I hypothesized that some polymorphisms (i.e., *BDNF* Val66Met; *SLC18A1* Thr136Ile) would confer protection against methamphetamine use disorder, while other variants (i.e., *SLC18A1* Pro4Thr) would confer a risk.

Table 5.1. Candidate genes investigated in this Chapter. Ala: alanine; Arg: arginine; Ile: isoleucine; Leu: leucine; M: major allele; m: minor allele; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Val: Valine.

Gene	Gene Name	Marker	Alleles (M>m)	Location	Gene function
SLC18A1	Solute carrier family 18 member 1	rs2270641	T>G Thr>Pro	8p21.3	Regulates monoamine transport from cytosol to synaptic vesicles
		rs2270637	C>G Ser>Thr		
		rs1390938	G>A Thr>Ile		
		rs17092104	G>C Leu>Val		
		rs17215801	C>A Arg>Leu		
		rs148468662	G>A Phe>Ser		
SLC18A2	Solute carrier family 18 member 2	rs363227	C>T	10q25.3	Regulates monoamine transport from cytosol to synaptic vesicles
		rs363224	C>A		
		rs363285	A>C		
		rs363333	T>C		
		rs363387	T>G Thr>Thr		
		rs363276	C>T		
BDNF	Brain-derived neurotrophic factor	rs6265	G>A Val>Met	11p14.1	Growth and differentiation of new neurons and synapses
DRD2	Dopamine receptor D2	rs1800497	A2>A1	11q23.2	Mediates dopamine signaling pathways
		rs1799732	Deletion		
COMT	Catechol-O-methyltransferase	rs4680	G>A Val>Met	22q11.21	Metabolizes catecholamine transmitters
NRG1	Neuregulin-1	rs62510682	G>T	8p12	Modulates major inhibitory and excitatory neurotransmitter systems
		rs35753505	T>C		
		rs2954041	G>T		
		rs10503929	T>C Met>Thr		
SGCZ	Sarcoglycan zeta	rs4427170	G>A	8p22	Bridges the extracellular matrix and the cytoskeleton
		rs12680924	A>T		
NPAS3	Neuronal PAS domain protein 3	rs10142034	C>G	14q13.1	Regulates hippocampal neurogenesis
		rs12434716	G>C Ala>Pro		
		rs12894058	G>A		

My secondary aims were to explore whether SNPs were associated with 1) drug use parameters, including age of onset and severity of methamphetamine use; and 2) cognitive performance in people with methamphetamine use disorder. Based on age-relevant results from Chapter 3, I focused on response inhibition (i.e., reduction in cue-induced response) and inhibitory control. I hypothesized that *BDNF* Val66Met would be associated with age of onset of methamphetamine use and cognition (Su et al., 2015, 2014). Based on our preclinical model, I also hypothesized that variants in *SLC18A1* would be associated with impaired response inhibition, and earlier age of methamphetamine use onset (Luikinga et al., unpublished observations). I fully acknowledge that due to the small sample size in the present Chapter, all analyses and results presented are preliminary, and should be interpreted with caution. Nonetheless, these findings can inform design of subsequent larger studies.

5.2. Methods

5.2.1. Participants, study design and procedure

Recruitment, eligibility criteria, and ethics approval were as described in Chapters 2 and 3. All participants were administered a clinical interview, a cognitive task battery, and provided a blood samples at the conclusion of the study. Blood samples from 28 people with methamphetamine use disorder and 29 controls were analyzed in this Chapter. All interviews, tasks, blood collection, and molecular work were performed by me, except for SNP genotyping which was performed at the Murdoch Children's Research Institute by Dr Kiymet Bozaoglu.

5.2.2. Measures

5.2.2.1. Background measures

Demographic questionnaire: As described in Chapter 3. In brief the demographic questionnaire collected information on key demographic and drug use characteristics (age, sex, ethnicity, and age of onset).

Wechsler Test of Adult Reading (WTAR): as described in Chapter 2. In brief, the WTAR assessed premorbid verbal IQ (Wechsler, 1997).

Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): as described in Chapter 2. In brief, the ASSIST assessed severity, frequency, and high-risk substance use (WHO ASSIST Working Group, 2010).

5.2.2.2. Cognitive measures

Inhibitory control: The Word-Color Stroop Task (Inhibition) was administered to measure inhibitory control. For more details on the task and performance index, please refer to Chapter 2 (**Table 1**). Note that as previously stated, a number of participants declined taking the test, or were unable to complete the task (e.g., due to color-blindness).

Response inhibition: methamphetamine-specific response inhibition was assessed using a cue reactivity paradigm. Details on paradigm development and validation are presented in Chapter 2. For the purpose of this Chapter, I investigated two outcome measures: physiological (i.e., $\Delta\text{SCR} = \text{SCR to methamphetamine cue} - \text{SCR to control cue}$) and subjective rating (i.e., craving). Indices were calculated as the response in the last block minus response in the first block of the task, thus a positive score indicated an increase in response and a negative score indicated a decrease in response (i.e., response inhibition).

5.2.3. Blood collection

Blood was collected by venipuncture at the completion of the study using a BD Vacutainer Safety-Lok blood collection set (Becton, Dickinson and Company (BD), NJ, USA) with a 22-gauge needle. Participants were instructed not to consume any food or hot drinks during the study session to ensure a 3-hour fast prior to collection. Four samples were collected in four different tubes in the following order: whole blood in a 6ml EDTA tube (BD) for SNP analyses; whole blood in a 9mL Tempus tube (Applied Biosystems, MA, USA) for gene expression analyses; serum in a 5mL SST tube (BD); and plasma in a second 6mL EDTA

tube (BD) for protein expression analysis. Due to the COVID-19 pandemic, this Chapter only analyzed SNPs. The EDTA and Tempus tubes containing whole blood were immediately stored at -80°C following collection.

5.2.4. Genotyping

5.2.4.1. DNA extraction

DNA was extracted from whole blood using a DNEasy Blood & Tissue Kit (Qiagen, Netherland). Whole blood in EDTA tubes were thawed at room temperature under a fume hood, then 200 µL of blood was pipetted into a 1.5 mL Eppendorf tube. 20 µL of Proteinase K (Qiagen) was slowly added to the whole blood to degrade proteins and avoid contamination. 200 µL of lysis buffer AL (Qiagen) was then added to the solution. Samples were vortexed thoroughly before being incubated at 56°C for 10 min. The samples were then briefly centrifuged, 200 µL of 100% ethanol was added, and samples were once again briefly centrifuged. The mixture was then pipetted into a DNeasy Mini spin column (Qiagen) placed in a 2 mL collection tube and centrifuged at 8000 rpm for 1 min at room temperature. The flow-through and collection tubes were discarded, and the spin column placed in a new 2 mL collection tube. 500 µL of washing buffer AW1 (Qiagen) was added to the spin column, before centrifuging at 8000 rpm for 1 min at room temperature. The flow-through and collection tube were once again discarded, and spin column placed a new 2 mL collection tube. 500 µL of washing buffer AW2 (Qiagen) was added to the spin column, before centrifuging at 14,000 rpm for 3 min at room temperature. Flow-through was discarded, and spin column placed back into the collection tube and centrifuged at 14,000 rpm for 1 min at room temperature. Flow-through and collection tubes were discarded, and spin column transferred to a 1.5 mL Eppendorf tube. 200 µL of elution buffer AE (Qiagen) was added to the center of the spin column. Samples were incubated at room temperature for 1 min, before being centrifuged at 8000 rpm for 1 min at room temperature. Spin columns were discarded, and DNA sample stored at 4°C.

5.2.4.2. Quality control

DNA concentration and purity were assessed using a NanoDrop Spectrophotometers (Thermo Fisher Scientific, MA, USA). A 260/230 ratio between 1.5 and 3 was considered 'pure' (Glase, 1995). Ratio ranged between 1.57-2.01 for samples analyzed in this Chapter. For genotyping, a DNA concentration of 5 ng/ μ L was required. Concentration ranged between 20.5-60.2 ng/ μ L (mean = 31.87; SEM = 2.04), which was diluted prior to genotyping. DNA integrity was assessed by gel electrophoresis. 5 μ L of each sample were aliquoted. Loading buffer was added to all samples. Samples were loaded on a 1% agarose in Tris/Borate/EDTA (TBE) gel. The gel was run for 20 to 30 min at 100 V and visualized using a ChemicDoc system (Bio-Rad Laboratories, CA, USA). Samples of high molecular weight with no impurities or contaminations were considered 'pure'. **Figure 5.1** is a representation of a DNA integrity gel.

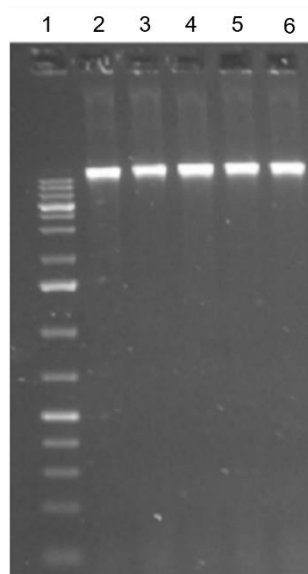


Figure 5.1. Representation of a DNA integrity gel. Lane 1 represents the DNA ladder. Lanes 2-6 represent pure, high molecular weight samples.

5.2.4.3. SNP Genotyping

SNP assays were designed using the Agena Assay Design Suite software (Agena San Diego, CA). Each SNP had two PCR primers (forward and reverse) as well as iPLEX extension primers specific for the SNP of interest (**Table 5.2**). The genotyping workflow is made up of three key steps, the PCR step, the Shrimp Alkaline Phosphatase treatment step and the iPLEX single base extension reaction. It should be noted that *FAAH* was not genotyped due to the COVID-19 pandemic.

Table 5.2. Primers for SNPs of interest

Gene	Marker	Forward Primer (5'→3')	Reverse Primer (3'→5')	Extend Primer (5'→3')
<i>SLC18A1</i>	rs2270641	ACGTTGGATGT CCTTCAGCAAC CGCTGGG	ACGTTGGATGACA GGACCTTCCCTAA GAGC	CGGGGAGCATCC AGAATGG
	rs2270637	ACGTTGGATGT TCAACAACAAC ACCGTGGC	ACGTTGGATGTGC TGGCAGTGTCATT CATC	TTGAAGAAAGCGT ACCTA
	rs1390938	ACGTTGGATGA ACAACGCTTG CAAGGCAC	ACGTTGGATGTTG AAGCAAACAGAAC CCCG	GTTTCTTGGAGGA AGAGA
	rs17092104	ACGTTGGATGG GTTCTCTGGC TCACAATA	ACGTTGGATGGA GGGCACTTCTTAC CTATG	TGGCTCACAATAT TTTTGGT
	rs17215801	ACGTTGGATGA TGCCGGAAGTT CCCCACAT	ACGTTGGATGAAC AGCCACGGTGTT GTTG	TTCCACCATCTTC TCCT
	rs14846866 2	ACGTTGGATGA TGGGTTGACCA GAAGTTGC	ACGTTGGATGAAG GCACAGGTTTCTT GGAG	AAACAGAACCCC GACC
<i>SLC18A2</i>	rs363227	ACGTTGGATGT GCTACAGAAAT CCAGACGG	ACGTTGGATGAAG ATGCTCTGGAAGC TGTC	TACAGGCCAGTG CACAC
	rs363224	ACGTTGGATGA ATGAAAGGAGG AACCTCGG	ACGTTGGATGTCA TCAGATTGCTGTG TGGG	AAATGTGCAGCAA GC
	rs363285	ACGTTGGATGT TGACAACCATC AGGACAAC	ACGTTGGATGTGA GATATGTAAC TAG CCAC	GATGGAAGGATAA CTTCCTGG
	rs363333	ACGTTGGATGA TGCACTTACGA AACCTGGC	ACGTTGGATGTAA AGAGGACCTCTTC CACC	GGGAAACCTGGC ATTTAGACA
	rs363387	ACGTTGGATGT GGTGATTCTTG CCCAAAGC	ACGTTGGATGGTT GGAGTCAGCATTT TATG	AGATTGTTCCAC TTGTC

	rs363276	ACGTTGGATGT GCATACTTAAC TGACCAGC	ACGTTGGATGAAA CTTCACCTGAAAC AGGC	TCCAAGCATTTGT CAATTCACC
BDNF	rs6265	ACGTTGGATGC TTCATTGGGGCC GAACTTTC	ACGTTGGATGGCT TGACATCATTGGC TGAC	GTTCTCATCCAA CAGCTCTTCTATC A
DRD2	rs1800497	ACGTTGGATGA CACAGCCATCC TCAAAGTG	ACGTTGGATGTGT GCAGCTCACTCCA TCCT	CCACCCTCAAAGT GCTGGTC
	rs1799732	ACGTTGGATGA AAGGAGCTGTA CCTCCTCG	ACGTTGGATGTCA AAACAAGGGATG GCGGA	ACCTACTCGGCG ATCCCCGGCCTG
COMT	rs4680	ACGTTGGATGT TTTCCAGGTCT GACAACGG	ACGTTGGATGCCA GCGGATGGTGGG TTTC	CCCACACCTTGTC CTTCA
NRG1	rs62510682	ACGTTGGATGT GTGCAAAGGTA GGCCTTCT	ACGTTGGATGGG TGGGTAAATGAAG GCTCT	AGGACTTCTTTAA ACAGCTCAGTA
	rs35753505	ACGTTGGATGA GGCATCAGTTT TCAATAGC	ACGTTGGATGGA GACTGGAAGCCA TGTATC	ACTAAAAAAGAGA TATATGATATTTG G
	rs2954041	ACGTTGGATGC ATTATTCATTGT TTGTTGC	ACGTTGGATGTTA AAGGGAACACAC ACTCG	AATGCATTGTTTG TTGCTGATGAAT
	rs10503929	ACGTTGGATGA AAGCTGCATGA CCGTCTTC	ACGTTGGATGTAG GATGGTGAGGCC CATTG	CCTGCGGTCTGA ACGAAACAATA
SGCZ	rs4427170	ACGTTGGATGC ATGGTTAAAGA GACATGC	ACGTTGGATGGG TCATTACTGAGAA ATAC	AGGGGTAAATGG TTTCATGCGG
	rs12680924	ACGTTGGATGG TCTGTCCTGCT AGAGAATG	ACGTTGGATGCCA CGGTTGTTGCTG GTTG	AGAGGTCACATGA AGAAAAACATA
NPAS3	rs10142034	ACGTTGGATGT CGCCTCCGACC CGCTGTCA	ACGTTGGATGCGT TCCCGGGGTGCT TGTC	CCCGCTCTCGGC GTC
	rs12434716	ACGTTGGATGG TAGCGCTCCAC CTTGATCT	ACGTTGGATGAG CACTCGGACTTTG AGAAC	CACCTTGATCTGC ATCG
	rs12894058	ACGTTGGATGG CTGTTCATAAG GTAAGGAG	ACGTTGGATGACT AATGTGCTTGTGT CCAG	CCCGTTTAAGTTT GCTGTAGTATTT

The DNA region of interest was amplified using standard PCR protocol and with the same conditions for all SNPs assayed (**Table 5.3**). All reactions were set up in a 384 well format, as required by the mass spectrometer.

Table 5.3. Protocol and thermocycling conditions for genotyping PCRs

Reagent	X 1		Temperature	Time	Number of cycles
Water	0.35 µl		95°C	15 min	1
10xPCR Buffer	0.625 µl		95°C	20 sec	45 cycles
25mM MgCl ₂	0.325 µl				
25 mM dNTP mix	0.1 µl				
Primer mix	0.5 µl		56°C	30 sec	1
Qiagen HotStarTaq (5 u/µl)	0.1 µl		72°C	3 min	1
5ng/µl GenomicDNA	3.0 µl		72°C	3 min	1
TOTAL VOLUME	5.0 µl		4°C	hold	1

Shrimp Alkaline Phosphatase Treatment

Following PCR amplification, Shrimp Alkaline Phosphatase (SAP) was added to the product to remove any unincorporated nucleotides and prevent interference in the extend reaction. The conditions for the SAP treatment are outlined below (**Table 5.4**).

Table 5.4. Protocol and thermocycling conditions for SAP Treatment

Reagent	X 1		Temperature	Time
Water	1.53 µl		37°C	40 minutes
SAP buffer (x10)	0.17 µl		85°C	5 minutes (to de-activate the enzyme)
SAP enzyme	0.30 µl		4°C	hold
TOTAL VOLUME	2.0 µL			

iPLEX Extend Reaction

Following the SAP treatment, the iPLEX single base extension reaction. This iPLEX reaction includes an extension primer which is designed immediately adjacent to the SNP of interest. The extend primer hybridizes directly adjacent to the SNP site and the extension occurs. A

termination mix containing all nucleotides, a sequence specific extend primer that flanks the SNP of interest and an enzyme are all combined in a mix and added to each sample. The incorporation of the nucleotides terminates the extension of the primer. Refer to **Table 5.5** for the reaction and the thermocycling conditions for the iPLEX extend reaction.

Table 5.5. Protocol and thermocycling conditions for iPLEX extend reaction

Reagent	X 1		Temperature	Time	Number of cycles
Water	0.7395 μ l		94°C	30 sec	1
iPLEX Buffer Plus (x10)	0.200 μ l		94°C	5 sec	40
iPLEX termination mix	0.100 μ l		52°C	5 sec	5
Primer mix (7 μ M; 14 μ M)	0.94 μ l		80°C	5 sec	5
iPLEX enzyme	0.0205 μ l		72°C	3 min	40
TOTAL VOLUME	2.0 μL		4°C	hold	1

Desalting and Spotting of reactions onto SpectroCHiPs

The extension products were then desalted using Clean Resin and then transferred from the microtiter plate onto a SpectroCHIP Array, via an automated nanodispenser where the products crystalized with a pre-spotted MALDI matrix. SpectroChips enabled an automated readout of genotype data and subsequent data analysis by a Compact MALDI-TOF mass-spectrometer (Agena).

Data analysis was performed on the Typer Software Module. This software automatically generates a report containing the SNP alleles in each sample (homozygous i.e., CC or heterozygous i.e., CG). All genotype calls are made in real time during MALDI-TOF analysis, however simultaneous visualization of all assays in a multiplex reaction are available immediately at the end of each run. The genotype data was then exported into an

excel spreadsheet and further analysis was performed as described in the data analysis section. Adherence to Hardy-Weinberg equilibrium and allele frequency were assessed to ensure validity of the results.

5.2.5. Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics 26 (IBM Corp., NY, USA). Independent samples t-tests and Chi-square analyses with Fisher's exact test were used to compare methamphetamine use disorder and control groups for all demographics and drug use characteristics. Analysis of covariance (ANCOVA) using significantly different demographic characteristics as covariates was used to assess differences in cognitive measures between the two groups. Chi-square analyses were used to calculate significance for genotype distribution between controls and people with methamphetamine use disorder for each SNP. Due to the exploratory nature of this Chapter, I followed up different genotype distribution at $p < 0.1$. For significant variants, odds ratios (OR) and 95% confidence intervals (CI) were calculated for alleles under all four inheritance models: co-dominant, dominant, recessive, and additive. Post-hoc power analyses were conducted for each model as described in Guerin et al. (2021). In brief, power was calculated using the total sample size and effect size w , using the minor allele frequency and total sample size for each marker. Power analyses were conducted in G*Power 3.1 (Erdfelder et al., 2009).

Secondary analyses were conducted in people with methamphetamine use disorder only. Due the exploratory nature of this aim, I investigated associations between genotypes and drug use parameters (age of onset and ASSIST score) and cognitive performance (inhibitory control and response inhibition) in variants with at least two participants per group. Differences were explored using analysis of variance (ANOVA), followed up by Tukey's multiple comparisons post hoc tests where appropriate. For this, Bonferroni correction was employed for each measure ($p < 0.025$ for drug use parameters; $p < 0.017$ for cognitive factors) to account for multiple comparisons. A multiple regression was conducted to assess

whether gene variants identified in this Chapter could predict age of onset of methamphetamine use. Other variables included in the regression were psychiatric and cognitive factors identified in **Chapter 3** (i.e. ASPD diagnosis and Stroop performance).

5.3. Results

5.3.1. Descriptive statistics

Note that due to the COVID-19 pandemic, not all participants from previous Chapters could be analyzed, hence sample size and results may slightly differ between Chapters. Demographic characteristics, drug use characteristics, and cognitive performance of people with methamphetamine use disorder and controls are described in **Table 5.6**. People with methamphetamine use disorder were on average older than controls ($p = 0.002$) and had lower premorbid IQ ($p = 0.003$). Therefore, age and IQ were used as covariate for subsequent group comparisons. There were no differences in sex and ethnicity distribution. Controls performed better than methamphetamine users in the Stroop task ($p = 0.012$). There were no other differences in cognitive performance when controlling for age and IQ.

Table 5.6. Demographic characteristics, drug use characteristics, and cognitive performance. *indicates a dependent variable with a significant group difference when age and IQ were included as covariates. &indicate a dependent variable with a significant group difference without covariate analysis. ANCOVAs were reported in this table. ASSISIT: Alcohol, Smoking, and Substance Involvement Screening Test; Methamphetamine: People with methamphetamine use disorder; ΔSCR: Differential Skin Conductance Response.

	Methamphetamine (n = 28)	Control (n = 29)	Test; p-value
Demographic:			
Age – years (±SD)*	32.14 (±6.78)	26.24 (±6.71)	$t=-3.301$; $p=0.002$
Females – no. (%)	12 (42.9)	19 (65.5)	$\chi^2=2.949$; $p=0.086$
IQ (±SD)*	105.04 (±10.07)	113.14 (±9.64)	$t=3.304$; $p=0.003$
Ethnicity – no. (%)			
Caucasian	21 (75.0)	19 (65.5)	$\chi^2=3.750$; $p=0.153$
Asian	5 (17.9)	10 (34.5)	
Aboriginal/Torres Strait Islander	2 (7.1)	0 (0)	
Drug Use:			
Age of Onset (±SD)	21.80 (±6.27)	-	-
ASSIST Amphetamine score (±SD)	31.46 (±5.85)	0 (±0)	-
Cognition:			
Stroop (Inhibition) (±SD)*	10.63 (±4.85)	18.25 (±17.1)	$F=6.752$; $p=0.012$

Δ SCR Index ($\pm$ SD) ^{&}	-0.07 ($\pm$ 0.14)	0.07 ($\pm$ 0.24)	F=3.846; p=0.057
Subjective Index ($\pm$ SD)	-1.68 ($\pm$ 2.59)	0 ($\pm$ 0)	-

5.3.2. Genotype and allele distribution

Table 5.7 lists the genotype frequencies for each gene variant investigated. The genotype distribution of *SLC18A1* rs2270641 (Pro4Thr) was significantly different between people with methamphetamine use disorder and controls ($\chi^2 = 8.208$; $p = 0.017$). There was a trend for *COMT* rs4680 (Val158Met) ($\chi^2 = 5.711$; $p = 0.058$) and *NRG1* rs35753505 ($\chi^2 = 4.965$; $p = 0.084$) between the two groups. There were no other significant differences.

Table 5.7. Genotype frequencies of variants of interest in people with methamphetamine use disorder and controls. Values in **bold** are statistically significant ($p < 0.05$). Values in *italics* are significant at $p < 0.1$. M: major allele; m: minor allele.

Gene	Marker	Methamphetamine				Control				p-value
		M/M	M/m	m/m	n	M/M	M/m	m/m	n	
<i>SLC18A1</i>	rs2270641	9	14	5	28	16	4	8	28	0.017
	rs2270637	20	8	0	28	21	6	1	28	0.519
	rs1390938	19	7	2	28	18	6	4	28	0.680
	rs17092104	22	5	1	28	20	5	2	27	0.814
	rs17215801	28	0	0	28	29	0	0	29	-
	rs148468662	28	0	0	28	29	0	0	29	-
<i>SLC18A2</i>	rs363227	17	10	1	28	21	5	3	29	0.215
	rs363224	5	12	11	28	3	14	11	28	0.721
	rs363285	9	2	0	11	11	2	0	13	0.855
	rs363333	21	6	1	28	23	4	1	28	0.782
	rs363387	23	4	1	28	25	3	0	28	0.542
	rs363276	15	11	2	28	19	5	2	26	0.266
<i>BDNF</i>	rs6265	14	9	2	25	18	9	1	28	0.717
<i>DRD2</i>	rs1800497	13	14	1	28	8	16	4	28	0.210
	rs1799732	21	7	0	28	23	6	0	29	0.698
<i>COMT</i>	rs4680	19	0	9	28	17	5	6	28	<i>0.058</i>
<i>NRG1</i>	rs62510682	12	13	3	28	15	8	2	25	0.459
	rs35753505	12	10	5	27	5	17	7	29	<i>0.084</i>
	rs2954041	27	1	0	28	23	5	1	29	0.137
	rs10503929	21	3	4	28	21	6	1	28	0.247
<i>SGCZ</i>	rs4427170	5	18	5	28	5	15	9	29	0.497
	rs12680924	11	14	3	28	11	15	2	28	0.889
<i>NPAS3</i>	rs10142034	19	9	0	28	21	7	1	29	0.514
	rs12434716	19	6	0	28	21	6	1	28	0.427
	rs12894058	12	12	4	28	15	10	4	29	0.780

Table 5.8 lists the allele frequencies, odds ratios (OR), 95% confidence intervals (CI), and post-hoc power for each gene variant that were differently observed at $p < 0.1$ between groups in **Table. 5.7**. For the co-dominant model, ORs were calculated by comparing distribution of heterozygous (M/m) and homozygous minor alleles (m/m) to the wild type homozygous major allele genotype (M/M). *SLC18A1* rs2270641 (Pro4Thr) was significantly different between methamphetamine users and controls under the co-dominant model of inheritance, with the heterozygous variant (Pro/Thr) conferring greater risk of methamphetamine use disorder (OR = 6.22). *COMT* rs4680 (Val156Met) was also significantly different under the co-dominant model, with the heterozygous variant protective against methamphetamine use disorder (OR = 0.47). In addition, *NRG1* rs35753505 was significantly different under both co-dominant and dominant models of inheritance. Since the dominant model assumes there is no differences between heterozygous and minor homozygous mutants, I will only focus only report the dominant model. The variant was protective against methamphetamine use disorder (OR = 0.26).

Table 5.8. Allele frequencies of variants of interest in people with methamphetamine use disorder and controls in four inheritance models. Values in **bold** are statistically significant ($p < 0.05$). CI: confidence interval; M: major allele; m: minor allele; Meth; methamphetamine; OR: odds ratio.

Gene Marker	Model	Allele	Meth	Control	OR	95% CI	p-value	Power (1 - β)
<i>SLC18A1</i> rs2270641 (Pro4Thr)	Co-dominant	M/M	9	16	1.00 (ref)			
		M/m	14	4	6.22	1.57-24.7	0.007	0.77
		m/m	5	8	1.11	0.28-4.43	0.881	0.05
	Additive	M	32	36	1.00 (ref)			
		m	24	20	1.35	0.63-2.89	0.439	0.12
	Dominant	M/M	9	16	1.00 (ref)			
		M/m+m/m	19	12	2.82	0.95-8.38	0.060	0.47
	Recessive	M/M+M/m	23	20	1.00 (ref)			
		m/m	5	8	0.54	0.15-1.93	0.342	0.16
<i>COMT</i> rs4680 (Val156Met)	Co-dominant	M/M	19	17	1.00 (ref)			
		M/m	0	5	0.47	0.33-0.67	0.027	0.60
		m/m	9	6	1.34	0.40-4.56	0.637	0.08
	Additive	M	38	39	1.00 (ref)			
		m	18	17	1.087	0.49-2.42	0.838	0.05
	Dominant	M/M	19	17	1.00 (ref)			
		M/m+m/m	9	11	0.73	0.24-2.19	0.577	0.09

<i>NRG1</i> rs35753505	Recessive	M/M+M/m	19	22	1.00 (ref)			
		m/m	9	6	1.74	0.52-5.78	0.365	0.15
	Co-dominant	M/M	12	5	1.00 (ref)			
		M/m	10	17	0.25	0.07-0.90	0.030	0.58
		m/m	5	7	0.30	0.06-1.40	0.119	0.34
	Additive	M	34	27	1.00 (ref)			
		m	20	31	0.51	0.24-1.09	0.081	0.42
	Dominant	M/M	12	5	1.00 (ref)			
		M/m+m/m	15	24	0.26	0.08-0.88	0.027	0.60
	Recessive	M/M+M/m	22	22	1.00 (ref)			
		m/m	5	7	0.71	0.20-2.60	0.609	0.08

5.3.3. Association between allele distribution, drug use parameters and cognition in people with methamphetamine use disorder

I examined associations between genotypes and drug use parameters (age of onset and ASSIST score) and cognition for SNPs with 2 or more participant for each genotype.

5.3.3.1. Drug use parameters

One-way ANOVA revealed a significance difference between *NRG1* rs10503929 (Thr289Met) genotype and age of onset of methamphetamine use [$F(2, 25) = 8.344$, $p = 0.002$]. Post-hoc test revealed that people carrying the heterozygous genotype (Met/Thr) started using methamphetamine later in life compared to homozygous major allele carriers (Met/Met; $p = 0.001$) and homozygous minor allele carriers (Thr/Thr; $p = 0.015$) (**Figure 5.2A**). There were no other significant genotypes differences in age of onset (**Table 5.9**). However, there was a trend for age of onset in *SLC18A1* rs2270641 (Pro4Thr) [$F(2, 25) = 2.970$, $p = 0.070$]. Post-hoc test revealed that people carrying the homozygous minor alleles genotype (Pro/Pro) started using methamphetamine later in life compared to homozygous major allele carriers (Thr/Thr; $p = 0.068$) (**Figure 5.2B**). There was also a trend in age of onset for *NPAS3* rs12894058 [$F(2, 25) = 2.277$, $p = 0.054$], with the homozygous minor allele carriers (A/A) more likely to have started using methamphetamine later in life compared to major allele carriers (G/G; $p = 0.084$) (**Figure 5.2C**).

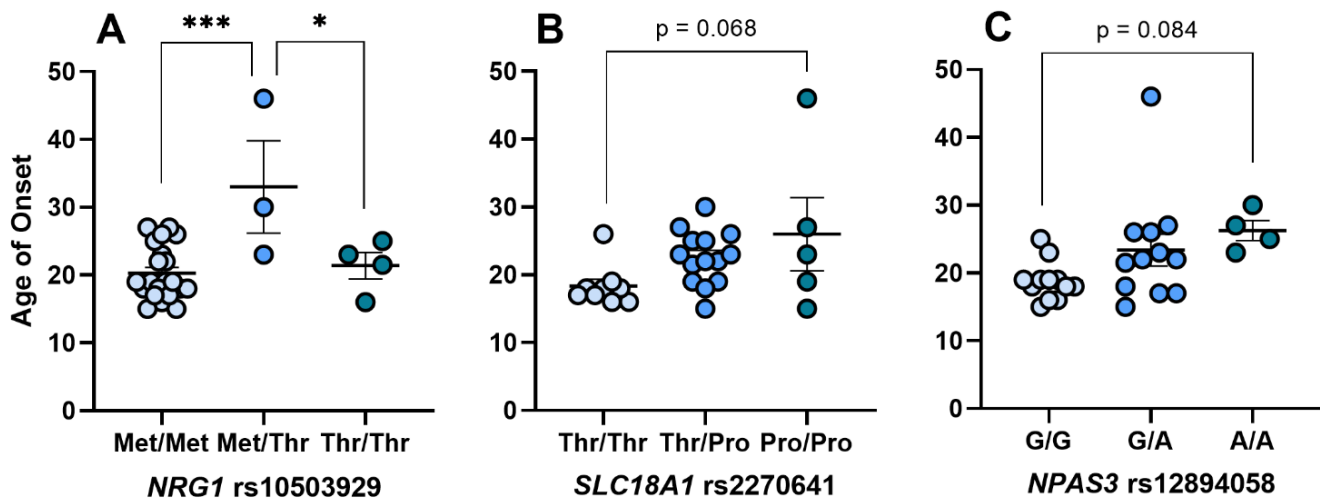


Figure 5.2. Genotype and age of onset of methamphetamine use. **A.** *NRG1* rs10503929. People carrying the Met/Thr genotype started using methamphetamine later in life compared to Met/Met and Thr/Thr carriers. **B.** *SLC18A1* Pro4Thr. People carrying the Pro/Pro genotype started using methamphetamine later in life compared to Thr/Thr carriers. **C.** *NPAS3* rs12894058. People carrying the A/A genotype started using methamphetamine later in life compared to G/G carriers. Data presented as individual values, mean $\pm$ SEM. * $p < 0.05$; *** $p < 0.001$

Table 5.9. Comparison between allele distribution and drug use parameters. Values in **bold** are significant ($p < 0.025$, Bonferroni corrected). Values in *italic* are significant at $p < 0.05$.

		n	Age of Onset		ASSIST Score	
			Mean ($\pm$ SD)	Test; p-value	Mean ($\pm$ SD)	Test; p-value
<i>SLC18A1</i> rs2270641 (Pro4Thr)	Thr/Thr	9	18.33 ($\pm$ 3.04)	F = 2.970; p = 0.070	33.67 ($\pm$ 3.81)	F = 1.278; p = 0.296
	Thr/Pro	15	22.56 ($\pm$ 3.96)		29.79 ($\pm$ 6.69)	
	Pro/Pro	5	26.00 ($\pm$ 12.04)		32.20 ($\pm$ 5.93)	
<i>SLC18A1</i> rs1390938 (Thr138Ile)	Ile/Ile	19	22.71 ($\pm$ 6.77)	F = 0.792; p = 0.464	30.74 ($\pm$ 6.44)	F = 0.674; p = 0.518
	Ile/Thr	7	20.57 ($\pm$ 5.38)		33.71 ($\pm$ 4.61)	
	Thr/Thr	2	17.50 ($\pm$ 0.71)		30.50 ($\pm$ 1.12)	
<i>SLC18A2</i> rs363224	C/C	5	20.00 ($\pm$ 4.00)	F = 0.447; p = 0.644	27.80 ($\pm$ 3.93)	F = 1.215; p = 0.314
	C/A	12	21.38 ($\pm$ 4.33)		32.17 ($\pm$ 3.93)	
	A/A	11	23.09 ($\pm$ 8.69)		32.36 ($\pm$ 4.37)	
<i>SLC18A2</i> rs363276	C/C	15	20.73 ($\pm$ 3.94)	F = 1.074; p = 0.357	33.60 ($\pm$ 4.40)	F = 2.944; p = 0.071
	C/T	11	22.23 ($\pm$ 8.64)		29.64 ($\pm$ 6.73)	
	T/T	2	27.50 ($\pm$ 3.54)		25.50 ($\pm$ 4.95)	
<i>BDNF</i> rs6265 (Val66Met)	Val/Val	14	21.18 ($\pm$ 4.09)	F = 1.231; p = 0.311	34.00 ($\pm$ 4.13)	F = 4.272 p = 0.027
	Val/Met	9	24.89 ($\pm$ 8.95)		28.11 ($\pm$ 7.18)	
	Met/Met	2	19.00 ($\pm$ 5.66)		26.00 ($\pm$ 2.83)	
<i>NRG1</i>	G/G	12	22.08 ($\pm$ 3.40)	F = 0.070; p = 0.933	32.75 ($\pm$ 4.99)	F = 4.439; p = 0.022
	G/T	13	21.35 ($\pm$ 8.25)		32.23 ($\pm$ 4.62)	

rs6251068 2	T/T	3	22.67 (± 7.51)		23.00 (± 8.72)	
NRG1 rs3575350 5	T/T	12	22.17 (± 8.97)	F = 0.044; p = 0.956	30.67 (± 7.16)	F = 0.317; p = 0.731
	T/C	10	21.55 (± 3.95)		32.50 (± 4.01)	
	C/C	5	21.76 (± 2.77)		30.40 (± 6.43)	
NRG1 rs1050392 9 (Thr289Met)	Met/Met	21	20.29 (± 3.96)	F = 8.344; p = 0.002	32.71 (± 4.97)	F = 2.958; p = 0.070
	Met/Thr	3	33.00 (± 11.79)		30.67 (± 3.79)	
	Thr/Thr	4	21.38 (± 3.86)		25.50 (± 8.66)	
SGCZ rs4427170	G/G	5	25.70 (± 11.88)	F = 1.711; p = 0.201	34.40 (± 3.91)	F = 0.790; p = 0.465
	G/A	18	21.61 (± 4.38)		31.00 (± 6.41)	
	A/A	5	18.60 (± 3.28)		30.20 (± 5.21)	
SGCZ rs1268092 4	A/A	11	20.81 (± 8.86)	F = 0.214; p = 0.809	32.90 (± 4.76)	F = 0.568; p = 0.574
	A/T	14	22.50 (± 4.09)		30.35 (± 6.74)	
	T/T	3	22.16 (± 4.53)		31.33 (± 5.77)	
NPAS3 rs1289405 8	G/G	12	18.75 (± 2.83)	F = 3.277; p = 0.054	30.66 (± 7.98)	F = 0.507; p = 0.608
	G/A	12	23.37 (± 8.12)		32.75 (± 3.98)	
	A/A	4	26.25 (± 2.98)		30.00 (± 2.16)	

One-way ANOVA revealed a significant difference between *NRG1* rs62510682 genotype and ASSIST score [$F(2, 25) = 4.439$; $p = 0.022$]. Post-hoc tests revealed that homozygous minor allele carriers (T/T) had significantly lower ASSIST scored compared to homozygous major allele carriers (G/G; $p = 0.021$) and heterozygous genotype carriers (G/T; $p = 0.028$) (**Figure 5.3A**). There was also a significant difference between *BDNF* rs6265 (Val66Met) genotype and ASSIST score [$F(2, 22) = 4.272$; $p = 0.027$]. Post-hoc tests revealed that people carrying the heterozygous genotype (Val/Met) had significantly lower ASSIST scored compared to homozygous major allele carriers (Val/Val; $p = 0.046$), although the significance did not survive Bonferroni correction (**Figure 5.3B**).

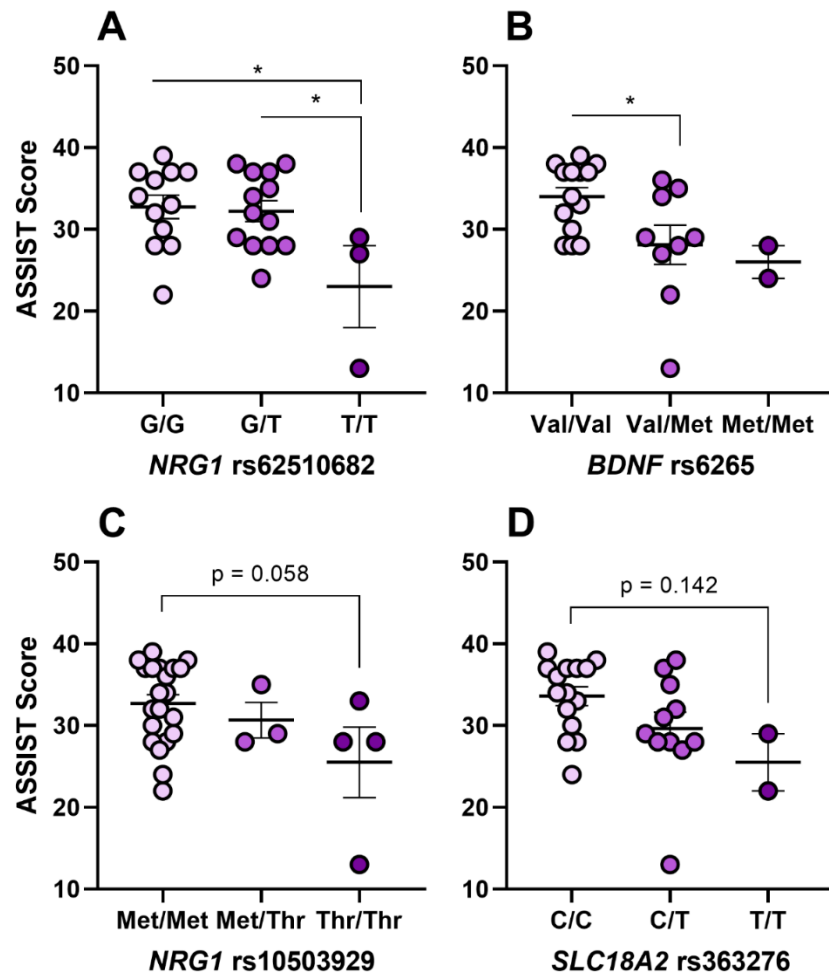


Figure 5.3. Genotype and severity of use. **A. *NRG1* rs62510682.** People carrying the T/T genotype had lower ASSIST scores compared to G/G and G/T carriers. **B. *BDNF* rs6265.** People carrying the Val/Met genotype had lower ASSIST scores compared to Val/Val carriers. **C. *NRG1* rs10503929.** People carrying the Thr/Thr genotype had lower ASSIST scores compared to Met/Met carriers. **D. *SLC18A2* rs363276.** People carrying the T/T genotype had lower ASSIST scores compared to C/C carriers. Data presented as individual values, mean $\pm$ SEM. * $p < 0.05$.

While there were no other significant differences between genotypes and ASSIST scores (**Table 5.9**), there was trend in *NRG1* rs10503929 (Thr289Met) [$F(2, 25) = 2.958$, $p = 0.070$]. Post-hoc test revealed that people carrying the homozygous minor alleles genotype (Thr/Thr) had a lower ASSIST score compared to homozygous major allele carriers (Met/Met; $p = 0.058$) (**Figure 5.3C**). There was also a trend in ASSIST score for *SLC18A2* rs363276 [$F(2, 25) = 2.944$, $p = 0.071$], with homozygous minor allele carriers (T/T) more likely to have lower ASSIST scores compared to major allele carriers (C/C; $p = 0.142$) (**Figure 5.3D**).

5.3.3.2. Cognition

There were no differences between any of the genotypes and performance in any of the cognitive tasks (**Table 5.10**). However, there was trend for Stroop performance in *NRG1* rs62510682 [$F(2, 23) = 2.744$, $p = 0.085$]. Post-hoc test revealed that people carrying the heterozygous genotype (G/T) performed better in the task compared to homozygous major allele carriers (T/T; $p = 0.073$) (**Figure 5.4A**). There was also a trend in subjective index for *NRG1* rs10503929 (Thr289Met) [$F(2, 25) = 2.910$, $p = 0.073$], with people carrying the heterozygous genotype (Met/Thr) having a higher index compared to minor allele carriers (Thr/Thr; $p = 0.068$) (**Figure 5.4B**).

Table 5.10. Comparison between allele distribution and cognition.

		n	Stroop (Inhibition)		Δ SCR Index		Subjective Index	
			Mean ($\pm$ SD)	Test; p-value	Mean ($\pm$ SD)	Test; p-value	Mean ($\pm$ SD)	Test; p-value
<i>SLC18A1</i> rs2270641 (Pro4Thr)	Thr/Thr	9	9.61 ($\pm$ 2.62)	$F = 2.249$; $p = 0.128$	-0.09 ($\pm$ 0.10)	$F = 1.192$; $p = 0.326$	-1.61 ($\pm$ 1.90)	$F = 0.010$; $p = 0.990$
	Thr/Pro	15	9.73 ($\pm$ 4.66)		-0.03 ($\pm$ 0.11)		-1.75 ($\pm$ 3.07)	
	Pro/Pro	5	14.56 ($\pm$ 6.76)		-0.16 ($\pm$ 0.28)		-1.60 ($\pm$ 2.70)	
<i>SLC18A1</i> rs1390938 (Thr138Ile)	Ile/Ile	19	11.71 ($\pm$ 5.12)	$F = 1.331$; $p = 0.284$	-0.06 ($\pm$ 0.15)	$F = 0.133$; $p = 0.876$	-1.21 ($\pm$ 2.80)	$F = 1.389$; $p = 0.268$
	Ile/Thr	7	8.87 ($\pm$ 4.01)		-0.07 ($\pm$ 0.14)		-3.07 ($\pm$ 1.39)	
	Thr/Thr	2	7.52 ($\pm$ 3.23)		-0.12 ($\pm$ 0.10)		-1.25 ($\pm$ 3.18)	
<i>SLC18A2</i> rs363224	C/C	5	7.53 ($\pm$ 3.94)	$F = 1.316$; $p = 0.288$	-0.01 ($\pm$ 0.03)	$F = 1.083$; $p = 0.360$	-2.00 ($\pm$ 1.58)	$F = 0.140$; $p = 0.870$
	C/A	12	11.57 ($\pm$ 5.43)		-0.12 ($\pm$ 0.17)		-1.37 ($\pm$ 3.16)	
	A/A	11	11.08 ($\pm$ 4.20)		-0.04 ($\pm$ 0.12)		-1.86 ($\pm$ 2.42)	
<i>SLC18A2</i> rs363276	C/C	15	10.77 ($\pm$ 3.14)	$F = 0.492$; $p = 0.618$	-0.02 ($\pm$ 0.11)	$F = 2.460$; $p = 0.133$	-2.40 ($\pm$ 2.36)	$F = 1.844$; $p = 0.179$
	C/T	11	11.04 ($\pm$ 6.66)		-0.12 ($\pm$ 0.16)		-0.54 ($\pm$ 2.59)	
	T/T	2	7.32 ($\pm$ 0.79)		-		-2.50 ($\pm$ 3.53)	
<i>BDNF</i> rs6265	Val/Val	14	11.6 ($\pm$ 4.54)	$F = 0.944$; $p = 0.406$	-0.06 ($\pm$ 0.17)	$F = 0.178$; $p = 0.839$	-2.03 ($\pm$ 2.13)	$F = 0.525$; $p = 0.599$

(Val66Met)	Val/Met	9	9.06 (±5.12)		-0.01 (±0.02)		-2.00 (±2.46)	
	Met/Met	2	13.54 (±8.31)		-0.07 (±0.00)		0.00 (±7.07)	
NRG1 rs62510682	G/G	12	10.98 (±4.38)	F = 2.746; p = 0.085	-0.02 (±0.09)	F = 2.185; p = 0.141	-0.83 (±3.09)	F = 1.708; p = 0.202
	G/T	13	11.73 (±4.98)		-0.14 (±0.17)		-2.00 (±2.03)	
	T/T	3	4.93 (±2.28)		-0.01 (±0.04)		-3.67 (±1.53)	
NRG1 rs35753505	T/T	12	8.96 (±3.97)	F = 2.342; p = 0.111	-0.04 (±0.11)	F = 0.637; p = 0.540	-2.66 (±2.38)	F = 2.302; p = 0.122
	T/C	10	12.91 (±5.79)		-0.11 (±0.18)		-0.40 (±2.64)	
	C/C	5	8.34 (±1.19)		-0.03 (±0.01)		-1.40 (±2.32)	
NRG1 rs10503929 (Thr289Met)	Met/Met	21	10.40 (±4.65)	F = 1.603; p = 0.223	-0.09 (±0.16)	F = 0.352; p = 0.708	-1.52 (±2.07)	F = 2.910; p = 0.073
	Met/Thr	3	14.73 (±6.94)		-0.04 (±0.05)		0.33 (±5.03)	
	Thr/Thr	4	8.01 (±2.21)		-0.02 (±0.09)		-4.00 (±1.87)	
SGCZ rs4427170	G/G	5	11.22 (±4.31)	F = 0.394; p = 0.679	-0.01 (±0.01)	F = 0.795; p = 0.467	-1.60 (±1.71)	F = 0.225; p = 0.800
	G/A	18	10.86 (±5.36)		-0.10 (±0.16)		-1.50 (±2.85)	
	A/A	5	8.26 (±1.65)		-0.02 (±0.09)		-2.40 (±2.63)	
SGCZ rs12680924	A/A	11	10.13 (±4.06)	F = 1.474; p = 0.350	-0.07 (±0.13)	F = 0.373; p = 0.694	-2.27 (±2.08)	F = 0.481; p = 0.624
	A/T	14	9.97 (±4.59)		-0.09 (±0.17)		-1.36 (±3.15)	
	T/T	3	15.06 (±7.89)		0.01 (±0.00)		-1.00 (±1.00)	
NPAS3 rs12894058	G/G	12	9.67 (±2.67)	F = 0.785; p = 0.468	-0.14 (±0.18)	F = 2.157; p = 0.145	-2.42 (±1.95)	F = 0.869; p = 0.432
	G/A	12	10.63 (±6.13)		-0.03 (±0.07)		-1.04 (±1.95)	
	A/A	4	13.25 (±5.86)		0.02 (±0.09)		-1.38 (±5.34)	

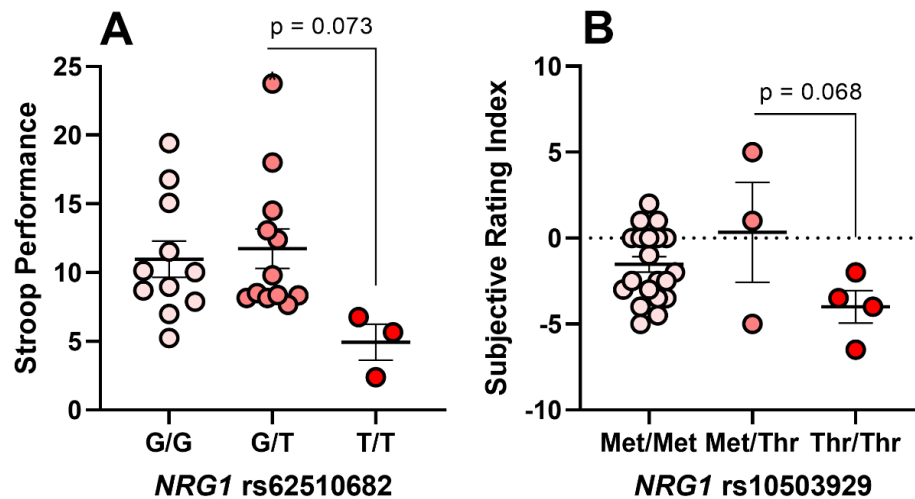


Figure 5.4. Genotype and cognition. A. *NRG1* rs62510682. People carrying the T/T genotype displayed poorer performance on the Stroop Task compared to G/T carriers. **B. *NRG1* rs10503929.** People carrying the Thr/Thr genotype displayed lower subjective rating (craving) index score compared to Met/Thr carriers. Data presented as individual values, mean $\pm$ SEM.

5.3.4. Multiple regression analysis

A multiple regression was run to predict the age of onset of methamphetamine use based on psychiatric and cognitive factors identified in **Chapter 3** (i.e. ASPD diagnosis and Stroop performance, respectively), and *NRG1* rs10503929, the only marker significantly associated with age of onset in the present Chapter (**Table 5.11**). The overall model was significant [$F(3, 25) = 10.846$; $p < 0.001$], and explained 59.7% of the variance in age of onset in this sample. Consistent with results in Chapter 3, comorbid diagnosis of ASPD was a significant predictor ($\beta = -0.386$, $p = 0.011$), whereas Stroop performance was not ($\beta = 0.165$, $p = 0.272$). In addition, carrying one of the homozygous genotypes of *NRG1* rs10503929 was the strongest predictor of age of onset of methamphetamine use ($\beta = 0.544$, $p < 0.001$).

Table 5.11. Multiple regression examining the predictive value of psychiatric, cognitive, and genetic factors on age of onset of methamphetamine use

Dependent Variables	Predictor Variables	R ²	b	SE b	β	p-value
Age of Onset	Model	0.288				
	Constant		14.883	4.486		
	ASPD		-4.865	1.758	-0.386	0.011
	Stroop (Inhibition)		0.218	0.193	0.165	0.272
	<i>NRG1</i> rs10503929		10.699	2.804	0.544	< 0.001

5.4. Discussion

The aim of the present Chapter was to investigate differences in polymorphism distribution in well-rationalized candidate genes between controls and people with methamphetamine use disorder. I found that *SLC18A1* rs2270641 conferred a risk for methamphetamine use disorder, whereas *COMT* rs4680 and *NRG1* rs35753505 were protective. In addition, the rs10503929 variant of *NRG1* was associated with age of onset of methamphetamine use, while rs62510682 was associated with severity of use as measured by the ASSIST. *BDNF* rs6265 was also found to be associated with severity of use. There were no significant differences in cognition between any genes and genotypes.

5.4.1. *SLC18A1*

In this study, I found that distribution of the *SLC18A1* rs2270641 polymorphism was significantly different between people with methamphetamine use disorder and controls (**Table 5.7**). Risk analyses revealed that carrying the heterozygous genotype (Pro/Thr) was associated with 6.2 greater odds of methamphetamine use disorder, relative to the wild type (Thr/Thr). This polymorphism is associated with schizophrenia in Caucasian people (Bly, 2005). *SLC18A1* codes for the vesicular monoamine transport 1 (VMAT1), a protein involved in neurotransmitter reuptake (Schuldiner et al., 1995). The rs2270641 polymorphism results in the substitution of a threonine (Thr) for a proline (Pro), and is located within the first 21 amino acid of VMAT1, in the cytosol (**Chapter 1, Figure 1.4**). While Bly (2005) speculates that rs2270641 may affect uptake and/or release of neurotransmitter from the vesicle (Bly, 2005), its biological function is still unknown.

Exploratory analyses revealed a trend in age of onset between rs2270641 genotypes. People carrying the wild type (Thr/Thr) were more likely to have started using methamphetamine earlier in life compared to those carrying the homozygous minor allele genotype (Pro/Pro) (**Figure 5.2B**). It is possible that wild type carriers are more likely to

develop methamphetamine use disorder if they are exposed to methamphetamine early in life. It is important to note that the trend did not reach statistical significance, and it is critical to replicate these findings in larger sample.

5.4.2. *COMT*

Carrying the heterozygous genotype (Val/Met) of *COMT* conferred protection against methamphetamine use disorder (OR = 0.47) relative to the wild type (Val/Val) (**Table 5.8**). This is in line with two Han Chinese studies (Jugurnauth et al., 2011; Li et al., 2004). *COMT* codes for the enzyme catechol-O-methyltransferase, which catalyzes the degradation of dopamine in the synaptic cleft (O'Leary et al., 2016). The rs4680 polymorphism is in the fourth exon and leads to the substitution of a valine (Val) for a methionine (Met). The Met allele is known to have about 25% of the enzymatic activity of the Val allele, resulting in homozygous Met/Met carriers having higher synaptic dopamine levels than Val carriers (Lachman et al., 1996).

People carrying the wild type Val/Val genotype have reported significantly greater total years of methamphetamine use compared to people carrying at least one Met allele (Bousman et al., 2010a). In people with schizophrenia, Met allele carriers displayed better neurocognitive performance compared to Val carriers (Bilder et al., 2002; Egan et al., 2001), including in inhibitory control. It is possible that carrying at least one Met allele is protective due to increased cognitive control, which may lead to reduced methamphetamine taking after experimentation. It should be noted that this is highly speculative, as I could not investigate the effect of the allele on cognition due to the low number of participants carrying it in the present study. Further research is warranted to delineate the mechanism underlying the protective effect of *COMT* rs4680 on methamphetamine use disorder vulnerability.

5.4.3. *NRG1*

5.4.3.1. rs35753505

Carrying the minor allele (C) of rs35753505 was found to be protective in a dominant model of inheritance (OR = 0.26), indicating lower number of C allele carriers in people with methamphetamine use disorder in this study (**Table 5.8**). This is in contrast with a previous study, which found no association between this polymorphism and methamphetamine use disorder (Okochi et al., 2009). Discrepancies between studies may reflect different factors. First, all participants in Okochi et al. (2009) were Japanese, whereas the sample in this study was predominantly of Caucasian descent. As previously noted, risk and protection conferred by polymorphisms may be ethnically divergent (Bousman et al., 2010b). Second, all participants in Okochi et al. (2009) had a comorbid diagnosis of methamphetamine-induced psychosis, whereas majority of participants in this study reported no psychotic symptoms. The C allele is associated with a risk for schizophrenia in Asian populations (Mostaid et al., 2017). In Okochi et al. (2009), it is possible that lower number of *NRG1* rs35753505 C allele carriers could not be detected because of potential increased number of C allele carriers due to comorbid methamphetamine-induced psychosis diagnosis in the methamphetamine users.

NRG1 codes for the neuregulin-1 protein, which binds to the Erb-B2 Receptor Tyrosine Kinase 4 (ErbB4) receptor. The *NRG1*-ErbB4 signaling pathway is involved in neural development (including neuron migration, axon guidance, myelination, and synapse formation), synaptic plasticity, and neuronal survival (Mei and Xiong, 2008). The rs35753505 polymorphism is located on the 5' end of *NRG1*. Some studies suggest that carrying the C allele is associated with better memory, visuospatial performance, and attention in other psychiatric disorders (Rolstad et al., 2015), whereas other studies found no effect of the allele on working or episodic memory (Krug et al., 2010). In this study, rs35753505 was not associated with cognitive performance.

5.4.3.2. rs10503929

A second *NRG1* polymorphism, rs10503929, was significantly associated with age of onset of methamphetamine use (**Figure 5.2A**). This polymorphism results in the substitution of a methionine (Met) for a threonine (Thr). People carrying the homozygous major (Met/Met) or homozygous minor alleles (Thr/Thr) were more likely to have started using methamphetamine earlier in life compared to heterozygous genotype carriers (Met/Thr). Notably, results from the multiple regression (**Table 5.11**) revealed that carrying a homozygous genotype was the single strongest predictor of age of onset out of all factors, including those identified in **Chapter 3**. While rs10503929 is a non-synonymous missense mutation, it does not appear to affect *NRG1* expression (Jagannath et al., 2017). The polymorphism may play a different role, for example contribute to change in affinity of the neuregulin-1 protein for its receptor. Dysregulation of neuregulin-1 signaling leads to disruption of hippocampal plasticity (Agarwal et al., 2014), which has been hypothesized to play a role in the development and maintenance of addiction (Kutlu and Gould, 2016). It is therefore possible that rs10503929 homozygous genotypes affect neuregulin-1 signaling, which in turn increase susceptibility for development of methamphetamine use disorder when exposed to methamphetamine early in life.

In addition, people carrying the Met/Met genotype were more likely to have a high ASSIST score compared to Thr/Thr carriers (**Figure 5.3C**). This indicates that Met/Met may be at risk factor for heightened severity and frequency of methamphetamine use (WHO ASSIST Working Group, 2010). In addition to lower ASSIST score, people carrying the Thr/Thr genotype had a lower subjective rating index compared to Met/Thr carriers (**Figure 5.4B**), indicating better response inhibition to methamphetamine-related cues. The risk of developing methamphetamine use disorder conferred by Thr/Thr is therefore unlikely to be associated with poor cognition or high risk and severe drug-taking behavior. Chronic stress is another well-known factor involved in the development of addiction (Sinha, 2008; Wemm and Sinha, 2019). It is possible that Thr/Thr plays a role in stress modulation. In fact, in my

sample, Thr/Thr carriers reported higher levels of anxiety and tension on the PANSS compared to heterozygous carriers ($p < 0.05$). Taken together, while both Met/Met and Thr/Thr appear to be age-dependent risk factors for early onset methamphetamine use, Met/Met may play a role in compulsive methamphetamine taking, as indicated by a higher ASSIST score, whereas Thr/Thr may do so by increasing stress response. It is however important to note that differences in ASSIST score and subjective index did not reach statistical significance ($p < 0.1$).

5.4.3.3. rs62510682

I identified a third *NRG1* polymorphism, rs62510682, which was associated with severity of methamphetamine use (**Figure 5.3A**). People carrying the homozygous minor alleles (T/T) were more likely to have lower ASSIST scores compared to both wild type (G/G) and heterozygous genotypes (G/T). This variant is located on the 5' end of *NRG1*, and its effect on gene or protein expression is unknown. Surprisingly, cognitive analyses revealed that T/T carriers displayed poorer performance in the Stroop task compared to G/T carriers (**Figure 5.4A**), though this was not significant. This indicates that T/T carriers may have poorer inhibitory control. The dissociation between severity of use and inhibition is consistent with results from Chapter 3, in which ASSIST score and Stroop performance did not correlate (**Table 3.8**).

Overall, it appears clear that *NRG1* variants play a role in susceptibility to methamphetamine use disorder, with one mutation conferring protection, one associated with earlier age of onset, and a third one with severity of methamphetamine use. Very little is known of the function of these variants, and future research should aim to delineate their role. Importantly, haplotype analysis should be carried out to examine the interplay between these variants.

5.4.4. *BDNF*

Contrary to my hypothesis, *BDNF* rs6265 was not associated with methamphetamine use disorder in this study (**Table 5.7**). This is in contrast with the results of the meta-analysis carried out as part of Chapter 4, which revealed that rs6265 is protective (Guerin et al., 2021a). It should be noted that in the current Chapter the sample size was low, with only two participants carrying the homozygous minor allele genotype. Nonetheless, I found an association between rs6265 and severity of use (**Figure 5.3B**), with participants carrying the homozygous major alleles (Val/Val) reporting higher ASSIST scores compared to heterozygous genotype carriers (Val/Met). This suggests that protection conferred by rs6265 may be due to lower severity of use in people carrying at least one minor allele. The Met allele is associated with anxiety disorders and anxiety-like behaviors (Chen et al., 2017; González-Castro et al., 2019; Moreira et al., 2015; Tomasi et al., 2020). It therefore unlikely that the Met allele exerts its protective effect by reducing methamphetamine-induced anxiety during withdrawal. Rather, decreased levels of the BDNF protein caused by the Met variant may reduce the rewarding effect of methamphetamine (Egan et al., 2003; Ghitza et al., 2010), and in turn reduce severity and frequency of use.

In contrast to previous reports (Su et al., 2015, 2014), there was no association between rs6265 and age of onset of methamphetamine use or cognition in this study. Discrepancies between studies may be due to differences in demographic characteristics. As previously mentioned, my sample primarily comprised Caucasian participants, whereas Su and colleagues (2014, 2015) exclusively recruited people of East Asian descent. Importantly, they only had between 16.5-21% female participants in their sample compared to 42.9% in the present study. There is evidence for sex-specific effects of genes on human disease (Ober et al., 2008). In addition, preclinical research suggests a sexually-dimorphic effect of BDNF deficiency on impulse behaviors in mice exposed to methamphetamine in young adulthood (Manning and van den Buuse, 2013), with *BDNF* genotypes having no

effect on prepulse inhibition in female animals. It is therefore possible that the association between *BDNF* rs6265 and age of onset and cognition may be specific to male participants. Due to the small sample size in the present study, I could not test this hypothesis. Further research is warranted to explore the effect of the relationship between sex and *BDNF* genotypes on drug use characteristics and cognition.

5.4.5. *SLC18A2*

I found a trend in ASSIST score for *SLC18A2* rs363276, with homozygous minor allele carriers (T/T) more likely to have lower ASSIST scores compared to major allele carriers (C/C) (**Figure 3D**). *SLC18A2* codes for VMAT2 (Schuldiner et al., 1995). Similar to VMAT1, VMAT2 regulates monoamine transport. While the biological function of rs363276 is unknown, it is possible that the C/C genotype reduces VMAT2 function, thus blocking reuptake of cytosolic dopamine. This may then lead to reduced release of dopamine in the synaptic cleft upon methamphetamine exposure. People with the rs363276 variant may therefore use of a higher dose to achieve the rewarding effect of the drug, which may eventually lead to the development of more severe methamphetamine use.

My results are in contrast with a previous report which found the T-allele (rather than C) to be associated with cocaine dependence (Levrán et al., 2015). This is surprising as cocaine and methamphetamine both exert their rewarding effect by interacting with VMAT2 (Eiden and Weihe, 2011). Discrepancies between studies may be due to ethnic divergence. Levrán and colleagues (2015) only found an association between the T-allele and cocaine dependence in African Americans, whereas there was no association in people of European and Middle Eastern (Levrán et al., 2015). In my study, most participants were Caucasian. It is therefore possible that risk conferred by rs363276 may differ between ethnicities. This further emphasize the need to stratify samples by ethnic background when investigating associations between gene variants and neuropsychiatric disorders (Bousman et al.,

2010b). Importantly, future work should aim to delineate and confirm the biological function of rs363276.

5.4.6. NPAS3

In the present study, there was a trend in age of onset of methamphetamine use and *NPAS3* rs12894058, with the homozygous G/G genotype carriers more likely to have started earlier compared to the A/A genotype (**Figure 5.2C**). rs12894058 was one of the top candidates identified in a GWAS in this population (Ikeda et al., 2013), indicating it may contribute to the genetic susceptibility for methamphetamine use disorder. *NPAS3* codes for the neuronal PAS domain protein 3 protein (NPAS3), a transcription factor localized to the nucleus (O'Leary et al., 2016). It regulates genes involved in hippocampal neurogenesis (Pieper et al., 2005). Variations in *NPAS3* may lead to reduced function and impairment of this process (Macintyre et al., 2010). Evidence from preclinical studies indicates that impaired hippocampal neurogenesis is associated with methamphetamine exposure (Cho and Kim, 2010; Mandyam et al., 2008; Venkatesan et al., 2011), which may in turn result in long-term cognitive deficits (Loxton and Canales, 2017). It is therefore possible that the rs12894058 variant of *NPAS3* causes pre-existing impairment in hippocampal neurogenesis, which are further exacerbated when methamphetamine is first taken early in life, eventually leading to the development of methamphetamine use disorder. Future work should aim to uncover the exact role of the G/G genotype in hippocampal neurogenesis to confirm this hypothesis.

5.4.7. Limitations

G*Power recommends a total sample size of least 108 participants to detect medium effect sizes using Chi-square analyses (Erdfelder et al., 2009). This is much larger than the current sample of 57. Post-hoc power analyses revealed that power ($1 - \beta$) for significant associations ranged between 0.58-0.77 in the present study. It is therefore possible that meaningful associations went undetected. All results presented and discussed in this

Chapter should therefore be taken with great caution. Nonetheless, these preliminary findings can inform design of subsequent hypothesis-driven studies. Due to the small sample size, I could not stratify the sample by sex and ethnicity. It is critical to do so as putative gene variant distribution may vary across population of different ethnic background (Bousman et al., 2010b), and sex (Ober et al., 2008). Lastly, I could not conduct haplotype analyses as some of the groups were too small ($n < 2$). Haplotype analyses are critical to better understand how different variants in the same gene (e.g., *NRG1*) contribute to susceptibility for methamphetamine use disorder.

5.4.8. Implications and future directions

The findings in the present study provide preliminary evidence of the involvement of certain gene variants in the susceptibility for methamphetamine use disorder. While the study was underpowered (power = 0.58-0.77), it provides proof-of-concept evidence for larger studies. Genes significantly associated with methamphetamine use disorder were primarily involved in dopamine neurotransmission (*SLC18A1* and *COMT*) and neuroplasticity (*NRG1* and *BDNF*). Notably, all these genes have been implicated in other substance use and comorbid neuropsychiatric disorders. The sample in the current study had a range of comorbidities (see **Chapter 3, Tables 3.2 and 3.3**), it is therefore possible that these variants are common genetic risk factors across psychiatric disorders.

Importantly, the interaction between variants remains to be investigated. Given the highly polygenic nature of substance use disorders (Ducci and Goldman, 2012), each variant is likely to have an extremely small contribution on the heritability of methamphetamine use disorder, and future studies should aim to replicate these findings in larger genome- and exome-wide association studies. While the ideal sample size will vary between disorders, populations and inheritance model examined, it is proposed that polygenic neuropsychiatric disorders such as methamphetamine use disorder require ~10,000 participants to uncover

their genetic architecture (Sullivan et al., 2018). I recognize that there are key practical limitations around recruiting people with methamphetamine use disorder. However, with the formation of international collaborations and pooling of datasets between different research groups, it would be feasible to obtain the required power. Alternatively, meta-analysis of smaller GWAS would be a cost-effective first step in elucidating the genetic structure of methamphetamine use disorder.

5.4.9. Conclusions

In conclusion, this study highlights a potential role for functional variants in *SLC18A1* and *COMT* in conferring a risk for or protection against methamphetamine use disorder, respectively. Consistent with literature, *BDNF* Val66Met was associated with reduced severity of methamphetamine use in this sample. In addition, three *NRG1* markers were identified to be involved in susceptibility for methamphetamine use disorder. A better understanding of the biological function and interplay between these variants is needed to elucidate the exact role of *NRG1* as a contributing factor for development of methamphetamine use disorder.

Chapter 6. General Discussion

The overall aim of this thesis was to investigate the association between psychiatric, cognitive, genetic factors, and age of onset of methamphetamine use in people with methamphetamine use disorder. Key findings from the present thesis are summarized in

Table 6.1.

Table 6.1. Summary of key associations between psychiatric, cognitive, and genetic factors and drug use parameters. ASPD: antisocial personality disorder; I.V.: intravenous; ΔSCR: differential skin conductance response.

	Age of Onset	Total Years of Use	Severity of use
Psychiatric	Comorbid ASPD → ↓ age of onset	Unassessed	Unassessed
Cognitive	↓ Stroop performance → ↓ age of onset	↑ average ΔSCR to methamphetamine cues → ↑ duration of use	I.V. use → ↑ average ΔSCR to methamphetamine cues
	↑ motor and cognitive impulsivity → ↓ age of onset		
Genetic	<i>NRG1</i> rs10503929 homozygous genotypes → ↓ age of onset	Unassessed	<i>NRG1</i> rs626510682 homozygous minor genotype → ↓ severity of use
			<i>BDNF</i> rs6265 heterozygous genotype → ↓ severity of use

6.1. Summary of findings

I first developed and validated a new cue reactivity paradigm to assess subjective and physiological response to methamphetamine-related cues in Chapter 2. I also examined potential modulators of cue reactivity. The paradigm elicited robust subjective craving in people with methamphetamine use disorder. Notably, poor inhibitory control was associated

with increased cue-induced craving. Use of sedatives was also significantly associated with craving response, highlighting the need to control for polydrug use in future studies. In addition, presentation of methamphetamine-related pictures elicited sweating over and above food-related pictures in people with methamphetamine use disorder but not in controls. Importantly, magnitude of the response was associated with primary route of administration and duration of methamphetamine use. People who used methamphetamine intravenously and for longer displayed more sweating to methamphetamine-cues, indicating a potential dose-dependent relationship between methamphetamine use and physiological response to cues. This cue reactivity paradigm provided a good platform to assess inhibition of objective and subjective cue reactivity in Chapters 3 and 5.

In Chapter 3, I investigated the association between comorbid psychiatric symptoms, performance in a cognitive task battery, and age of onset of methamphetamine use and other drug use characteristics. There was a significant negative association between duration of methamphetamine use and performance on the symbol coding and verbal fluency tasks. This may indicate a dose-dependent relationship between methamphetamine use and deficits in attention, speed of processing, and verbal fluency, suggesting that impairment on these domains are a consequence of methamphetamine use. In addition, I observed a strong positive association between poor inhibitory control performance (measured by the Stroop task) and age of onset of methamphetamine use, suggesting that such deficits may be a risk factors for the development of methamphetamine use disorder. Lastly, using a hierarchical multiple regression model, I identified comorbid diagnosis of antisocial personality disorder (ASPD) as the strongest predictor of age of onset of methamphetamine use in my sample.

In Chapter 4, I systematically reviewed gene-association studies in people with methamphetamine use disorder to identify promising targets and pathways underlying the disorder. While I identified several key limitations in the literature, meta-analytic approach

revealed that functional variants in *FAAH* and *BDNF* were associated with a risk for and protection against methamphetamine use disorder, respectively.

In Chapter 5, I investigated the association between candidate gene polymorphisms and risk for methamphetamine use disorder. I also explored associations between these polymorphisms and drug use parameters and cognition. A variant in *SLC18A1* was identified as a potential risk factor for methamphetamine use disorder, with the heterozygous genotype associated with 6.22 greater odds of methamphetamine use disorder in this study. Variants in *COMT* and *NRG1* were identified as possibly conferring protection against methamphetamine use disorder. Exploratory analyses revealed an association between a different *NRG1* polymorphism and age of onset of methamphetamine use, whereas a third variant was associated with severity of use. Lastly, consistent with results from Chapter 4, a *BDNF* variant had a protective effect on severity of use in the present thesis. It is important to note that candidate gene analyses in this thesis were not adequately powered, and should be treated as preliminary results.

Taken together, my thesis identified key psychiatric, cognitive, and genetic factors associated with methamphetamine use disorder. Findings in Chapter 2 are consistent with the Pavlovian conditioning model of addiction. That is, subjective and physiological response to drug-related cues is experience-dependent. Importantly, while cue-induced craving appears to be a conditioned response, their magnitude may be modulated by impairment in inhibitory control. This is consistent with the impaired Response Inhibition and Salience Attribution model of addiction (Zilverstand et al., 2018). Results in this Chapter offer an alternative view on the “gas and brakes” model of motivation and decision making (Casey et al., 2011), which suggests that these processes are in competition to initiate, maintain, and terminate drug-seeking behavior. In this study, Pavlovian-based cue reactivity and inhibitory control interact, such that cue-induced responses are modulated by cognitive control. Furthermore, Chapter 3 provides evidence of inhibitory and impulse control is

associated with the age of onset of methamphetamine use. This is consistent with literature suggesting that the adolescents are more impulsive and more likely to engage in risky behaviors compared to adults (Gardner and Steinberg, 2005; Scott, 1992; Steinberg et al., 2008). This Chapter also suggests that personality disorders may contribute to poor impulse control and predict the age of onset of methamphetamine use, a concept supported by large epidemiological studies (Hopfer et al., 2013; Masroor et al., 2019). Lastly, Chapter 5 is the first study to provide preliminary evidence that age onset of methamphetamine use may also have a genetic basis. Overall, the work presented in this thesis suggests that psychiatric, cognitive, and genetic factors may all contribute to the susceptibility of developing methamphetamine use disorder and may interact with one another. Findings from this thesis have added to our knowledge of the disorder, specifically highlighting risk factors associated with age of onset of methamphetamine use, and will potentially inform the development of early interventions targeting these factors to treat people with early onset methamphetamine use and prevent their transition into methamphetamine use disorder.

6.2. Cognitive consequence of methamphetamine use: neural interpretation

In Chapter 2, I found that physiological response (differential skin conductance response; Δ SCR) to methamphetamine-related cues was positively associated with duration of methamphetamine use. In addition, Δ SCR was on average higher in people who use methamphetamine intravenously compared to those who smoked methamphetamine. Given that cues were balanced in terms of route of administration in the paradigm (i.e., same number of needles vs. pipes), the effect of mode of use is unlikely to be related to the cues themselves, but rather indicates overall higher sweating to methamphetamine-related cues in people who inject methamphetamine. Intravenous use is associated with higher methamphetamine bioavailability and more severe methamphetamine dependence (Cruickshank and Dyer, 2009; McKetin et al., 2008). It should be noted there was no

correlation between the ASSIST amphetamine score, another measure of dependence severity, and cue reactivity in this sample. This may be due to the ASSIST also assessing the risk of current and future harm (WHO ASSIST Working Group, 2010), and may not be specific to current dependence severity. Nonetheless, these results indicate a potential dose-dependent effect of methamphetamine on increasing physiological cue reactivity.

The prefrontal cortex (PFC) and ventral striatum play a critical role in drug-related cue processing (Chase et al., 2011; Jasinska et al., 2014). Gray matter reduction is observed in brain regions underlying this process in people with methamphetamine use disorder, particularly in the frontal lobes (Kim et al., 2006; London et al., 2015; Lyoo et al., 2015; Morales et al., 2012; Nakama et al., 2011; Ruan et al., 2018). Notably, total years of methamphetamine use are associated with reduced gray matter in frontal and temporal cortices (Okita et al., 2018; Ruan et al., 2018). In contrast, *increased* striatal gray matter volume is observed in this population (Berman et al., 2008).

Such neurotoxicity caused by chronic methamphetamine use may alter brain structure in regions involved in cognitive processes, including cue processing, leading to deficits in this domain. Importantly, growing evidence suggests that discontinuation of use and protracted abstinence may help the recovery of structural deficits in these brain regions (Kim et al., 2006; London et al., 2015; Morales et al., 2012; Nie et al., 2020; Ruan et al., 2018). This is in line with evidence from small longitudinal studies also indicating that certain cognitive abilities may recover with abstinence (Iudicello et al., 2010; Proebstl et al., 2019; Zhong et al., 2016). It is important to note that due to the cross-sectional nature of this study, causation cannot be confirmed. It is possible that heightened physiological response to methamphetamine-related cues lead to longer duration of methamphetamine use. To validate the direction of the relationship between these factors and drug use parameters, it is critical to replicate these findings in prospective cohort studies.

In contrast, it is unlikely that increased reactivity to methamphetamine cues predates intravenous methamphetamine use. It is therefore safe to assume that using methamphetamine intravenously does in fact lead to heightened cue reactivity. To the best of my knowledge, no studies have directly compared the effect of intravenous and smoked methamphetamine on the human brain. However, higher methamphetamine bioavailability and peak plasma concentration associated with intravenous use may lead to greater neurotoxicity (Cruickshank and Dyer, 2009). Importantly, intravenous use is associated with repeated relapses during treatment, and treatment non-completion in people with methamphetamine use disorder over and above other route of administration (Hillhouse et al., 2007). Taken together, it is possible that intravenous use causes greater structural alterations in brain regions associated with cue processing that ultimately leads to heightened response to methamphetamine-related cues in people who inject methamphetamine, therefore resulting in poorer treatment outcomes compared to people who smoke methamphetamine. It is therefore important to take route of administration into account when designing treatment plans for people with methamphetamine use disorder.

6.3. Factors associated with age of onset of methamphetamine use

6.3.1. Inhibition and impulse control

In Chapter 3, I found that early-onset methamphetamine use was associated with greater deficits in inhibitory control measured by the Stroop task and lower impulse control measured by Barratt's Impulsiveness Scale. Evidence from longitudinal studies indicates that poor inhibition does not recover with abstinence (Fitzpatrick et al., 2021; Proebstl et al., 2019). Taken together, this strongly suggests that lack of inhibition and high impulsivity predate the onset of methamphetamine use and are therefore risk factors related to younger age of onset.

Performance on these tasks share overlapping neural correlates, both relying heavily on the PFC (Dalley et al., 2011; Munakata et al., 2011). In addition, dopaminergic signaling

plays a key role in modulation of inhibition and impulsivity (Jentsch et al., 2014; Jentsch and Pennington, 2014). Cortical regions are known to undergo dramatic changes and maturation in adolescence (Kolb et al., 2012; Spear, 2000), and dopaminergic innervation in these regions mature very slowly (Kalsbeek et al., 1988; Naneix et al., 2012). Importantly, poor neuronal integrity in the anterior cingulate cortex, a subregion of the PFC, is directly associated with poorer Stroop performance in early-onset (10-14 years old) methamphetamine use (Kim et al., 2016). Therefore, ongoing maturation and development of cortical regions during adolescence may result in pre-existing reduction in inhibitory and impulse control abilities for some individuals, which may facilitate the transition from casual to problematic methamphetamine use. Taken together with my findings, methamphetamine use early in life combined with premorbid deficits in inhibition and impulsivity may increase the risk of developing a formal methamphetamine use disorder.

6.3.2. Predicting age of onset of methamphetamine use using psychiatric, cognitive, and genetic factors

In Chapter 3, I conducted a hierarchical multiple regression to investigate whether adding psychiatric and cognitive factors improved the prediction of age of onset of methamphetamine use over and above demographic characteristics in people with methamphetamine use disorder. In Chapter 5, I conducted a separate multiple linear regression, this time including genetic factors identified in the Chapter. The only gene marker significantly associated with age of onset of methamphetamine use was *NRG1* rs10503929, with the homozygous genotypes associated with earlier onset. It was therefore included in the model, along with comorbid diagnosis of ASPD and Stroop performance. In the final model, diagnosis of ASPD was a significant predictor, whereas Stroop performance was not. Importantly, carrying one of the homozygous genotypes of *NRG1* rs10503929 was the strongest predictor of age of onset of methamphetamine use. While no studies have investigated the association between *NRG1* variants and ASPD, evidence suggests that

antisocial traits may have a genetic basis (South, 2015). In addition, *NRG1* is associated with other personality disorders, including schizotypal personality disorder in adolescents (Lin et al., 2005). In this thesis *NRG1* rs10503929 was not associated with ASPD, though this is most likely due to the small sample size. It would be informative to further investigate the relationship between ASPD and *NRG1* in larger studies given they were the strongest predictors of age of onset of methamphetamine use in this thesis.

6.4. Potential model of adolescent vulnerability for methamphetamine use disorder

Based on my findings I propose the following model of adolescent vulnerability for methamphetamine use disorder (**Figure 6.1**). PFC maturation in adolescence may result in pre-existing deficits in inhibitory and impulse control. When methamphetamine is experimented with during this period, these deficits may perpetuate methamphetamine-taking. Given that poor inhibition is associated with an increase in cue-induced craving, these deficits may also lead to repeated relapse after abstinence (Carter and Tiffany, 1999). This eventually will result in the development of a formal methamphetamine use disorder later in life.

In addition, I identified comorbid diagnosis of ASPD as a predictor of early onset methamphetamine use. Results in this thesis along with evidence from large epidemiological studies showing the impact of personality and conduct disorders on subsequent development of substance use disorders (Hopfer et al., 2013; Masroor et al., 2019) strongly suggest that diagnosis of ASPD may predate onset of methamphetamine use. Notably, key symptoms of ASPD include high impulse behaviors (American Psychiatric Association, 2013). Therefore, in addition to predating and predicting younger methamphetamine use onset, comorbid ASPD may also play a role in premorbid impulse control deficits previously identified as a risk factor.

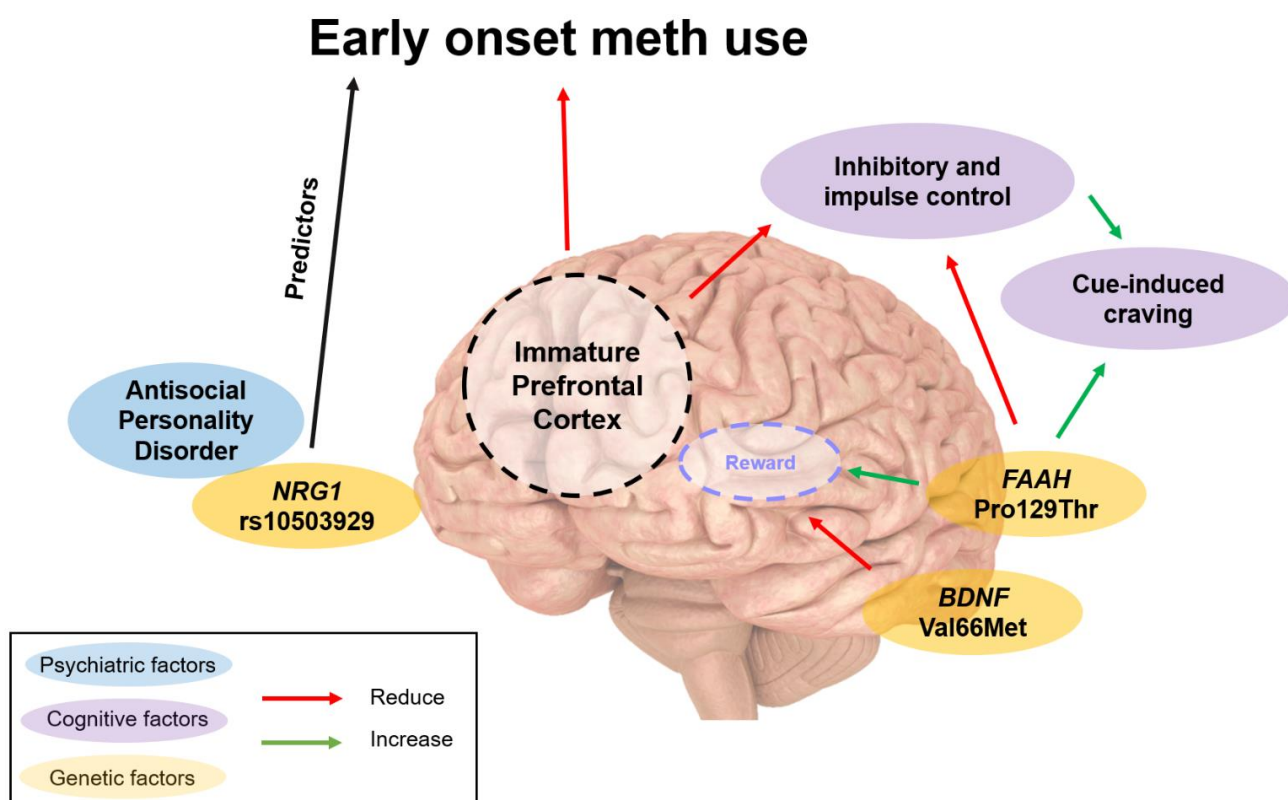


Figure 6.1. Schematic of a potential model of adolescent vulnerability for methamphetamine use disorder. Blue bubbles represent psychiatric factors. Purple bubbles represent cognitive factors. Yellow bubbles represent genetic factors. Red arrows indicate that a factor reduces function of subsequent factor. Green arrows indicate that a factor increases function of subsequent factor.

A number of preliminary genetic factors were also identified in this thesis. In the final regression model, the homozygous genotypes of *NRG1* rs10503929 were identified as the strongest predictors of age of onset of methamphetamine use. This suggests that people carrying the genotypes are more likely to develop methamphetamine use disorder when exposed to methamphetamine early in life. While little is known of the function of rs10503929, it may disrupt *NRG1* signaling. Dysregulation of *NRG1* may affect hippocampal plasticity (Agarwal et al., 2014), which plays a role in the development of substance use disorders (Kutlu and Gould, 2016). Importantly, results from this chapter were preliminary, and future research should aim to replicate the association of rs10503929 with early-onset methamphetamine use. In Chapter 4, two other gene polymorphisms were identified to play

a role in methamphetamine use disorder susceptibility: *FAAH* Pro129Thr and *BDNF* Val66Met. *FAAH* Pro129Thr may confer a risk for methamphetamine use disorder by increasing the subjective effect of methamphetamine, reward-related brain activity, and impulse behaviors (Hariri et al., 2009b; Patel et al., 2018). In contrast, *BDNF* Val66Met may be protective against methamphetamine use disorder by reducing the rewarding effect of methamphetamine (Ghitza et al., 2010), which in turn may lead to less severe methamphetamine use as observed in Chapter 5.

In summary, early onset methamphetamine use in combination with premorbid cognitive deficits can lead to development of methamphetamine use disorder later in life. In addition, psychiatric and genetic factors may predict the age of onset of methamphetamine use and susceptibility to methamphetamine use disorder. It is clear that targeting factors associated with early onset methamphetamine use is critical to develop new interventions to prevent young people from transitioning from casual to problematic methamphetamine use.

6.5. Clinical implications

6.5.1. Targeting inhibition and impulse control

Deficits in inhibitory and impulse control appear to be key factors involved with risk for methamphetamine use disorder. These deficits are also associated with unemployment, treatment non-completion, and relapse in this population (Pattij and De Vries, 2013; Weber et al., 2012; Winhusen et al., 2013). Targeting inhibitory and impulse control in early onset methamphetamine users is therefore essential to ensure the best clinical outcomes.

Cognitive remediation therapy (CRT) may be a promising psychosocial treatment option. CRT uses a range of methods, including drill and practice exercises, cognitive strategies training, and group discussions to improve cognitive processes in the long term (Bryce et al., 2016; McGurk et al., 2007). In schizophrenia, CRT results in improvement of cognitive abilities of medium effect sizes (McGurk et al., 2007; Wykes et al., 2011), and has

been well received by participants (Bryce et al., 2018; Contreras et al., 2016). Due to the shared cognitive characteristics between schizophrenia and methamphetamine use disorder (Guerin et al., 2019), CRT may also be beneficial to treat methamphetamine use disorder. In fact, preliminary evidence from a small clinical trial (N = 35) indicates that working memory training can significantly improve impulse control compared to treatment as usual in people with methamphetamine use disorder (Brooks et al., 2017). This effect may be due to the normalization of frontostriatal structure and function which are affected by methamphetamine use (Brooks et al., 2016).

CRT is currently receiving worldwide attention, with several trials underway in people with methamphetamine use disorder. In Australia, a feasibility study testing the efficacy of goal management training in improving executive functions and impulsivity is anticipated to start shortly (ACTRN12621000172808). In the US, a new tool, Neurocognitive Empowerment for Addiction Treatment (NEAT), is currently being trialed to test whether cognitive training can reduce self-reported craving and improve cognition in people with amphetamine use disorder (NCT03922646). If proven to be successful, these novel approaches can improve cognitive functioning in young casual methamphetamine users and prevent them from developing a methamphetamine use disorder.

There is currently no FDA- or TGA-approved medication to treat methamphetamine use disorder. In addition to psychosocial therapies, efforts are needed for the development or repurposing of already approved pharmacological agents. A recent meta-analysis of pharmacotherapies for methamphetamine use disorder revealed that methylphenidate is one of the most promising interventions (Chan et al., 2019). Two trials reported reduced methamphetamine use following treatment with methylphenidate compared to placebo (Konstenius et al., 2014; Tiihonen et al., 2007), as measured by the number of negative drug urine tests at follow-up. Evidence suggests that methylphenidate can increase inhibitory control performance in healthy participants (Roberts et al., 2020), most likely by improving

frontostriatal cognitive functioning (Spencer et al., 2015). This may at least partially explain why it is efficacious in reducing methamphetamine use. It should be noted that both studies presented high risk of bias (Chan et al., 2019). It is therefore critical to conduct larger, well controlled trials to ascertain the efficacy of methylphenidate in reducing methamphetamine use. Nonetheless, methylphenidate is already approved for use in adolescents to treat attention-deficit hyperactivity disorder in Australia (Therapeutic Goods Administration, 2020). It is therefore well-characterized and safe for use in young people.

In sum, CRT and methylphenidate may be promising treatments targeting deficits in inhibitory and impulse control, which can improve treatment retention, and reduce methamphetamine use and relapse in people with methamphetamine use disorder. Notably, best practice in problematic substance use treatment should include both pharmacological and psychosocial interventions (Ray et al., 2020). Therefore, methylphenidate could be used as adjunct to CRT to ensure the best treatment outcomes.

6.5.2. Neuromodulation

In addition to psychosocial and pharmacological interventions, non-invasive neuromodulation has shown promising results in reducing a range of outcomes in people with methamphetamine use disorder. Three randomized controlled trials have assessed the efficacy of repetitive transcranial magnetic stimulation (rTMS) targeting the dorsolateral PFC. Active rTMS led to significant reduction in craving and withdrawal symptoms compared to sham and/or control participants (Liang et al., 2018; Liu et al., 2019; H. Su et al., 2017). Notably, both high and low frequency of rTMS reduced levels of cue-induced craving (Liu et al., 2017). Likewise, repeated sessions of transcranial direct current stimulations (tDCS) have shown promises in reducing cue-induced craving for methamphetamine (Rohani Anaraki et al., 2019). In addition, bilateral tDCS significantly improved executive function and cognitive control after 10 sessions compared to sham stimulation (Alizadehgoradel et

al., 2020). Importantly, this was associated with a long-lasting reduction in craving for methamphetamine. Taken together, neuromodulation of the PFC, a brain region involved in executive function and cue-response processes, appears to improve cognitive performance, and reduce craving in people with methamphetamine use disorder. Future studies to trial such intervention in young people to assess whether it can prevent the transition to problematic methamphetamine use are warranted.

6.5.3. Targeting dual diagnosis

In the present thesis, antisocial personality disorder (ASPD) was one the most frequent comorbid psychiatric disorders present in our sample, and one of the strongest predictors of young age of onset of methamphetamine use. Consistent with the latter finding, antisocial behaviors are likely to develop prior to initiation of methamphetamine use (Degenhardt et al., 2007; Embry et al., 2009; Kramer et al., 2009).

Comorbid personality disorders are associated with higher severity of dependence and poorer treatment outcome in other drugs (Parmar and Kalojiya, 2018; Westermeyer and Thuras, 2005). A range of psychosocial interventions, including dialectical behavioral therapy, dynamic deconstructive psychotherapy, and dual-focused schema therapy have shown some promise in the treatment of dual diagnosis in adults (Parmar and Kalojiya, 2018), though the number of trials remain low and the current evidence is inadequate (Kienast et al., 2014). Importantly, there is currently no treatment studies focusing on adolescents with methamphetamine use disorder and comorbid ASPD (Kuitunen-Paul et al., 2020). It is therefore clear that identifying and treating personality disorders in young people is essential to prevent the onset of casual methamphetamine use because this population appears more likely to transition into having a formal methamphetamine use disorder. The recently published report from the Royal Commission into Victoria's Mental Health System recommends the establishment of a new statewide service for people living with mental

illness and substance use disorder, and undertaking of dedicated research into dual diagnosis (State of Victoria, 2021). This will ideally include more research and development of new early interventions targeting personality disorders and substance use in young people.

6.5.4. Gene variants and treatment response

Gene variants can interfere with both psychosocial and pharmacological treatments in psychiatric populations (Jiménez et al., 2018; Pickar and Rubinow, 2001). In this study, variants in three genes were associated with methamphetamine use disorder: *SLC18A1* rs2270641, *NRG1* rs35753505, and *COMT* rs4680. The latter is particularly noteworthy. As described above, methylphenidate is one of the most promising medications to reduce methamphetamine use (Chan et al., 2019). Evidence from attention-deficit hyperactivity disorder literature suggests that carrying the *COMT* Val/Val variant is associated with increased number of adverse events in response to methylphenidate (Joensen et al., 2017), though it does not appear to impact treatment outcomes (Pagerols et al., 2017). In addition, Val carriers are less likely to respond to antipsychotic and antidepressant treatment compared to Met carriers (Amare et al., 2017; Huang et al., 2016). This may partly explain why these classes of medication have failed to reduce methamphetamine use in previous clinical trials (Chan et al., 2019). When investigating response to new pharmacotherapies, it is therefore important to consider the interplay between the drug and gene variants involved in the pathway targeted. In addition, it is critical to take into account gene variants to ensure prescription of the right medication or therapy to clients when presenting with the disorder (Hamburg and Collins, 2010).

6.6. Strengths, limitations, and future directions

The present study is one of the first to systematically investigate psychiatric, cognitive, and genetic factors in people with methamphetamine use disorder. For this thesis, I developed a new cue reactivity paradigm comprising methamphetamine-related cues and control cues. All methamphetamine cues were first validated with a focus group comprising inpatients with a current diagnosis of methamphetamine use disorder. In addition, non-drug cues were included as they serve as critical control stimuli to understand drug-specific cue reactivity. In fact, without control cues and a control group it is impossible to determine whether an increase in reactivity to methamphetamine cues is actually related to the associative memory created during methamphetamine use. I also recorded two measures of reactivity: subjective self-reported craving, and physiological response (SCR). While subjective measures are an easy and quick way to assess response to cues, they may be unreliable as participants may report strong levels of craving due to their understanding of what they are expected to report in the study (Carter and Tiffany, 1999). On the other hand, SCR is less affected by demand characteristics, and is a good predictor of craving in other drugs (Bordnick et al., 2005; Kaag et al., 2016). The physiological component of the study was designed and analyzed in accordance with the “Publication recommendations for electrodermal measurements”, published by Boucsein and colleagues (2012). All physiological data were processed using a MATLAB code, thus reducing experimenter bias during analyses. Lastly, it is the first study to identify factors modulating both subjective and physiological response to methamphetamine-related cues. This is critical to gain a better understanding of cue-induced relapse vulnerability.

There is however a key limitation to note in addition to those already discussed in Chapter 2. While presentation of drug-related cues alone can elicit a response (Carter and Tiffany, 1999; Drummond, 2000), context also plays a critical role in the expression of conditioned response. Drug craving can be triggered by environments associated with the

drug-taking experience (O'Brien et al., 1992; Wikler, 1973). To fully understand the conditioned response and craving triggered by memories of the methamphetamine use experience, it is important that future studies take context into account. The use of virtual reality has proven to be a promising avenue to build such environments, and has been trialed in people with methamphetamine use disorder (Culbertson et al., 2010; Wang et al., 2018). In a similar fashion to the focus group conducted in this study, virtual environments should be designed and implemented in collaboration with people with lived experience to ensure maximum ecological validity.

I also used a range of structured clinical interviews (Kay et al., 1987; Sheehan et al., 1998), and a validated cognitive task battery (Delis et al., 2001; Kern et al., 2008) to comprehensively assess psychiatric and cognitive factors in people with methamphetamine use disorder in this thesis. The thorough assessment of nine different clusters of neuropsychiatric disorders is a strength of this thesis. Most studies in adolescent-onset methamphetamine use have focused on a limited selection of comorbidities (Kuitunen-Paul et al., 2020). I also rigorously assessed not only positive, but also negative and general psychotic symptoms in Chapter 3. While most studies to date have focused on positive symptoms as they are more commonly associated with methamphetamine use (Bell, 1973; Lecomte et al., 2013; McKetin et al., 2016), studies suggest that at least 20% of people with methamphetamine use disorder also report negative symptoms (Srisurapanont et al., 2003). Notably, recent evidence suggests that negative symptoms may not be related to methamphetamine use in this population, but rather may be due to polysubstance use, and in particular comorbid sedative use (Voce et al., 2019). While not directly associated with methamphetamine use, it is critical to assess frequency and distribution of negative symptoms in this population, as polysubstance use is common in people with methamphetamine use disorder (Connor et al., 2014). In fact, over 50% of the sample in this thesis were people with a polysubstance use disorder. Considering the association between

negative symptoms, poor functional outcomes (Correll and Schooler, 2020), and short- and long-term treatment response in other neuropsychiatric disorders (Carbon and Correll, 2014), it is critical to further assess the interaction between other substance use and negative symptoms in people who use methamphetamine.

To predict age of onset of methamphetamine use, I conducted a hierarchical multiple regression. The main advantage of using this statistical tool is the ability to control for nuisance variable such a demographic characteristics (Gelman and Hill, 2006). However, due to the cross-sectional nature of the study, the directionality of the relationship between variables of interest and drug use parameters could not be confirmed. While previous studies do suggest that poor inhibition and impulse control, as well as personality and conduct disorders may predate the onset of methamphetamine use (see **Sections 6.3 and 6.4** of this Chapter, respectively), an alternative explanation of the findings is that methamphetamine use early in life may be more harmful to brain regions involved in modulation of inhibitory and impulse controls. Likewise, It is possible that early methamphetamine use may contribute to the development of the clinical symptomatology of ASPD, including impulsivity, failure to plan ahead, and recklessness, irritability, and aggressiveness (American Psychiatric Association, 2013). In fact, methamphetamine use is associated with increased odds of aggression (McKetin et al., 2019), and impulsive behaviors (Guerin et al., 2019; Potvin et al., 2018).

In addition, the sample size in this Chapter was smaller than planned due to the COVID-19 pandemic. While the correlation analyses were adequately powered, the hierarchical multiple regression did not have statistical power to detect appropriate effect sizes. In fact, the sample could only detect very large effect sizes ($f^2 = 0.30$), which is unlikely to be the case in such an heterogenous sample. Results from the regression model should therefore be seen as preliminary and should be replicated in a larger sample. Future prospective cohort studies should aim to recruit and follow young people who use

methamphetamine across several years to identify which factors lead to the development of a formal methamphetamine use disorder, and which factors are a consequence of early methamphetamine use. To this date, very few longitudinal studies have examined psychiatric factors in young people (Degenhardt et al., 2007), and no studies have assessed changes in cognition over time in adolescents who use methamphetamine.

Another important limitation of this work is the potential confounding effect of polysubstance use on psychiatric and cognitive outcomes. In fact, over 50% of the sample in this thesis met criteria for a polysubstance use disorder, with cannabis and nicotine use disorders the most prevalent. Concurrent use of other drugs is associated with higher risk of development of psychotic symptoms in people who use methamphetamine (Bramness and Rognli, 2016; McKetin et al., 2010). In addition, data from a large twin study (n = 1419) suggest that cannabis use disorder is also strongly associated with ASPD (Gillespie et al., 2018). People who use multiple substances also display poorer performance in executive function tasks compared to people only using one drug (Lewis et al., 2020), with comorbid ketamine use the strongest predictor of poor cognitive performance in people who use methamphetamine (Cheng and Wang, 2016). Therefore, results in the present thesis could partly be attributed to cannabis and other drugs use, and not only to methamphetamine use. To investigate the potential confounding effect of polysubstance use in this population, future studies should have three groups: a methamphetamine-naïve group, a polysubstance use group, and a methamphetamine-only group. It should be noted, however, that while other drug use may be a confound, recruiting people who use drugs other than methamphetamine is more ecologically valid, as polysubstance use is common in this population (Connor et al., 2014).

In Chapter 4, I systematically reviewed candidate gene association studies in people with methamphetamine use disorder. Strengths included publication of the study protocol prior to the commencement of the review on PROSPERO (CRD420201778260). In addition,

for the first time in the field of genetics of methamphetamine use disorder, a rigorous assessment of quality and risk of bias of every study included in the review was conducted. This allowed for the identification of critical limitations in the current literature. This work will ensure that future studies avoid major design pitfalls. Lastly, a robust meta-analytic approach was used to identify potential markers involved in the genetic susceptibility for methamphetamine use disorder. Rigorous sensitivity analyses based on the quality of studies were carried out to ensure the results from the meta-analysis were reliable.

Lastly, in Chapter 5 I conducted candidate gene analyses on well-rationalized gene variants. Selection was based on previous literature, robust preclinical rodent data, and results from Chapter 4. Other strengths included the evaluation of different models of inheritance (co-dominant, dominant, recessive, and additive). Most studies to date have only focus on additive inheritance (Guerin et al., 2021a), which assumes the disease severity increases proportionally to the number of polymorphic alleles. I also conducted exploratory analyses to identify any relationship between gene variants and drug use characteristics such as age of onset and severity of use, and cognition. This provided some preliminary clues into the mechanism of risk and/or protection of key polymorphisms in methamphetamine use disorder.

As stated in Chapter 5, the study was underpowered to detect appropriate effect sizes. This was in part caused by the abrupt suspension of study recruitment in March 2020 due to the COVID-19 pandemic. Future larger studies in this population are needed to replicate these findings. In addition, while there was a strong rationale for variant selection, little is known of the biological relevance of *SLC18A1* and *NRG1* polymorphisms, and future studies should aim to delineate their function. First, it is critical to understand the relationship between the mutations and both gene- and protein-expression to uncover the molecular consequences of the variants (i.e., up- or down-regulation). Second, the bio-behavioral function of the variant needs to be assessed. This can be done using a range of preclinical

and *in vitro* tools. For example, using rodent knock-in models can help identify the role of each *SLC18A1* genotype on neurotransmitter brain levels by combining it with microdialysis and also testing for methamphetamine self-administration (Chefer et al., 2009). *In vitro* work can also determine effect of variants on binding affinity between receptors and their ligands (de Jong et al., 2005).

Comparably to the sample in Chapter 3, over 50% of the sample in this Chapter had a comorbid polysubstance use disorder. This is important as all significant genes identified above have been implicated in other substance use disorders. Therefore, an alternative explanation for the findings in this thesis is that these variants confer a shared genetic risk for certain symptoms observed across substance use disorders, rather than specifically be associated with methamphetamine use disorder. As previously noted, future studies should aim to compare people who exclusively use methamphetamine and people with polysubstance use disorder to uncover the genetics architecture of the disorder.

6.7. Conclusions

Methamphetamine use disorder represents a significant health threat globally, with an ever-expanding market and increase of users worldwide. More alarming, experimentation with methamphetamine begins early in life. This is a major concern as adolescents show heightened vulnerability to developing substance use disorders. The work presented in this thesis systematically investigated psychiatric, cognitive, and genetic factors associated with methamphetamine use disorder and identified key factors associated with early onset of methamphetamine use. Findings from this work suggest when methamphetamine is experimented with during adolescence, pre-existing cognitive deficits caused by the maturation of key brain regions may perpetuate methamphetamine-taking, eventually leading to the development of a formal methamphetamine use disorder. Comorbid diagnosis

of antisocial personality disorder and variants in genes involved in addiction neurocircuitry may further confer a risk for the disorder.

In conclusion, the work presented in this thesis will enable the conception and design of future larger prospective cohort studies and clinical trials to further assess and target the factors identified in people who started using methamphetamine early in life, preventing them from developing a formal methamphetamine use disorder, and thus alleviating the rising burden of disease.

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