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Genes, Neurocognition, and HIV Risk Behaviors in the Context of Methamphetamine and HIV

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

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by

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DEDICATION

There is a well known African proverb which states that "It takes a village to raise a child" and I believe it also takes a village to complete a dissertation. Thus, I would like to dedicate this dissertation to my village of friends and family for their unconditional support and love. In particular, my parents Dan and Carol Bousman whom provided a solid foundation from which I could build a successful education and my partner and best friend Jennifer Terpstra who provided encouragement and love that allowed me to persevere when times were tough.

EPIGRAPH

For every complex problem there is a simple solution which is wrong.

George Bernard Shaw

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ABSTRACT OF THE DISSERTATION

Genes, Neurocognition, and HIV Risk Behaviors in the Context of Methamphetamine and HIV

by

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University of California, San Diego, 2009
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Relative to other substances of abuse (e.g. alcohol, tobacco) the mechanisms which underlie the onset and progression of methamphetamine use disorders (METH) and their consequences (e.g. HIV risk behavior) are unclear. The purpose of this dissertation was to elucidate potential mechanisms using a systems biology and ecological approach in which, genetic, neurocognitive, and behavioral data were examined and explored in the context of METH and HIV. First a synthesis of the current genetic association literature was conducted to provide a systematic and quantitative review of the genetic epidemiology of METH use disorders. Then utilizing results

obtained from the synthesis and existing data on 400 adults with and without HIV-infection and/or METH dependence, a series of analyses were undertaken to replicate putative genetic markers for METH dependence as well as to examine and explore genetic, neurocognitive and contextual factors for HIV risk behaviors. All subjects underwent a comprehensive clinical characterization that included: demographic information, standardized medical examination, neurocognitive and psychiatric assessment as well as HIV risk behavior assessment. Available blood samples were used for DNA extraction and genotyping. Synthesis of the literature uncovered 39 genes, of which 18 were found to have a putative genotypic, allelic, and/or haplotypic association with METH use disorders, predominately in Asian populations. Case-control analysis among a diverse sample of African-Americans, Hispanics, and Caucasians for six of the putative and three novel genes suggested considerable ethnic divergence for METH dependence. Cross-sectional analyses revealed several complex associations between genetic [*i.e*. catechol-o-methyltransferase (*COMT*)], neurocognitive (*i.e.* executive functioning), and contextual (*i.e.* METH, HIV) factors on HIV risk behavior. Analyses suggested that dependent on a subjects *COMT* genotype and context, executive functioning and HIV risk behavior profiles can be significantly different, respectively. Findings provide support for further validation of candidate genes for METH dependence reported among Asian populations across other ethnic/ancestral groups as well as, examination of gene-context (i.e. gene-environment) associations and neurocognitive factors to better understand the complexity of HIV risk behavior.

CHAPTER 1

INTRODUCTION

INTRODUCTION

With increasing rates of methamphetamine (METH) dependence in the United States, especially among individuals infected with human immunodeficiency virus (HIV) (Bing et al., 2001; Woody et al., 1999), greater attention has been given to the causes and consequences of METH dependence. However, only recently have investigations into the genetic etiology of METH dependence been initiated; with few of these genetic associations being replicated in diverse ethnic populations. Furthermore, the combined influence of genetic and neurocognitive variation on HIV risk behavior within the context of METH dependence and HIV-infection is currently unknown.

According to previous research, interactions between METH use and HIV-infection are a major public health concern for a variety of reasons. First, evidence from cell cultures and animal models suggests that replication of feline immunodeciency virus in the context of METH is accelerated and may be amplified in the central nervous system (Gavrilin, Mathes, & Podell, 2002). In addition, increased HIV viral loads among METH users have been linked to poor adherence to highly active antiretroviral therapy (HAART) (Ellis et al., 2003; Reback, Larkins, & Shoptaw, 2003) as well as reduced access to medical care and over utilization of emergency departments (Peck, Shoptaw, Rotheram-Fuller, Reback, & Bierman, 2005; Richards et al., 1999). Furthermore, it has been suggested that METH is consumed at greater frequency among HIV-infected individuals to self-manage HIV-related depression, fatigue, and neuropathic pain (Robinson & Rempel, 2006). Thus, not only does it appear that METH dependence and HIV-infection attribute to strains on individuals and communities but also interact in a complex and potentially synergistic manner.

In the ensuing chapters, I will present five distinct but thematic studies (each an independent chapter) that collectively contribute to understanding the complex relationships between genotypic variation, neurocognitive functioning, and HIV risk behavior in the context of METH dependence and HIV-infection (Figure 1.1). Specifically, in Chapter 2, I begin by providing a background of METH use disorders followed by a review and synthesis of the genetic epidemiological literature related to METH use disorders. In Chapter 3, I build on Chapter 2 by presenting a study in which I selected six putative and three novel single nucleotide polymorphisms (SNPs) derived from the synthesis and examine them for association with METH dependence in an ethnically diverse population. Then in Chapter 4, I attempt to illustrate how genotype-phenotype relationships can be context dependent via results of a study that examined the relationship between a common single nucleotide polymorphism (SNP) located in catechol-o-methtransferase (*COMT*) gene (*i.e.* Val158Met) and executive functioning (*e.g.* behavioral planning, decision making) among those with and without METH dependence and/or HIV-infection. In Chapter 5, I describe and examine the contextual influence of METH dependence, HIV-infection and their combination on HIV risk behavior as well as negative mood (e.g. depression, anxiety) among a sub-sample of men who have sex with men (MSM). Finally, in Chapter 6, I present a study that examines the full model presented in Figure 1.1 by building on data presented in Chapter 4 and specifically looks at the combined and independent influences of contextual factors (*i.e.* METH, HIV), *COMT* genotype, and executive functioning on HIV risk behaviors. Collectively, these chapters provide observations that could be used to better

understand the influence of genetic, neurocognitive, and contextual interactions on complex phenotypes such as HIV risk behavior.

Conceptual Framework

My methodological approach and data interpretation were guided by systems biology (Boogerd, 2007) and ecological (Bronfenbrenner, 1979; Hancock, 1985; Sallis & Owen, 2002) frameworks. These frameworks recognize that complex behaviors and subsequent consequences of those behaviors are rarely a result of a single factor, rather a host of factors ranging from the molecular to societal levels. In addition, these frameworks are inherently interdisciplinary and emphasize the complex integration and interaction of biological and non-biological networks while accounting for the context in which these networks operate. Thus, particular associations present in one context may be absent or altered when examined in another context. The specific aims of this dissertation fit well into these frameworks in that I proposed to examine the interaction between genetic factors, neurocognitive factors and HIV risk behavior while accounting for contextual factors such as HIV-infection and METH dependence.

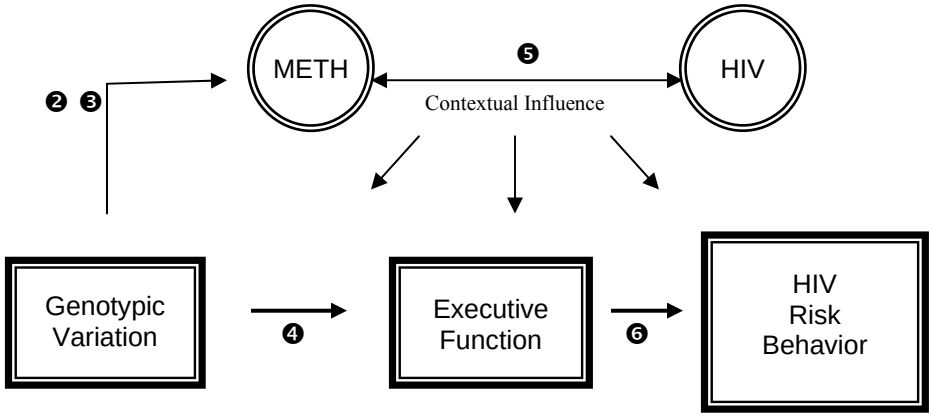

Figure 1.1 Conceptual Diagram of Genotypic Variation Affects on Executive Function and HIV Risk Behavior in the Context of METH and HIV

Chapters:

❷ Review and synthesis of genetic association studies of METH Use Disorders
❸ Associations of putative and novel genetic variants for METH dependence
❹ Impact of *COMT* genotype on executive functioning in the context of HIV-infection and METH dependence
❺ Sexual behavior and negative mood in the context of HIV-infection and METH dependence
❻ *COMT* genotype, executive dysfunction and sexual risk behavior in the context of HIV-infection and METH dependence

METH = Methamphetamine; HIV = Human Immunodeficiency Virus

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CHAPTER 2

REVIEW AND SYNTHESIS OF GENETIC ASSOCIATION STUDIES OF METHAMPHETAMINE USE DISORDERS

ABSTRACT

Efforts to understand the biological processes that increase susceptibility to methamphetamine (METH) use disorders (*i.e.*, abuse, dependence, and psychosis) have uncovered several putative genotypic variants. To date a synthesis of this information has not been conducted. Thus, systematic searches of the current literature were undertaken for genetic-association studies of METH use disorders. Each gene's chromosomal location, function, and examined polymorphic markers were extracted. Frequencies, odds ratios and 95% confidence intervals for risk alleles, as well as sample size and power, were calculated. We uncovered 38 studies examining 39 genes, of which 18 were found to have a significant genotypic, allelic, and/or haplotypic association with METH use disorders. Three genes (*COMT, DRD4,* and *GABRA1*) were associated with METH abuse, nine (*ARRB2, BDNF, CYP2D6, GLYT1, GSTM1, GSTP1, PDYN, PICK1,* and *SLC22A3*) with METH dependence, two (*AKT1* and *GABRG2*) with METH abuse/dependence, and four (*DTNBP1, OPRM1, SNCA,* and *SOD2*) with METH psychosis. Limitations related to phenotypic classification, statistical power, and potential publication bias in the current literature were noted. Similar to other behavioral, psychiatric, and substance use disorders, the genetic epidemiology of METH use disorders is complex and likely polygenic. National and international collaborative efforts are needed to increase the availability of large population-based samples and improve upon the power to detect genetic associations of small magnitude. Further, replication of the findings reviewed here along with further development of more rigorous methodologies and reporting protocols will aid in delineating the complex genetic epidemiology of METH use disorders.

INTRODUCTION

Consequent to the rise of methamphetamine (METH) consumption, researchers and clinicians from various disciplines have taken interest in the pathology of METH use disorders. This interest has been prominently marked in Southeast Asia and North America where the majority of METH production and consumption occurs (United Nations Office on Drugs and Crime, 2007). More recently, facilitated by the completion of the human genome, addiction scientists are focusing on potential genetic influences on METH use, abuse, dependence, and psychosis. Thus, the objective of this systematic review was to provide a synthesis of genetic studies of METH use disorders, with particular emphasis given to the results of genetic association studies.

First, we provide a brief introduction to the prevalence and effects of METH use, as well as define and provide a review of the heritability of METH use disorders. Next, we extend previous reviews of the genetics of METH in the literature (Barr et al., 2006; Costa & Eaton, 2006) by systematically reviewing results from genetic association studies and arriving at quantitative estimates of the effect of each genetic marker on the risk for METH use disorders. The review concludes with a summary of putative risk genes for METH use disorders, limitations of the current research and future directions for further investigation.

Prevalence and Effects of Methamphetamine Use

METH is a potent synthetic psychostimulant that can be injected, smoked, snorted, ingested (Anglin, Burke, Perrochet, Stamper, & Dawud-Noursi, 2000) or administered transrectally (Cantrell, Breckenridge, & Jost, 2006). Use of METH has been reported on every continent and its global prevalence is estimated at 15 to 16 million

people (United Nations Office on Drugs and Crime, 2007). Although a global problem, the vast majority of METH users reside in East and Southeast Asia as well as North America, with isolated pockets of high usage in parts of Europe such as the Czech Republic (United Nations Office on Drugs and Crime, 2007). In China, the largest METH market in the world, prevalence of METH use among new drug users is estimated at 5.6% surpassed only by heroin use (Lu, Fang, & Wang, 2008). In neighboring Japan, 0.3% of the general population and 6.8% of juvenile offenders are METH users (Miura, Fujiki, Shibata, & Ishikawa, 2006). Furthermore, other countries in East and Southeast Asia, such as Cambodia, Indonesia, Laos, Myanmar, Thailand, and the Philippines have also reported increased rates of METH use in recent years (Kulsudjarit, 2004). In North America, Mexico has become the largest producer of METH as a result of restrictions on precursor chemicals in the US and Canada. However, the west coasts of the Untied States and Canada have been greatly impacted by METH use (Maxwell & Rutkowski, 2008; National Drug Intelligence Center, 2006). The latest estimated annual prevalence rates in the general population for METH use in Canada were 0.8% in 2004, 1.4% in the United States in 2006, and 0.1% in Mexico in 2002 (Maxwell & Rutkowski, 2008).

The acute and chronic effects of METH are dependent on several factors including the amount and length of time the drug is consumed, route of administration, and purity of the drug. The acute effects range from euphoria and increased energy to loss of appetite, insomnia, and irritability. Prolonged use of METH may result in mood disturbances, tooth decay, cardiovascular problems, neurocognitive problems and onset of psychotic symptoms. Additionally, METH use has been linked to impulsive and risky behavior as well as higher rates of HIV seroconversion, hepatitis C infection, and other

sexually transmitted infections (see Barr et al., 2006; Quinton & Yamamoto, 2006; Scott et al., 2007 for comprehensive reviews of the effects of METH use).

Methamphetamine Use Disorder Phenotypes

In any research, defining the phenotype of interest is imperative for sound conclusions to be drawn and causal inferences to be made; however, unlike many medical disorders which have a blood, tissue or other confirmatory test, psychiatric disorders such as METH use disorders do not currently have these objective assessments (Costa & Eaton, 2006). The term "METH use disorder" represents a collection of at least three related phenotypic classifications that includes METH abuse, dependence and psychosis. These classifications each have unique criteria used to assign individuals to a diagnostic category based on relevant similarities. In general, classification can be of two types, including: 1) monothetic type, in which all members are identical on all characteristics; and 2) polythetic type, in which all members are similar, but not identical (Millon, 1991). METH use disorder classification systems are primarily polythetic.

In present-day psychiatric practice, phenotypic classifications of METH use disorders are governed by two major classification systems: (1) Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association, 1994) and (2) International Classification of Diseases (ICD) (World Health Organization, 1992). These classification systems stem from similar scientific and conceptual roots, but disagreement on certain aspects and details do exist despite attempts to bring the two systems into accord. Table 2.1 provides a summary of the similarities and differences of the two most commonly used versions of these classification systems.

In general, both classification systems concur on the diagnosis of METH dependence. However, for METH abuse and METH psychosis, divergence can be seen in the criteria related to the pattern of METH use and duration/persistence of psychotic symptoms, respectively. The ICD-10 criteria for METH abuse are more stringent in that they require persistent use of METH for at least one month or repeatedly over twelve months, whereas the DSM-IV allows for criteria to be met anytime within a twelve-month period. Likewise, ICD-10 criteria for METH psychosis are more stringent than the DSM-IV in that they require an earlier onset of symptoms (2 weeks vs. 1 month) and minimum duration of symptoms (48 hours vs. not specified). However, the ICD-10 does permit a longer maximum duration of psychotic symptoms than the DSM-IV (6 months vs. 1 month). Thus, research on the genetic epidemiology of these three phenotypic classifications of METH use disorders requires knowledge of not only the specific phenotype under investigation but also the classification system utilized.

Heritability of Methamphetamine Use Disorders

Heritability is defined as the proportion of the total phenotypic variance in a trait that is due to genetic factors, and non-zero heritability may justify the search for specific genetic factors in METH use disorders. In fact, according to a recent proposed genetic epidemiological framework (Burton, Tobin, & Hopper, 2005), calculations of heritability estimates are one of the first steps in a systematic approach to gene identification for any complex disorder. Accordingly, over the past two decades, a number of twin (Grove et al., 1990; Gynther, Carey, Gottesman, & Vogler, 1995; Kendler & Prescott, 1998; Kendler, Jacobson, Prescott, & Neale, 2003; Tsuang et al., 1996; Tsuang et al., 1998) and adoption (Cadoret, Troughton, O'Gorman, & Heywood, 1986; Cadoret, Yates,

Troughton, Woodworth, & Stewart, 1995) studies of substance use disorders have been conducted. Generally, these studies have reported substantial heritability for substance abuse/dependence, and consequently demonstrated the importance of genetic factors in the etiology of these disorders (see Vanyukov & Tarter, 2000) for a comprehensive review of the heritability of substance abuse).

To date, no study has provided specific heritability estimates for the abuse of or dependence on METH; however, the drug class known as "stimulants", which includes METH as well as cocaine, has been examined (Kendler & Prescott, 1998; Kendler et al., 2003; Tsuang et al., 1996; Tsuang et al., 1998). In these studies, heritability estimates ranged from .33 to .44 among male twin-pairs and .79 among female twin-pairs. Thus, dependent on sex, 33% to 79% of the variance in stimulant abuse can be attributed to genetic factors, and between 21% and 67% can be attributed to environmental factors. Collectively, these studies affirmatively answer a critical question in the genetic epidemiology of METH use disorders, which is "Is the risk of METH use disorders inherited?" However, these studies are not able to elucidate what specific genetic risk factors are inherited.

To assist with this process, linkage-based genome scans have been conducted to identify chromosomal regions (loci) that may confer vulnerability to METH use disorders. For example, one recent study (Uhl, Liu, Walther, Hess, & Naiman, 2001) looked specifically at illicit substance addiction, including stimulants. Results from this study provide evidence for potential substance abuse loci on chromosomes 3, 4, 9, 10, 11, 12, 13 and X from which candidate polymorphisms could be selected. In a more recent linkage analysis for cocaine dependence as well as cocaine-induced paranoia, Gelernter

and associates (Gelernter et al., 2005) found "suggestive" linkage (lod score < 2.2) on chromosomes 3 and 10 for cocaine dependence and "evidence" of linkage (lod score > 3.3) on chromosome 9 for cocaine-induced paranoia. Interestingly, they also reported evidence of linkage on chromosomes 12 and 18, two chromosomes not identified by Uhl and colleagues (2002). However, as Uhl et al. (Uhl, Liu, & Naiman, 2002) explain, none of the identified loci in their study displayed evidence for linkage that was strong enough to deem it a major genetic influence on substance abuse vulnerability; moreover, this study was not exclusively focused on METH use disorders, leading to the suggestion that selection of genes at other loci (*e.g.*, through candidate gene or genome-wide association studies (GWAS)) may be warranted in order to avoid false-negative and false-positive results.

To date, two GWAS have been conducted for stimulant use disorders (Uhl et al., 2008; Yu et al., 2008). Uhl and associates (2008) examined two independent samples, including 380 Japanese and 200 Han-Chinese, to identify concordant genes for METH dependence. Results revealed several convergent chromosomal regions and candidate genes implicated in cell adhesion, enzymatic, transcriptional regulation, and other processes among the two samples after adjustment for chance observations. On the other hand, Yu and colleagues (2008) examined cocaine dependence and cocaine-induced paranoia among a sample of African-Americans and European-Americans. Results indicated a number of candidate genes for cocaine dependence and cocaine-induced paranoia, of which the synaptotagmin XIII (*SYT13*) and alpha-endomannosidase (*MANEA*) genes were most noteworthy, respectively. Although other regions on chromosomes 1, 2, 4, 6, 7, 9, 11, 15, 16, 18, and 22 were also implicated.

Clearly, linkage and genome-wide association studies in the field of METH use and related disorders have supplied a tentative roadmap from which candidate genes and polymorphisms have been selected, and have provided further justification for additional association studies to commence. In fact, since the time of the first linkage studies several genetic association studies have been undertaken. However, since the first genetic association study of METH use disorders appeared in 2001, there has not been (to our knowledge) an attempt to systematically review and synthesize this body of literature. Thus, we conducted a systematic review of the genetic association literature related to METH use disorders in an effort to: (1) provide a current synthesis of the genetic association research to date, and (2) stimulate further genetic investigations of METH use disorders.

METHOD

Search Strategy

Systematic searches were conducted utilizing Medline and PsychINFO, and were limited to articles written in English and published between January, 2000 and June, 2008. Search terms included 'methamphetamine' along with various combinations of the following terms: 'twin', 'adoption', 'linkage', 'association', 'gene', 'allele', 'heritability' and 'human'. In addition, a blanket search was conducted with only the term 'methamphetamine' to ensure no relevant studies were missed. Next, an extensive review of the references of relevant articles was carried out and additional articles were acquired. Studies were included in this review if they met both of the following inclusion criteria: 1) reported the results of an association study between a gene and a METH use disorder;

and 2) reported sufficient data to enable calculation of genotype and allele frequencies, odds ratios, and confidence intervals, as well as power.

Synthesis Approach

All studies were reviewed with special attention to: candidate gene, sample size, sample characteristics (*i.e.,* gender ratio, mean age, ancestry), phenotype, and phenotypic classification criteria. For each study, additional information on each gene's location (*i.e.* chromosome), attributed function, marker(s) and variant type(s) (*e.g.,* single nucleotide polymorphism, SNP) were reviewed. If more than one gene or marker on a gene were examined in the same study, each association was reviewed separately. For each study selected, only published genotype and allele frequencies were extracted. When applicable, haplotype data were also extracted. Gene function was derived from each study author's interpretation of the link between the gene and the specific phenotype under investigation; however, most evaluated genes subserve numerous molecular functions and biological processes.

Reported genotype and allele frequencies for cases and controls were abstracted by each marker and study. Chi-square analysis was used to compute significance levels for genotype and allele frequencies, and odds ratios and 95% confidence intervals were calculated for minor alleles. In addition, power and population attributable risk percent (PAR%) was calculated using reported minor allele frequencies and sample sizes for cases and controls for each marker by study. All analyses were conducted using STATA software, version 10 (College Station, TX).

RESULTS

Characteristics of Genetic Association Studies of METH Use Disorders

Our review of the literature uncovered 38 gene-association studies for METH use disorders (Table 2.2). Studies examining METH dependence (k = 22; 58%) were most prevalent in the literature; however, studies of METH abuse (k = 6; 16%) and psychosis (k = 13; 34%) were represented. Notably, several studies (J. Chen et al., 2004; Ikeda et al., 2006; Nishiyama et al., 2005) recruited subjects for more than one of the three METH use disorder phenotypes. In contrast, two studies (Aoyama et al., 2006; S. K. Lin, Chen, Ball, Liu, & Loh, 2003) reported exclusion of polysubstance abuse, and two other studies (Hong, Cheng, Shu, Yang, & Tsai, 2003; Tsai et al., 2002) excluded any history of psychosis.

ICD-10-DCR criteria for phenotypic classification of METH use disorders was utilized more frequently than DSM-IV criteria (k = 25; 67% vs. k = 14; 37%) and in one study (Ikeda, 2007), it was reported that both classification systems were used. Japanese populations (k = 26; 68%) were the most commonly studied, followed by Han-Chinese (k = 10; 26%), Korean (k = 1; 3%), and Czech (k = 1; 3%) populations. Notably, one study reported results from both a Japanese and Han-Chinese population (Nakamura et al., 2006). Four studies (Cheng et al., 2005; Koizumi et al., 2004; S. K. Lin et al., 2003; Tsai et al., 2002) examined exclusively male or female subjects and three studies did not report gender data (C. K. Chen et al., 2007; Hong et al., 2003; Iwata et al., 2004); thus, for these studies gender ratios were not calculated. However, among the remaining 31 studies, gender ratios revealed that, on average, one female case was selected for every four male cases; among controls, one female was selected for every three males selected.

Importantly, the ratio of male:female cases was very different from the ratio of male:female controls in several studies. Three studies (C. K. Chen et al., 2007; J. Chen et al., 2004; Iwata et al., 2004) did not report age distributions for cases or controls; however among the remaining 35 studies the mean age of cases was 33.3 (SD = 4.6) years and for controls 34.5 (SD = 4.3) years. In contrast to the data on gender-matching, case and control samples were often closely matched on age within each study.

Genes Examined in the Literature

Table 2.3 provides a comprehensive list of the 39 genes examined in the genetic association literature for METH use disorder, along with information on each gene's chromosomal location, stated function, and study citations. The chromosomal location of the genes examined was diverse; however, genes located on chromosomes 2, 10, 12, 13, 15, 18, 19, and 21 were not represented in the current body of literature. Gene function was also diverse; however, the majority of genes were implicated in neurotransmitter reception, signaling, or metabolism (72%), or METH detoxification or metabolism (13%).

Genotypic and Allelic Associations with METH Use Disorders

Table 2.4 provides genotype and allele frequencies with accompanying significance levels, odds ratios (ORs), 95% confidence intervals (95% CIs), sample sizes, and power calculations for every examined marker of each gene in Table 2.3. For some genes, several markers have been examined by several studies whereas other genes are represented by only a single marker and/or study. When possible and appropriate, reference SNP (rs) numbers have been provided. The most common type of variant examined in the METH use disorder literature has been the SNP; however, studies have

also examined variable number tandem repeats (VNTRs), insertion/deletions (I/D), other restriction fragment length polymorphisms (RFLPs), and other sequence variations.

Of the 39 examined genes, 17 were shown to have a significant ($p < 0.05$) genotypic and/or allelic association with METH use disorders (*SOD2* had only a haplotypic association). Minor alleles for markers in seven of these genes (*AKT1, ARRB2, DTNP1, GSTP1, OPRM1, PDYN, SNCA*) conferred significant risk (OR > 1) for a METH use disorder whereas minor alleles located in *BDNF, COMT*, *CYP2D6, GABRA1*, and *PICK1* were found to have a significant protective effect (OR < 1). Interestingly, minor alleles for markers examined in *GLYT1* and *SLC22A3* conferred both significant risk and protective effects. For *GLTY1* rs2248829 conferred a protective effect (OR = .72, p = .04) but rs2486001 conferred a significant risk (OR = 1.67, p = .0002) (Morita et al., 2007). For *SLC22A3* markers rs3106164 and rs4709426 conferred a significant protective (OR = .75, p = .03) and risk (OR = 1.29, p = .03) effect, respectively (Aoyama et al., 2006). In addition, *GABRG2* marker rs211013 (S. K. Lin et al., 2003) and the *GSTM1* gene deletion (Koizumi et al., 2004) were found to be significantly associated with a METH use disorder in females at the genotypic level but these findings did not translate to the allelic level, suggesting a mechanism of action other than additive. Although not independently associated with any of the three METH use disorders, *DRD4* was found to have a significant interaction with *COMT* for METH abuse (Li et al., 2004).

Of the 17 genes with significant genotype and/or allele effects, the largest effects were identified for *DTNBP1* in that two SNPs (rs2619538 and rs3213207) in this gene conferred a 2.58 (p = 0.03) and 7.13 (p = 0.001) greater odds of METH psychosis,

respectively(Kishimoto, Ujike, Motohashi et al., 2008). Another gene housing SNPs (rs1372520, rs3756059, rs3756063) with large effects for METH psychosis was *SNCA* (OR = 2.67; p = 0.04), albeit only among female subjects (Kobayashi et al., 2004). For METH abuse/dependence, both *PDYN* (OR = 1.83, p = 0.002) and *AKT1* (OR = 1.59, p = 0.02) conferred the largest effects (Ikeda et al., 2006; Nomura et al., 2006).

Total sample sizes for the studies under investigation (including sub-analyses) on average were 471 (SD = 179) and ranged from 71 to 879. Case:control ratios ranged from .3 to 2.4, with a mean of .90 and a standard deviation of .35. Power analyses revealed that a majority of the published gene-association studies of METH use disorders were underpowered ($1-\beta < .80$) to detect reasonable effect sizes, given the reported sample sizes and minor allele frequencies. Power among the examined studies ranged from .00 to .97. Among studies finding a significant allelic association, power ranged from .40 to .97 ($M = .58$, SD = .20) whereas, among non-significant studies the range was .00 to .80 ($M = .14$, SD = .14). Population attributable risk percent (PAR%) estimates revealed that approximately 12% of METH use disorder cases could be accounted for by the minor alleles of the variants reviewed. Among variants with significant allelic associations it was estimated that the PAR% was 5%.

Haplotype Associations for METH Use Disorders

In addition to reporting single-marker associations, several of the reviewed studies also conducted and reported haplotypic associations (Table 2.5). The number of SNPs included in each haplotype ranged from two to six markers. Significant haplotypic associations were reported for *AKT1, ARRB2, DTNBP1, DRD4, GABRG2, GLYT1,*

PICK1 and *SOD2*. For all these genes, significant genotype and/or allele associations were also found (see above), with the exception of *SOD2*.

DISCUSSION

This review systematically uncovered 38 genetic association studies in the literature and applied a quantitative synthesis approach to arrive at estimates of the genetic effects on the vulnerability to METH use disorders. The remainder of our discussion focuses on the 18 genes that were found to be significantly associated (either by genotype, allele, and/or haplotype) with METH use disorders in the literature. In addition, we will discuss the current limitations of the literature and conclude with potential future directions for further investigation of METH use disorders.

Putative Genetic Associations for METH Use Disorders

Eighteen genes in the current literature have shown significant associations with METH use disorders. Of these 18 genes, three (*COMT, DRD4, GABRA1*) were associated with METH abuse, nine (*ARRB2, BDNF, CYP2D6, GLYT1, GSTM1, GSTP1, PDYN, PICK1, SLC22A3*) with METH dependence, two (*AKT1, GABRG2*) with METH abuse/dependence, and four (*DTNBP1, OPRM1, SNCA, SOD2*) with METH psychosis. Although the remaining 21 genes examined in the literature (*ACE, AGT, CART, DRD1, DRD2, DRD3, FAAH, GABRA6, GABRB2, GHRL, NQO1, NQO2, OPRD1, PAI1, PIK4CA, PLAT, PLG, SIGMAR1, SLC6A3, SLC6A4*, and *XBP1*) were not found to have a significant association with METH use disorders, it should be noted that most of these markers were examined in underpowered studies and thus could reflect type II errors to which further replication is required prior to dismissing them as potential candidate markers.

Genetic Associations with METH Abuse and/or Dependence

A large majority (n = 14) of the genes found to be significantly associated with METH use disorders have been specifically linked to METH abuse and/or dependence. Although these two disorders are phenotypically different, we discuss this group of associations in the aggregate, recognizing that genes conferring a risk for one particular phenotype may not be applicable to the other. Due to a paucity of reported replications for any of the associations reported in this review and the high probability that many of the significant associations reviewed represent false-positives, our discussion will pay particular attention to those associations observed in adequately powered ($1-\beta > .80$) investigations.

Of the 14 significant genes for abuse/dependence *ARRB2* and *PDYN* were identified by adequately powered studies. *ARRB2* has recently been implicated in the *AKT-GSK3B* signaling cascade in which *ARRB2* subserves as a mediator of behavioral response to dopaminergic drugs (Beaulieu et al., 2005). In a Japanese sample it was found that three variants (rs1045280T>C, rs2036657A>G, rs4790694C>A) in this gene as well as a haplotype comprising these variants was significantly associated with METH dependence (Ikeda et al., 2007). In this study, the authors also reported exploration of gene-gene interactions between *ARRB2*, *AKT1* and *GSK3B*; however, no significant interactions were found. As this is the only study to our knowledge that has examined *ARRB2*, further work clearly is necessary.

PDYN encodes prodynorphin, which is a precursor molecule for a host of endogenous opioids, including neuropeptides that assist in regulation of perception, behavior, and memory (Rockman et al., 2005). Additionally, it has been shown that

PDYN interacts with psychostimulants (Daunais, Roberts, & McGinty, 1993; Hurd, Brown, Finlay, Fibiger, & Gerfen, 1992; Pfeiffer, Brantl, Herz, & Emrich, 1986; Shippenberg & Rea, 1997) resulting in reductions of basal levels of dopamine in the extracellular fluid of the nucleus accumbens and dopaminergic tone in the tuberoinfundibular system (Kreek, Schluger, Borg, Gunduz, & Ho, 1999). In the current literature, a 68-base-pair repeat polymorphism in the promoter region (Zimprich et al., 2000) of the *PDYN* gene was examined among METH-dependent Japanese men and women (Nomura et al., 2006). In this study, it was reported that the 3- or 4-repeat (H) alleles were significantly more frequent in cases versus controls. Conversely, in a study predating the Japanese study among European-, African-, and Hispanic-American cocaine users it was found that the 1- or 2-repeat (L) alleles were more frequent in cases versus controls (A. C. Chen et al., 2002). Nomura et al. (Nomura et al., 2006) explain that this divergence may be a result of recent work (Turchan, Maj, Przewlocka, & Przewlocki, 2002) that suggests a differential influence of acutely and chronically administered cocaine and amphetamine on the biosynthesis of *PDYN*.

In addition to *ARRB2* and *PDYN* other significant genes identified in this review have also been implicated in the dopaminergic system (*AKT1, BDNF, COMT, DRD4, PICK1, SLC22A3*). *AKT1* has been implicated in several transduction-signaling pathways that are important in the developing and adult central nervous system (Emamian, Hall, Birnbaum, Karayiorgou, & Gogos, 2004) and has been shown to interact with neuronal dopaminergic signaling(Beaulieu, Gainetdinov, & Caron, 2007; Bonci & Hopf, 2005). *BDNF* is one of several neurotrophic factors that influence the development, maintenance and survival of dopaminergic neurons in the central nervous system (Hyman et al., 1991).

Although the precise pathways and mechanisms by which *BDNF* acts are unclear, demonstrations of *BDNF'*s role in survival and differentiation of dopamine neurons (Hyman et al., 1991; Spina, Squinto, Miller, Lindsay, & Hyman, 1992) has suggested a putative role of *BDNF* in the addiction process (Bolanos & Nestler, 2004; Tsai, 2007). *COMT* is a major mammalian enzyme involved in the metabolic degradation of dopamine released in the brain and has been linked to neurocognition (Bruder et al., 2005; Malhotra et al., 2002; Rosa et al., 2004), novelty seeking (Golimbet, Alfimova, Gritsenko, & Ebstein, 2007; Hosak, Libiger, Cizek, Beranek, & Cermakova, 2006), amphetamine response (Mattay et al., 2003), and psychiatric disorders (Egan et al., 2001; Glatt, Faraone, & Tsuang, 2003; Karayiorgou et al., 1997; Qian et al., 2003) in addition to drug abuse (Horowitz et al., 2000; Vandenbergh, Rodriguez, Miller, Uhl, & Lachman, 1997). *DRD4* is one of three dopamine receptors (D2 and D3 are the others) that constitute the D2 receptor family, and is involved in a variety of functions such as cognition, locomotor activity, emotion, food intake, positive reinforcement and endocrine regulation(Missale, Nash, Robinson, Jaber, & Caron, 1998). *PICK1* has been implicated in the targeting and localization of synaptic membrane proteins (Deken, Beckman, & Quick, 2001) as well as surface clustering of the dopamine transporter (Torres et al., 2001). Finally, *SLC22A3* encodes the organic cation transporter 3 (*OCT3*) which has been implicated in the transport and subsequent regulation of concentrations of METH as well as dopamine and serotonin in the brain (Grundemann, Schechinger, Rappold, & Schomig, 1998; Wu et al., 1998).

Beyond the dopamenergic system, several of the identified significant genes in the review have been implicated in the glutamatergic system (*GABRG2*, *GLYT1*) as well as

drug metabolism (*CYP2D6, GSTM1, GSTP1*). Within the glutamatergic system, *GABRG2* is a subunit in the gamma-aminobutyric acid $(GABA)_A$ gene cluster which serves as a receptor for major inhibitory neurotransmission in the brain (Venter & Olsen, 1986; Wilcox et al., 1992) and has been linked to alcoholism with comorbid antisocial personality disorder (Loh et al., 2000). Whereas, *GLYT1* encodes one of two subfamilies of glycine transporters that are involved in regulation of glycine concentrations around N-methyl-D-aspartate (NMDA) receptors and extracellular glycine at synapses within the glutamatergic system (Sato, Adams, Betz, & Schloss, 1995; Smith, Borden, Hartig, Branchek, & Weinshank, 1992). Among the drug metabolism genes, *CYP2D6* codes for a liver enzyme responsible for oxidative metabolism of a number of psychoactive substances, including METH (L. Y. Lin et al., 1997). *GSTM1* and GSTP1 on the other hand, belong to a family of enzymes (glutathione S-transferases) involved in xenobiotic detoxification by means of conjugating glutathione (McLellan et al., 1997). *GSTM1* is the one of the most frequently studied genes related to lung cancer susceptibility (Carlsten, Sagoo, Frodsham, Burke, & Higgins, 2008) and recent work has also implicated it in schizophrenia (Harada, Tachikawa, & Kawanishi, 2001; Pae et al., 2004).

Genetic Associations for METH Psychosis

METH-induced psychosis has been considered a pharmacological model of schizophrenia given its phenotypic similarities (Bell, 1965; Snyder, 1973). Based on this rationale 13 studies have examined genetic factors that may confer a vulnerability to psychosis subsequent to onset of METH administration. Of these studies, four significant genetic associations have been reported of which one study examining *DTNB1*

(Kishimoto, Ujike, Motohashi et al., 2008) had adequate power and will be discussed in detail.

DTNBP1 or dysbindin-1 is a gene encoding a coiled-coil containing protein that in the brain is found primarily in axon bundles and mossy fiber synaptic terminals in the cerebellum and hippocampus (Benson, Newey, Martin-Rendon, Hawkes, & Blake, 2001; Talbot et al., 2004). Currently *DTNBP1* is one of the most promising candidate genes for schizophrenia (Benson, Sillitoe, & Blake, 2004; Williams, O'Donovan, & Owen, 2005). Thus, based on the aforementioned resemblance to schizophrenia, Kishimoto and colleagues (Kishimoto, Ujike, Motohashi et al., 2008) examined *DTNBP1* among Japanese subjects with METH dependence with psychosis. In this review, two SNPs (rs2619538A>T and rs3213207A>G) conferred an approximate 2.6- and 7.1-times greater odds of METH dependence with psychosis, respectively. In addition, two significant haplotypes (*DTNBP1* haplotype 1 and 3 in Table 2.5) were reported in which haplotype 1 (C-A-A) implied a substantial protection and haplotype 3 (C-G-T) conferred a significant risk for METH dependence with psychosis.

Other gene associations for METH psychosis included *OPRM1* which codes for the μ-opioid receptor and is one of three known receptors in the opioid family (along with kappa and delta receptors). *OPRM1* is the most frequently studied candidate gene for opioid dependence due to its clear involvement in mediating the physiological effects of endogenous and exogenous opioids (including heroin) (Glatt et al., 2007). It has also been shown to be involved in the susceptibility to other drugs of abuse such as nicotine (Berrettini & Lerman, 2005) and alcohol (Oroszi & Goldman, 2004; Town, Schinka, Tan, & Mullan, 2000; Zhang et al., 2006). In addition, *SNCA* or alpha-synuclein was

significantly associated with METH psychosis. *SNCA* is a member of the synuclein family, which also includes beta- and gamma-synuclein and is predominately concentrated in presynaptic nerve terminals (Jakes, Spillantini, & Goedert, 1994). *SNCA* has been implicated in the modulation of dopamine transmission (Xu et al., 2002) and has received attention for its potential role in neurodegenerative diseases as well as alcohol dependence (Bonsch et al., 2005). Finally, a single haplotype (T-A-B-T-T) of *SOD2* (see Table 2.5 for details) was identified for METH psychosis (Nakamura et al., 2006). *SOD2* encodes a mitochondrial protein that plays a critical role in cellular defense against oxidative damage (Cadet et al., 1994; Macmillan-Crow & Cruthirds, 2001) and mutations in this gene have recently been implicated in Alzheimer's disease (Marcus, Strafaci, & Freedman, 2006), radiotherapy response (Burri et al., 2008), brain tumor risk (Rajaraman et al., 2008) and prostate cancer (Kang et al., 2007).

Limitations of Genetic Association Studies of METH Use Disorders

Collectively, the reviewed studies may provide a partial molecular basis for the previously demonstrated heritability of stimulant dependence in general, and the genetic vulnerability to METH abuse, dependence and psychosis in particular; however, several limitations in the literature should be noted. Throughout the course of this review, three overarching limitations of genetic association studies of METH use disorders surfaced. First, phenotypic classification and subsequent description of the particular METH use disorder under study was often insufficient. Second, sample sizes and consequent power to detect associations were, for many studies, below adequate levels. Finally, although not formally measured, the potential for publication bias and/or presence of the "file drawer" phenomenon is of concern and thus will be discussed.

Phenotypic Classification of METH Use Disorders

As discussed above, precise phenotypic classification is of the utmost importance in genetic association studies and is required for accurate generalizations. The studies reviewed here utilized one of two commonly used METH use disorder classification systems (DSM-IV or ICD-10) and in some instances independent studies examining genetic markers in the same gene utilized different classification systems to ascertain participant phenotype. Thus, it is possible that ascertainment of different phenotypes, albeit slight, could result in divergent findings and subsequently blur the hypothesized associations under investigation. Further efforts to bring the DSM and ICD criteria for the three METH use disorder phenotypes in concordance with each other are needed, especially for the phenotype of METH psychosis.

Another concern related to phenotype involves selection of control participants for genetic association studies of METH use disorders. One of the major remaining questions of the significant conclusions made in the reviewed literature is: what do these markers tell us about the specific phenotype under investigation? Unfortunately, the answer is not clear because controls selected in the current literature have presumably never been exposed to METH (in most studies, this was never explicitly stated). Thus, markers reported as being associated with METH dependence may actually be associated with initiation, abuse or progression from abuse to dependence. Likewise, markers for METH psychosis may actually be markers for general psychosis and be completely unrelated to METH use. Though speculative, these are formidable concerns about the current literature. Thus, future investigation into genetic associations of METH abuse/dependence should explore the inclusion of controls with histories of experimental

use of METH in addition to super-controls who have never been exposed to METH. Likewise, studies examining METH psychosis may find it advantageous to include controls that have a history of METH use but have never met criteria for a psychotic episode. Utilization of these selection methods may elucidate putative genetic associations and improve our understanding of the genetic epidemiology of METH use disorders.

Power Concerns

A study's ability to recruit an adequate sample size to ensure sufficient power to detect a significant genetic effect for a complex disorder such as METH use disorders has been implicated as one of the main reasons for a paucity of replications of initially positive gene-disease associations. In our review we found that many of the studies were not adequately powered to detect reasonable genotype, allele, and/or haplotype differences between cases and controls (Table 2.4) and subsequently the likelihood of false-negative associations is highly probable. In a previous review addressing problems with reporting genetic associations (Colhoun, McKeigue, & Davey Smith, 2003) it was suggested that reasons such as: 1) underestimation of effect size, 2) heterogeneity among cases, and 3) misclassification of outcomes are all probable reasons for false-negative findings. For the studies in this review all of these reasons may be applicable. In particular, underestimation of effect and subsequent inadequate sample sizes was evident. The range of sample sizes among the reviewed articles in this synthesis was 71 - 879 total participants (Mean = 430, SD = 186). Given that the typical size of an effect in genetic association studies is relatively small (OR < 1.5) (Colhoun et al., 2003), large sample sizes are required. In fact, even when exploring common genetic variants (>30%) sample

size requirements have been shown to exceed 500 participants in case-control designs (Hattersley & McCarthy, 2005) and at minimum 5000 participants for nested case-control designs (Davey Smith et al., 2005). Thus, the majority of the studies included in this review were underpowered. It should be noted that many studies did not report their power analysis procedures and thus it is unknown to what degree these concerns were considered. Nevertheless, reporting *a priori* the estimated effect size and anticipated power while also addressing potential heterogeneity among cases and misclassification in diagnosis requires considerably more attention from investigators as well as journal reviewers and editors in regard to future genetic association studies of METH use disorders.

Publication Bias and the File Drawer

It is generally known that a great deal of research is conducted but never submitted (*i.e.*, file-drawer phenomenon) or accepted (*i.e.*, publication bias) for publication. For genetic association studies both these circumstances are of concern, as they can negatively influence future research and bias systematic reviews of the literature. Statistical methods for assessing publication bias have been developed but no single method is adequate and large numbers of studies are needed to make accurate estimates (Munafo, Clark, & Flint, 2004). Thus, in the current review of the METH use disorder literature formal calculations of publication bias were not undertaken. However, the potential for publication bias related to genetic association research conducted on METH use disorders is presumed to be at least as prevalent as that of other disorders. Solutions to publication bias and the file drawer phenomenon have been described in detail elsewhere (Calnan, Smith, & Sterne, 2006; Colhoun et al., 2003; Little et al., 2002)

and include internet-based reporting, pooling data, brief-format publications as well as editor and reviewer education strategies. Although these solutions would undoubtedly reduce publication bias and increase reporting of negative findings, few of them have been implemented to date. Future access to and knowledge of negative findings in well-powered studies would greatly improve systematic reviews (such as this one) and further elucidate plausible biological as well as environmental pathways by which METH use disorders develop.

Future Directions

Our results concur with other genetic reviews of substance abuse (Barr et al., 2006; Saxon, 2006; Vanyukov & Tarter, 2000) as well as Bipolar Disorder (Craddock, Dave, & Greening, 2001) and Major Depression (Levinson, 2006) in that, to date, a majority of the genes examined for association with METH use disorders are implicated in dopamine and other neurotransmitter receptor signaling pathways. As aforementioned, METH psychosis has been viewed as a pharmacological model of schizophrenia (BELL, 1965; Snyder, 1973); however METH use has also been posited as a pharmacological model of mania (Jacobs & Silverstone, 1986; Mamelak, 1978) and during the withdrawal period a model for depression (Kitanaka, Kitanaka, & Takemura, 2008; Seltzer & Tonge, 1975). Thus, studies examining the genetic architecture of METH use disorders may also provide links to understanding the genetic complexities of a variety of other psychiatric disorders. However, it should be noted that most if not all of the genes examined also subsevere other functions and belong to other pathways. In fact, exploration of the 39 genes in this review utilizing Ingenuity Pathways Analysis version 6.3-1402 (Ingenuity Systems, Inc; Redwood City, CA), found that the examined genes comprise a total of 4

networks (Table 2.6) representing 72 canonical pathways (not shown). These networks may be useful in the selection of additional candidate genes for future investigations and also provide further support of the genetic complexity and probable polygenic nature of METH use disorders.

To this point, the focus of this review has been on synthesizing the genetic association literature for METH use disorders which, to date, has been based almost exclusively on hypothesis-driven candidate-gene studies. This approach is highly feasible and preferable when putative relationships between a gene and disorder are already supported by current knowledge. This approach also assumes the disorder is heritable; however, as aforementioned specific heritability estimates for METH use disorders have not been reported. Twin as well as family studies of METH use disorders, excluding other substances of abuse, are warranted. As evidence of a genetic effect, we estimated that 12% of METH use disorders could be attributed to all the variants in the reviewed studies and 5% attributed to those with a significant allelic association. However, these estimates are tempered by the fact that the first published estimates of a particular variant usually over-estimate the actual genetic effect. In addition, there may be redundancy between markers (i.e., they may not be independent risk factors), thus these population attributable risk estimates should be interpreted as an absolute upper maximum that will likely be refined downward as additional studies emerge.

Given our current poor understanding of the molecular mechanisms underlying METH use disorders, high-throughput approaches to hypothesis-generation may be more useful for advancing the field (Hattersley & McCarthy, 2005). This realization has very recently led to the application of genome-wide association scan (GWAS) technology to

the study of METH disorders. The GWAS approach is discovery-driven rather than hypothesis-driven and as such, requires very large sample sizes, application of statistical approaches that correct for multiple testing, and replication of findings in independent samples. As aforementioned, to date, one GWAS has been conducted for METH use disorders (Uhl et al., 2008). Interestingly, a number of the candidate genes identified overlapped with those identified in other GWASs of addiction (Bierut et al., 2007; Johnson et al., 2006; Q. R. Liu et al., 2006) to other drugs and thus support the notion of shared genetic vulnerabilities across different substances. However, none of the candidate genes presented by Uhl et al. (2008) implicated any of the genes in the present review. The lack of overlap between GWA and candidate-gene findings for METH use disorders may result form phenotypic differences and/or methodological factors. In fact, Uhl et al. (2008) report that their samples included both polysubstance abusers as well as subjects with psychosis; thus, direct comparison with many of the candidate-gene studies reported in this review may not be appropriate. In addition, GWASs only assay single nucleotide polymorphisms (SNPs) and do not include other types of polymorphisms (e.g. VNTRs) reported in the candidate-gene literature which further excludes comparison with many of the studies in this review. Nevertheless, additional GWASs utilizing larger sample sizes with more rigorously defined phenotypes are required to assist in identification of chromosomal regions and genes that harbor variants that may predispose to METH use disorders.

One of the major limitations mentioned by authors in nearly all the studies reviewed was a smaller than desired sample size. Unfortunately, as aforementioned, ascertaining the required numbers of participants needed to achieve adequate power to

detect significant differences between cases and controls is not always feasible for individual research groups. Two potential strategies to address this limitation in the future are meta-analysis and large-scale collaborations. Meta-analytic techniques assist in providing evidence of an association between a gene polymorphism and phenotype by increasing sample size and power to detect and estimate an effect (Zintzaras & Lau, 2008). To date, this approach has been employed for several psychiatric disorders including alcoholism; however, no meta-analysis has been undertaken for METH use disorders. The paucity of this approach for METH use disorders is a direct reflection of the limited number of studies available for any particular gene variant. However, as the literature base in METH use disorders continues to build, future meta-analyses will be able to commence. The second strategy to address sample size concerns involves development of national and international collaborations by which large population-based research biobanks can be formed and used to facilitate investigations into genetic and environmental predictors of disease, as well as their interplay with one another (Davey Smith et al., 2005). Pooling of samples and resources will undoubtedly reduce the current limitations on power and assist in elucidating mechanistic pathways to METH use disorders as well as other complex diseases. However at the same time, development of statistical strategies to lessen concerns about false-positive and false-negative findings as well as methodological procedures for improving phenotypic characterization will also need to be addressed before the genetic knowledge can be useful in the clinical and public health arena.

Conclusion

It is evident that more work is required to understand the complex nature of METH abuse, dependence, and psychosis and the work completed to date should serve as a roadmap for future investigations in the rapidly evolving field of addiction genetics. In fact, as a testament to this rapid growth two new studies linking GSTT1 (Hashimoto et al., 2008) and FZD3 (Kishimoto, Ujike, Okahisa et al., 2008) with a METH use disorder were published after the inclusion cut-off date for this review.

In this review we systematically examined genetic association studies for METH use disorders and synthesized the results of these studies to stimulate and aid in future investigations. It is also our hope that the limitations discussed will be addressed in future work and steps toward national and international collaborations be taken to understand and ultimately, prevent and treat METH use disorders using the obtained knowledge of its genetic underpinnings.

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Table 2.1 Comparison of ICD-10-DCR and DSM-IV phenotype criteria for METH abuse/dependence and METH psychosis

Phenotype	Classification Type	
Criteria	ICD-10-DCR	DSM-IV
METH Abuse/Harmful use[1]		
Use resulting in dysfunctional behavior	X	X
Use despite persistent problems		X
Pattern of use:		
1 month or repeatedly within 12 months	X	
Occurring within a 12 month period		X
Never met criteria for dependence	X	X
METH Dependence (3 or more required)		
Tolerance	X	X
Withdrawal state	X	X
Impaired capacity to control use	X	X
Preoccupation with drug	X	X
Persistent use despite harm	X	X
Important activities given up/reduced		X
Desire/compulsion to take drug	X	
Desire/unsuccessful efforts to control use		X
METH Psychosis		
Onset of psychosis during/within:		
2 weeks of use	X	
1 month of use		X
Persistence of psychotic Sx >48 hours	X	
Duration psychosis does not exceed:		
6 months	X	
1 month		X

[1] The ICD-10-DCR requires all criteria are met whereas the DSM-IV specifies 1 or more are needed for diagnosis (not including never meeting criteria for dependence; ICD-10-DCR = International Classification of Disease, 10th revision, Diagnostic Criteria for Research; DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, 4th edition

Table 2.2 Summary of gene-association studies of METH use disorders (N = 38)

Author	Date	METH Phenotype	Phenotype Criteria	Gender ratio (M/F)		Mean age		Ancestry
				METH	Cont.	METH	Cont.	
Aoyama	2006	Dependence[a]	ICD-10-DCR	4.6	0.7	37	41	Japanese
Chen	2004	Abuse or Psychosis	DSM-IV	1.7	1.3	–	–	Han-Chinese
Chen	2007	Dependence/psychosis	DSM-IV	–	–	–	–	Han-Chinese
Cheng	2005	Dependence	DSM-IV	Males only		28	30	Han-Chinese
Hashimoto	2005	Dependence	ICD-10-DCR	3.7	3.7	37	37	Japanese
Hong	2003	Dependence[b]	DSM-IV	–	–	29	28	Han-Chinese
Ide	2004	Dependence	ICD-10-DCR	3.5	2.9	36	34	Japanese
Ide	2006	Dependence/psychosis	ICD-10-DCR	3.4	3.5	36	35	Japanese
Ikeda	2006	Abuse or Dependence	DSM-IV	4	0.9	37	34	Japanese
Ikeda	2007	Dependence	DSM-IV/ICD-10	4.5	0.8	37	37	Japanese
Inada	2004	Dependence/psychosis	ICD-10-DCR	3.6	3.6	36	37	Japanese
Itoh	2005	Dependence	ICD-10-DCR	3.9	3.6	37	37	Japanese
Iwata	2004	Dependence/psychosis	ICD-10-DCR	–	–	–	–	Japanese
Kanahara	2008	Dependence/psychosis	ICD-10-DCR	4.1	3.8	39	37	Japanese
Kishimoto	2008	Dependence/psychosis	ICD-10-DCR	4.6	3.9	38	37	Japanese
Kobayashi	2004	Dependence/psychosis	ICD-10-DCR	4.3	1.1	38	39	Japanese
Kobayashi	2006	Dependence/psychosis	ICD-10-DCR	4.2	2.4	38	35	Japanese
Koizumi	2004	Dependence	ICD-10-DCR	Males only		37	37	Japanese
				Females only		28	36	Japanese
Li	2004	Abuse	DSM-IV	1.7	1.3	27	34	Han-Chinese
Lin	2003	Abuse[a]	DSM-IV	Males only		28	35	Han-Chinese
				Females only		25	33	Han-Chinese
Liu	2004	Dependence	DSM-IV	4	11	31	30	Han-Chinese
Liu	2006	Abuse	DSM-IV	2.6	1.4	27	35	Han-Chinese
Matsuzawa	2007	Dependence	ICD-10-DCR	4.7	4.1	37	39	Japanese
Morio	2006	Dependence	ICD-10-DCR	4.5	3.9	37	39	Japanese
Morita	2005a	Dependence	ICD-10-DCR	4.3	4.3	38	37	Japanese
Morita	2005b	Dependence	ICD-10-DCR	4.3	4.3	38	37	Japanese
Morita	2007	Dependence	ICD-10-DCR	4.5	3.5	37	37	Japanese

(Table 2.2 Continued) Summary of gene-association studies of METH use disorders (N = 38)

Author	Date	METH Phenotype	Phenotype Criteria	Gender ratio (M/F)		Mean age		Ancestry
				METH	Cont.	METH	Cont.	
Nakamura	2006	Dependence/psychosis	ICD-10-DCR	4.5	3.2	36	37	Japanese
Nakamura	2006	Dependence/psychosis	ICD-10-DCR	3.2	1.1	27	29	Han-Chinese
Nishiyama	2005	Abuse or Dependence	DSM-IV	4.2	1.1	38	34	Japanese
Nomura	2006	Dependence	ICD-10-DCR	3.9	3.4	36	37	Japanese
Ohgake	2005	Dependence	ICD-10-DCR	3.8	3.5	37	37	Japanese
Otani	2008	Dependence	ICD-10-DCR	4.8	4.1	37	37	Japanese
Sery	2001	Dependence	DSM-IV	1.4	0.9	22	21	Czech
Suzuki	2006	Dependence/psychosis	ICD-10-DCR	4.5	3.4	36	37	Japanese
Tsai	2002	Dependence[b]	DSM-IV	Males only		29	28	Han-Chinese
Ujike	2003	Dependence/psychosis	ICD-10-DCR	4.5	3.6	36	36	Japanese
Yoon	2005	Dependence	DSM-IV	6.9	4	35	47	Korean

a = polysubstance excluded; b = psychosis excluded

Table 2.3 Genes Examined in the METH Use Disorder Literature

Gene	Gene Name	Chr.	Function[1]	Reference(s)
ACE	angiotensin I-converting enzyme	17q	Regulation of dopamine transmission	Sery, 2001
AGT	angiotensin I	1q	Regulation of dopamine transmission	Sery, 2001
AKT1	v-akt murine thymoma viral oncogene homolog 1	14q	Mediates dopamine-associated behavior	Ikeda, 2006
ARRB2	arrestin, beta 2	17p	Mediates dopamine signaling pathways	Ikeda, 2007
BDNF	brain-derived neurotrophic factor	11p	Modulates dopaminergic functions	Cheng, 2005; Itoh, 2005
CART	cocaine- and amphetamine-regulated transcript	5q	Roles in reward, feeding, and stress	Morio, 2006
COMT	catechol-o-methyltransferase	22q	Metabolism of catecholamine transmitters	Li, 2004; Suzuki; 2006
CYP2D6	cytochrome P450, subfamily 2D, polypeptide 6	22q	Methamphetamine metabolism	Otani, 2008
DRD1	dopamine receptor D1	5q	Mediates psychostimulant response	Liu, 2006
DRD2	dopamine receptor D2	11q	Mediates psychostimulant response	Chen, 2004; Sery, 2001; Tsai, 2002
DRD3	dopamine receptor D3	3q	Mediates psychostimulant response	Chen, 2004
DRD4	dopamine receptor D4	11p	Mediates psychostimulant response	Chen, 2004; Li, 2004; Tsai, 2002
DTNBP1	dystrobrevin-binding protien 1 (dysbindin)	6p	Modulates glutamate/dopamine systems	Kishimoto, 2008
FAAH	fatty acid amide hydrolase	1p	Regulator in endocannabinoid system	Morita, 2005a
GABRA1	gamma-aminobutyric acid receptor A1	5q	Major inhibitory neurotransmitter receptor	Lin, 2003
GABRA6	gamma-aminobutyric acid receptor A6	5q	Major inhibitory neurotransmitter receptor	Lin, 2003
GABRB2	gamma-aminobutyric acid receptor B2	5q	Major inhibitory neurotransmitter receptor	Lin, 2003
GABRG2	gamma-aminobutyric acid receptor G2	5q	Major inhibitory neurotransmitter receptor	Lin, 2003; Nishiyama, 2005
GHRL	ghrelin	3p	Involved in hormone-release and appetite	Yoon, 2005
GLYT1	glycine transporter	1p	Modulate NMDA receptor signaling	Morita, 2007
GSTM1	glutathione S-transferase M1	1p	Detoxification of xenobiotics	Koizumi, 2004
GSTP1	glutathione S-transferase P1	11q	Detoxification of xenobiotics	Hashimoto, 2005
NQO1	NAD(P)H-quinone oxidoreductase 1	16q	Protect agianst redox cycling/oxidative stress	Ohgake, 2005
NQO2	NRH-quinone oxidoreductase 2	6p	Protect agianst redox cycling/oxidative stress	Ohgake, 2005
OPRD1	delta-opioid receptor 1	1p	Mediates opiate response	Kobayashi, 2006
OPRM1	mu-opioid receptor 1	6q	Mediates opiate response	Ide, 2005
PAI1	plasminogen activator inhibitor-1	7q	Acute regulation of dopamine release	Iwata, 2004
PDYN	prodynorphin	20p	Regulates dopamine release	Nomura, 2006
PICK1	protein interacting with C Kinase 1	22q	Interacts with dopamine transporters	Matsuzawa, 2007
PIK4CA	phosphatidylinositol 4-kinase catalytic, alpha	22q	Involed in dopamine regulation	Kanahara, 2008
PLAT	plasminogen activator, tissue	8p	Acute regulation of dopamine release	Iwata, 2004

Table 2.3 (Continued) Genes Examined in the METH Use Disorder Literature

Gene	Gene Name	Chr.	Function[1]	Reference(s)
PLG	plasminogen	6q	Acute regulation of dopamine release	Iwata, 2004
SIGMAR1	sigma receptor 1	9p	Interacts with psychotomimetic drugs	Inada, 2004
SLC22A3	extraneuronal monoamine transporter	6q	Transport of neurotransmitters	Aoyama, 2006
SLC6A3	dopamine transporter	5p	Mediates the active reuptake of dopamine	Ujike, 2003; Hong, 2003; Liu, 2004
SLC6A4	serotonin transporter	17q	Mediates the active reuptake of serotonin	Chen, 2007; Hong, 2003
SNCA	alpha-synuclein	4q	Involved in dopamine uptake	Kobayashi, 2004
SOD2	superoxide dismutase 2	6q	Free radical scavenger	Nakamura, 2006
XBP1	x-box binding protein 1	22q	Involved in endoplasmic reticulum stress response	Morita, 2005b

Chr = Chromosome; 1 = Gene function represents author's interpretation of the most plausible link between the gene and the phenoype under study

Table 2.4 Genotype and allele frequences, odds ratios, 95% confidence intervals and overall power for published gene-association studies of METH use disorders

	Variant				Sample Size		METH			Control				m Allele		Allele-wise		
Gene	**Marker**	**Type**	**Author**	**Type**	**METH**	**Cont.**	**M/M**	**M/m**	**m/m**	**M/M**	**M/m**	**m/m**	***p***	**METH**	**CNT**	**OR(m)**	***p***	**Power**
ACE	rs4340	RFLP	Sery	D	87	126	0.20	0.59	0.22	0.26	0.48	0.25	0.32	0.51	0.50	1.06	0.75	0.03
AGT	rs699	SNP	Sery	D	89	125	0.36	0.52	0.12	0.33	0.46	0.21	0.27	0.38	0.44	0.79	0.23	0.21
AKT1	4-SNPs	Hap[1]	Ikeda	A/D	182	437	–	–	–	–	–	–	–	–	–	–	–	–
	rs1130214	SNP	Ikeda	A/D	182	437	0.70	0.28	0.02	0.72	0.25	0.03	0.97	0.16	0.16	1.01	0.97	0.00
	rs2494732	SNP	Ikeda	A/D	182	437	0.47	0.43	0.09	0.49	0.44	0.08	0.59	0.31	0.30	1.07	0.59	0.05
	rs2498799	SNP	Ikeda	A/D	182	437	0.22	0.54	0.24	0.28	0.48	0.24	0.81	0.51	0.48	1.12	0.35	0.15
	rs2498804	SNP	Ikeda	A/D	182	437	0.35	0.51	0.15	0.32	0.47	0.20	0.20	0.40	0.44	0.85	0.22	0.23
	rs3730358	SNP	Ikeda	A/D	182	437	0.75	0.24	0.02	0.83	0.16	0.01	**0.02**	0.13	0.09	1.59	**0.02**	0.53
	rs3803300	SNP	Ikeda	A/D	182	437	0.35	0.50	0.15	0.28	0.54	0.18	0.15	0.40	0.45	0.83	0.15	0.34
ARRB2	3-SNPs	Hap[1]	Ikeda	D	177	546	–	–	–	–	–	–	**0.02**	–	–	–	–	–
	rs1045280	SNP	Ikeda	D	177	546	0.66	0.31	0.03	0.76	0.22	0.01	**0.01**	0.19	0.12	1.61	**0.004**	0.88
	rs2036657	SNP	Ikeda	D	177	546	0.73	0.25	0.02	0.80	0.19	0.01	0.12	0.15	0.11	1.43	**0.04**	0.48
	rs4790694	SNP	Ikeda	D	177	546	0.78	0.22	0.00	0.86	0.14	0.00	**0.02**	0.11	0.07	1.61	**0.02**	0.62
BDNF	132C>T	SNP	Itoh	D	189	202	0.93	0.07	0.00	0.91	0.09	0.00	0.59	0.04	0.05	0.77	0.49	0.07
	rs6265	SNP	Cheng	D	103	122	0.30	0.55	0.15	0.19	0.56	0.25	**0.05**[c]	0.42	0.53	0.64	**0.02**[c]	0.61
			Itoh	D	189	202	0.37	0.51	0.12	0.35	0.53	0.12	0.89	0.38	0.39	0.95	0.71	0.04
CART	-156A>G	SNP	Morio	D	203	233	0.46	0.41	0.12	0.48	0.41	0.10	0.78	0.33	0.31	1.10	0.51	0.08
	rs2239670	SNP	Morio	D	199	238	0.53	0.40	0.07	0.53	0.40	0.08	0.97	0.27	0.28	0.97	0.83	0.04
COMT	rs4680	SNP	Li	A	410	390	0.56	0.37	0.08	0.46	0.44	0.09	**0.04**	0.26	0.32	0.77	**0.02**	0.74
				A/P	154	252	0.48	0.38	0.07	0.59	0.34	0.08	0.38	0.26	0.25	1.17	0.36	0.04
			Suzuki	D/P	143	200	0.40	0.45	0.15	0.47	0.43	0.11	0.31	0.37	0.32	1.28	0.12	0.25
					117	155	0.40	0.45	0.15	0.51	0.38	0.11	0.20[c]	0.37	0.30	1.38	0.08[c]	0.37
					26	45	0.38	0.46	0.15	0.33	0.58	0.09	0.93[d]	0.38	0.38	1.03	0.94[d]	0.00
CYP2D6	na	SNP	Otani	D	202	336	–	–	–	–	–	–	**0.02**	0.13	0.18	0.62	**0.02**[e]	0.55
DRD1	-48A>G	SNP	Liu	A	135	425	0.73	0.26	0.01	0.71	0.26	0.03	0.32	0.14	0.16	0.86	0.45	0.10
				A[b]	228	425	0.67	0.31	0.02	0.71	0.26	0.03	0.25	0.18	0.16	1.15	0.36	0.14
DRD2	TaqI A	RFLP	Chen	A	416	435	0.34	0.50	0.16	0.31	0.52	0.16	0.75	0.41	0.42	0.95	0.58	0.06

Table 2.4 (Continued) Genotype and allele frequences, odds ratios, 95% confidence intervals and overall power for published gene-association studies of METH use disorders

Gene	Marker	Variant Type	Author	Type	Sample Size METH	Sample Size Cont.	METH M/M	METH M/m	METH m/m	Control M/M	Control M/m	Control m/m	*p*	m Allele METH	m Allele CNT	Allele-wise OR(m)	Allele-wise *p*	Power
				A/P	154	252	0.30	0.52	0.18	0.37	0.49	0.14	0.29	0.44	0.39	1.25	0.12	0.27
			Sery	D	93	132	0.61	0.37	0.02	0.57	0.40	0.03	0.77	0.20	0.23	0.85	0.50	0.09
			Tsai	D[b]	116	112	0.41	0.45	0.14	0.35	0.46	0.19	0.46[c]	0.36	0.42	0.78	0.21[c]	0.23
DRD3	rs6280	SNP	Chen	A	412	467	0.50	0.42	0.08	0.46	0.44	0.09	0.58	0.29	0.28	0.96	0.70	0.06
				A/P	150	252	0.50	0.43	0.07	0.50	0.41	0.09	0.81	0.29	0.30	0.96	0.79	0.04
DRD4	120bp promoter	VNTR	Li	A	415	415	0.47	0.43	0.10	0.53	0.39	0.08	0.24	0.31	0.28	1.19	0.11	0.25
				A/P	154	252	0.45	0.40	0.07	0.48	0.45	0.11	0.84	0.27	0.33	0.88	0.41	0.40
	120bp/48bp	Hap[1]	Li	A	416	435	–	–	–	–	–	–	**0.003**	–	–	–	–	–
	exon III	VNTR	Chen	A	393	414	0.91	0.09	0.00	0.89	0.11	0.00	0.25	0.04	0.06	0.76	0.22	0.41
			Chen	A/P	139	244	0.91	0.09	0.00	0.91	0.09	0.00	0.99	0.05	0.05	1.04	0.91	0.03
			Tsai	D[b]	116	112	0.85	0.15	0.00	0.90	0.09	0.01	0.11[c]	0.07	0.05	1.40	0.39[c]	0.10
DRD4/	120bp/rs4680	Inter	Li	A	416	435	–	–	–	–	–	–	**0.01**	–	–	–	–	–
COMT	48bp/rs4680	Inter	Li	A	416	435	–	–	–	–	–	–	**0.00**	–	–	–	–	–
DTNBP1	3-SNPs	Hap[1]	Kishimoto	D/P	197	232	–	–	–	–	–	–	–	–	–	–	–	–
	rs2619538	SNP	Kishimoto	D/P	197	232	0.92	0.08	0.00	0.97	0.03	0.00	**0.05**	0.04	0.02	2.58	**0.03**	0.34
	rs2619539	SNP	Kishimoto	D/P	190	240	0.41	0.49	0.09	0.49	0.45	0.06	0.17	0.34	0.29	1.30	0.07	0.32
	rs3213207	SNP	Kishimoto	D/P	197	243	0.89	0.11	0.00	0.98	0.02	0.00	**0.00**	0.06	0.01	7.13	**0.00**	0.97
FAAH	rs57947754	SNP	Morita	D	153	200	0.69	0.28	0.03	0.70	0.29	0.02	0.57	0.17	0.16	1.10	0.64	0.04
GABRA1	rs2279020	SNP	Lin	A[a]	141	244	0.31	0.53	0.16	0.33	0.48	0.18	0.63[c]	0.42	0.43	0.98	0.91[c]	0.04
					105	188	0.37	0.40	0.23	0.27	0.44	0.29	0.15[d]	0.43	0.51	0.71	**0.05**[d]	0.43
GABRA6	rs4480562	SNP	Lin	A[a]	142	248	0.54	0.33	0.13	0.50	0.39	0.12	0.53[c]	0.30	0.31	0.95	0.74[c]	0.04
					105	187	0.51	0.38	0.10	0.44	0.43	0.13	0.45[d]	0.30	0.34	0.80	0.22[d]	0.14
GABRB2	rs2229944	SNP	Lin	A[a]	142	247	0.94	0.06	0.00	0.93	0.06	0.00	0.71[c]	0.03	0.04	0.77	0.54[c]	0.07
					106	190	0.91	0.08	0.01	0.93	0.07	0.00	0.35[d]	0.05	0.03	1.54	0.30[d]	0.17
GABRG2	1128+99C>A	SNP	Nishiyama	A/D	178	288	0.31	0.44	0.24	0.28	0.44	0.28	0.60	0.46	0.50	0.86	0.28	0.20
	315C>T	SNP	Nishiyama	A/D	178	288	0.49	0.42	0.09	0.55	0.39	0.06	0.37	0.30	0.26	1.23	0.16	0.24
	2-SNPs	Hap[1]	Nishiyama	A/D	178	288	–	–	–	–	–	–	-	–	–	–	–	–
	rs211013	SNP	Lin	A[a]	142	248	0.48	0.42	0.10	0.49	0.41	0.10	0.98[c]	0.31	0.30	1.03	0.87[c]	0.04

Table 2.4 (Continued) Genotype and allele frequences, odds ratios, 95% confidence intervals and overall power for published gene-association studies of METH use disorders

Gene	Marker	Variant Type	Author	Type	Sample Size METH	Cont.	METH M/M	M/m	m/m	Control M/M	M/m	m/m	*p*	m Allele METH	CNT	Allele-wise OR(m)	*p*	Power
					106	190	0.40	0.55	0.06	0.50	0.39	0.11	**0.02**[d]	0.33	0.31	1.12	0.53[d]	0.06
	rs211014	SNP	Lin	A[a]	140	246	0.31	0.44	0.25	0.25	0.48	0.27	0.42[c]	0.47	0.51	0.84	0.26[c]	0.17
					106	190	0.27	0.55	0.18	0.30	0.44	0.26	0.14[d]	0.45	0.48	0.89	0.50[d]	0.09
	rs4480617	SNP	Lin	A[a]	142	247	0.93	0.07	0.00	0.94	0.06	0.00	0.70[c]	0.04	0.03	1.02	0.95[c]	0.09
					106	190	0.85	0.14	0.01	0.94	0.06	0.00	**0.02**[d]	0.08	0.03	2.92	**0.005**[d]	0.70
GHRL	rs61564875	SNP	Yoon	D[c]	118	144	0.75	0.24	0.01	0.72	0.23	0.06	0.11	0.13	0.17	0.71	0.17	0.21
GLYT1	2-SNPs	Hap[1]	Morita	D	204	210	–	–	–	–	–	–	-	–	–	–	–	–
	rs2248632	SNP	Morita	D	204	210	0.60	0.34	0.06	0.54	0.39	0.08	0.45	0.23	0.27	0.81	0.20	0.24
	rs2248829	SNP	Morita	D	204	210	0.58	0.37	0.05	0.52	0.36	0.11	**0.05**	0.23	0.30	0.72	**0.04**	0.60
	rs2486001	SNP	Morita	D	204	210	0.52	0.40	0.08	0.66	0.30	0.04	**0.03**	0.28	0.19	1.67	**0.002**	0.85
GSTM1	na	Del	Koizumi	D	125	157	0.46	0.45	0.09	0.43	0.48	0.09	0.82[c]	0.31	0.33	0.92	0.63[c]	0.07
					32	43	0.75	0.22	0.03	0.49	0.49	0.02	**0.03**[d]	0.14	0.27	0.45	0.06[d]	0.40
GSTP1	rs1695	SNP	Hashimoto	D	189	199	0.76	0.22	0.02	0.84	0.16	0.00	**0.03**	0.13	0.08	1.70	**0.03**	0.58
NQO1	rs1800566	SNP	Ohgake	D	191	207	0.36	0.51	0.13	0.41	0.46	0.13	0.58	0.38	0.36	1.11	0.47	0.07
NQO2	na	I/D	Ohgake	D	191	207	0.61	0.31	0.08	0.59	0.36	0.05	0.34	0.23	0.23	1.03	0.84	0.00
OPRD1	IVS1+18G>C	SNP	Kobayashi	D/P	170	260	0.82	0.16	0.02	0.85	0.15	0.00	0.10	0.10	0.08	1.29	0.30	0.15
	rs2234918	SNP	Kobayashi	D/P	170	260	0.59	0.37	0.04	0.70	0.27	0.03	0.09	0.22	0.17	1.41	0.06	0.41
	rs4654327	SNP	Kobayashi	D/P	170	260	0.64	0.32	0.04	0.73	0.24	0.03	0.14	0.20	0.15	1.42	0.06	0.44
OPRM1	4-SNPs	Hap[1]	Ide	D/P	128	179	–	–	–	–	–	–	0.30	–	–	–	–	–
	rs1799971	SNP	Ide	D/P	128	213	0.38	0.42	0.20	0.31	0.46	0.22	0.43	0.41	0.45	0.83	0.23	0.15
			Ide	D	131	213	0.38	0.43	0.19	0.31	0.46	0.22	0.43	0.40	0.45	0.82	0.21	0.23
	IVS1-A4980G	SNP	Ide	D	138	187	0.94	0.06	0.00	0.95	0.05	0.00	0.99	0.03	0.03	1.09	0.86	0.00
	rs2075572	SNP	Ide	D/P	128	232	0.57	0.34	0.09	0.66	0.31	0.03	**0.01**	0.26	0.18	1.60	**0.01**	0.68
	rs598682	SNP	Ide	D/P	128	179	0.79	0.20	0.01	0.86	0.13	0.01	0.21	0.11	0.08	1.50	0.15	0.21
	rs599548	SNP	Ide	D/P	128	179	0.75	0.22	0.03	0.75	0.23	0.02	0.70	0.14	0.13	1.08	0.74	0.04
PAI1	rs1799889	I/D	Iwata	D/P	184	288	0.47	0.43	0.09	0.40	0.48	0.12	0.25	0.31	0.36	0.79	0.10	0.33
PDYN	68bp promoter	VNTR	Nomura	D	143	209	0.59	0.34	0.07	0.71	0.28	0.00	**0.001**	0.24	0.15	1.83	**0.002**	0.82
PICK1	6-SNPs	Hap[1]	Matsuzawa	D	208	218	–	–	–	–	–	–	–	–	–	–	–	–
	rs11089858	SNP	Matsuzawa	D	208	218	0.80	0.19	0.01	0.83	0.17	0.00	0.71	0.10	0.09	1.17	0.49	0.06

Table 2.4 (Continued) Genotype and allele frequences, odds ratios, 95% confidence intervals and overall power for published gene-association studies of METH use disorders

Gene	Variant Marker	Type	Author	Type	Sample Size METH	Cont.	METH M/M	M/m	m/m	Control M/M	M/m	m/m	*p*	m Allele METH	CNT	Allele-wise OR(m)	*p*	Power
	rs2076369	SNP	Matsuzawa	D	208	218	0.35	0.48	0.17	0.38	0.51	0.11	0.23	0.41	0.37	1.19	0.21	0.20
	rs713729	SNP	Matsuzawa	D	208	218	0.80	0.18	0.02	0.69	0.29	0.02	**0.03**	0.11	0.17	0.63	**0.02**	0.68
	rs737622	SNP	Matsuzawa	D	208	218	0.41	0.45	0.14	0.41	0.49	0.10	0.35	0.37	0.35	1.10	0.51	0.08
PIK4CA	4-SNPs	Hap[1]	Kanahara	D/P	232	233	–	–	–	–	–	–	-	–	–	–	–	–
	rs2072513	SNP	Kanahara	D/P	232	233	0.23	0.56	0.21	0.26	0.52	0.23	0.62	0.49	0.48	1.01	0.95	0.03
	rs165862	SNP	Kanahara	D/P	232	233	0.23	0.54	0.22	0.25	0.50	0.24	0.68	0.50	0.50	0.99	1.00	0.00
	rs165793	SNP	Kanahara	D/P	232	233	0.37	0.50	0.13	0.39	0.46	0.15	0.52	0.38	0.38	0.97	0.83	0.00
	rs165789	SNP	Kanahara	D/P	232	233	0.24	0.55	0.22	0.26	0.51	0.24	0.68	0.49	0.49	0.99	1.00	0.00
PLAT	na	I/D	Iwata	D/P	185	288	0.26	0.52	0.22	0.27	0.51	0.23	0.97	0.48	0.48	1.01	0.95	0.00
PLG	2-SNPs	Hap[1]	Iwata	D/P	184	288	–	–	–	–	–	–	0.75	–	–	–	–	–
SIGMAR1	rs1799729	I/D	Inada	D/P	143	181	0.49	0.43	0.08	0.50	0.43	0.07	0.92	0.30	0.29	1.05	0.78	0.04
	rs1800866	SNP	Inada	D/P	143	183	0.43	0.45	0.12	0.47	0.45	0.08	0.40	0.35	0.30	1.22	0.25	0.25
SLC22A3	2-SNPs	Hap[1]	Aoyama	D[a]	210	442	–	–	–	–	–	–	0.18	–	–	–	–	–
	rs3088442	SNP	Aoyama	D[a]	210	438	0.24	0.44	0.32	0.27	0.47	0.25	0.17	0.54	0.49	1.23	0.08	0.37
	rs3106164	SNP	Aoyama	D[a]	207	440	0.57	0.36	0.07	0.47	0.45	0.08	**0.05**	0.25	0.31	0.75	**0.03**	0.58
	rs4709426	SNP	Aoyama	D[a]	211	442	0.28	0.51	0.21	0.37	0.45	0.18	0.06	0.46	0.40	1.29	**0.03**	0.52
	rs509707	SNP	Aoyama	D[a]	210	442	0.44	0.44	0.12	0.51	0.40	0.09	0.19	0.34	0.29	1.26	0.07	0.43
	rs7745775	SNP	Aoyama	D[a]	207	425	0.56	0.41	0.03	0.58	0.34	0.08	0.06	0.24	0.25	0.93	0.62	0.05
SLC6A3	1342A>G	SNP	Ujike	D/P	124	159	0.81	0.18	0.01	0.81	0.16	0.03	0.80	0.10	0.11	0.89	0.69	0.04
	242C>T	SNP	Ujike	D/P	105	106	0.98	0.02	0.00	0.98	0.02	0.00	0.97	0.01	0.01	1.03	0.98	0.00
	exon 15	VNTR	Hong	D[b]	101	109	0.83	0.16	0.01	0.84	0.16	0.00	0.58	0.09	0.08	1.16	0.68	0.04
			Liu	D	30	72	0.83	0.13	0.03	0.82	0.18	0.00	0.26	0.10	0.09	1.12	0.83	0.03
			Liu	D/P	88	30	0.74	0.26	0.00	0.83	0.13	0.03	0.09	0.13	0.10	1.35	0.53	0.05
			Ujike	D/P	124	160	0.85	0.15	0.01	0.86	0.13	0.01	0.63	0.08	0.08	1.08	0.80	0.00
	2319G>A	SNP	Ujike	D/P	124	157	0.56	0.39	0.06	0.59	0.36	0.05	0.88	0.25	0.23	1.10	0.63	0.07
SLC6A4	na	I/D	Chen	D/P	150	238	0.51	0.45	0.04	0.49	0.44	0.07	0.44	0.39	0.29	1.33	0.06[f]	0.80
			Hong	D[b]	102	112	0.58	0.32	0.10	0.54	0.31	0.14	0.60	0.26	0.30	0.82	0.37	0.13
	intron 2	VNTR	Hong	D[b]	103	112	0.83	0.16	0.01	0.89	0.11	0.00	0.32	0.09	0.05	1.69	0.17	0.30
SNCA	rs1372520	SNP	Kobayashi	D/P	170	161	0.84	0.16	0.01	0.88	0.11	0.01	0.39	0.09	0.07	1.27	0.41	0.12

Table 2.4 (Continued) Genotype and allele frequences, odds ratios, 95% confidence intervals and overall power for published gene-association studies of METH use disorders

Gene	Marker	Variant Type	Author	Type	Sample Size METH	Sample Size Cont.	METH M/M	METH M/m	METH m/m	Control M/M	Control M/m	Control m/m	*p*	m Allele METH	m Allele CNT	Allele-wise OR(m)	Allele-wise *p*	Power
					138	83	0.86	0.13	0.01	0.86	0.13	0.01	0.93[c]	0.07	0.08	0.92	0.82[c]	0.04
					32	78	0.72	0.28	0.00	0.90	0.09	0.01	**0.03[d]**	0.14	0.06	2.67	**0.04[d]**	0.41
	rs2870027	SNP	Kobayashi	D/P	170	161	0.35	0.52	0.13	0.34	0.47	0.19	0.28	0.39	0.43	0.86	0.33	0.16
					138	83	0.33	0.55	0.12	0.33	0.48	0.19	0.27[c]	0.39	0.43	0.84	0.38[c]	0.11
					32	78	0.44	0.38	0.19	0.36	0.45	0.19	0.72[d]	0.38	0.42	0.84	0.57[d]	0.06
	rs3756059	SNP	Kobayashi	D/P	170	161	0.83	0.16	0.01	0.88	0.11	0.01	0.32	0.09	0.07	1.32	0.34	0.12
					138	83	0.86	0.14	0.01	0.86	0.13	0.01	0.93[c]	0.08	0.08	0.97	0.93[c]	0.00
					32	78	0.72	0.28	0.00	0.90	0.09	0.01	**0.03[d]**	0.14	0.06	2.67	**0.04[d]**	0.41
	rs3756063	SNP	Kobayashi	D/P	170	161	0.82	0.17	0.01	0.88	0.11	0.01	0.26	0.09	0.07	1.37	0.28	0.12
					138	83	0.85	0.14	0.01	0.86	0.13	0.01	0.91[c]	0.08	0.08	1.02	0.96[c]	0.00
					32	78	0.72	0.28	0.00	0.90	0.09	0.01	**0.03[d]**	0.14	0.06	2.67	**0.04[d]**	0.41
	T10A7	Seq Var	Kobayashi	D/P	170	161	–	–	–	–	–	–	0.68	–	–	–	–	–
					138	83	–	–	–	–	–	–	0.68[c]	–	–	–	–	–
					78	32	–	–	–	–	–	–	0.99[d]	–	–	–	–	–
SOD2	5-SNPs	Hap[1]	Nakamura[g]	D/P	116	186	–	–	–	–	–	–	-	–	–	–	–	–
					135	204	–	–	–	–	–	–	-	–	–	–	–	–
	rs2758357	SNP	Nakamura[g]	D/P	116	186	0.66	0.32	0.02	0.70	0.25	0.05	0.22	0.17	0.18	1.02	0.94	0.03
					135	204	0.59	0.40	0.01	0.63	0.35	0.02	0.68	0.21	0.19	1.11	0.58	0.04
	rs4880	SNP	Nakamura[g]	D/P	116	186	0.72	0.26	0.02	0.80	0.18	0.02	0.21	0.11	0.15	1.41	0.16	0.09
					135	204	0.66	0.33	0.01	0.74	0.23	0.03	0.15	0.18	0.14	1.33	0.18	0.08
	rs2855116	SNP	Nakamura[g]	D/P	116	186	0.78	0.21	0.01	0.81	0.17	0.02	0.69	0.10	0.11	1.10	0.73	0.03
					135	204	0.68	0.31	0.01	0.74	0.24	0.02	0.34	0.17	0.14	1.27	0.27	0.06
	rs2758330	SNP	Nakamura[g]	D/P	116	186	0.23	0.46	0.30	0.29	0.45	0.26	0.50	0.53	0.48	1.22	0.22	0.08
					135	204	0.22	0.47	0.31	0.21	0.52	0.27	0.59	0.55	0.53	1.07	0.68	0.04
	rs2842980	SNP	Nakamura[g]	D/P	116	186	0.30	0.46	0.24	0.27	0.45	0.28	0.61	0.47	0.51	0.84	0.31	0.06
					135	204	0.31	0.46	0.23	0.28	0.52	0.21	0.59	0.46	0.46	0.99	0.95	0.00
XBP1	116C>G	SNP	Morita	D	153	200	0.47	0.42	0.11	0.40	0.44	0.16	0.28	0.32	0.38	0.77	0.10	0.35

1 = More detailed haplotype information can be found in Table 5; Bolded values are statistically significant at $p < 0.05$

A = Abuse; A/P = Abuse-psychosis; D = Dependence; D/P - Depedence-psychosis

Table 2.4 (Continued) Genotype and allele frequences, odds ratios, 95% confidence intervals and overall power for published gene-association studies of METH use disorders

a = polysubstance excluded; b = psychosis excluded; c = males only; d = females only e = Extensive vs. Intermediate metabolizers; f = METH psychosis vs. METH non-psychosis; g = Japanese samples are listed in the first row and Han-Chinese samples in the second row
I/D = Insertion/Deletion; RFLP = Restriction Fragment Length Polymorphism; SNP = Single Nucleotide Polymorphism; VNTR = Variable Number Tandem Repeat

Table 2.5 Haplotype frequencies among published studies of METH use disorders

Gene Haplotype	Haplotype Markers				Frequency METH	Frequency Control	p-values	Ref
AKT1[1]	rs3803300	rs1130214	rs3730358	rs2498799				Ikeda, 2006
1	A	G	C	G	0.28	0.20	0.02	
2	A	G	T	A	0.07	0.05	**0.05**	
3	G	G	C	G	0.12	0.21	0.003	
ARRB2[1]	rs1045280	rs2036657	rs4790694					Ikeda, 2007
1	T	A	C		0.80	0.85	**0.02**	
2	C	G	A		0.09	0.05	**0.02**	
DTNBP1	rs2619539	rs3213207	rs2619538					Kishimoto, 2008
1	C	A	A		0.61	0.71	**0.001**	
2	G	A	A		0.33	0.27	0.08	
3	C	G	T		0.03	0.002	**0.001**	
4	C	G	A		0.02	0.002	0.11	
5	C	A	T		0.006	0.007	0.83	
6	G	G	A		0.009	0.00	0.15	
7	G	A	T		0.00	0.004	0.18	
GABRG2	315C>T	1128+99C>A						Nishiyama, 2005
1	C	C			0.28	0.31	0.52	
2	C	A			0.43	0.43	1.00	
3	T	C			0.26	0.19	**0.03**	
4	T	A			0.04	0.07	0.12	
GLYT1	rs2486001	rs2248829						Morita, 2007
1	C	G			0.48	0.54	0.08	
2	C	A			0.24	0.27	0.30	
3	T	G			0.28	0.16	**0.00**	
4	T	A			0.00	0.03	–	
OPRM1	rs1799971	rs2075572	rs599548	rs598682			0.30	Ide, 2006
1	A	C	A	A	0.00	0.002	–	
2	A	C	A	G	0.005	0.02	–	
3	A	C	G	A	0.00	0.01	–	
4	A	C	G	G	0.34	0.33	–	

Table 2.5 (Continued) Haplotype frequencies among published studies of METH use disorders

Gene Haplotype	Haplotype Markers						Frequency METH	Frequency Control	p-values	Ref
5	A	G	A	A			0.00	0.005	–	
6	A	G	A	G			0.12	0.08	–	
7	A	G	G	A			0.11	0.05	–	
8	A	G	G	G			0.03	0.03	–	
9	G	C	A	G			0.02	0.006	–	
10	G	C	G	G			0.38	0.44	–	
11	G	G	A	G			0.005	0.02	–	
12	G	G	G	A			0.00	0.004	–	
13	G	G	G	G			0.004	0.00	–	
PICK1	rs737622	rs3026682	rs11089858	rs713729	rs3952	rs2076369				Matsuzawa, 2007
1	C	G	G	T	A	T	0.34	0.35	0.63	
2	G	A	G	T	G	G	0.32	0.32	0.85	
3	C	G	G	A	A	G	0.09	0.15	**0.02**	
4	C	G	A	T	A	G	0.07	0.08	0.66	
5	C	G	G	T	A	G	0.09	0.06	0.09	
6	G	A	G	T	G	T	0.04	0.01	**0.01**	
7	C	G	G	A	A	T	0.02	0.01	0.66	
8	G	A	G	A	G	G	0.004	0.01	0.40	
PIK4CA[1]	rs2072513	rs165862	rs165793	rs165789						Kanahara, 2008
1	G	C	T	A			0.45	0.46	1.00	
2	A	A	C	G			0.36	0.35	1.00	
3	A	A	T	G			0.11	0.11	0.99	
4	A	C	T	A			0.05	0.05	1.00	
SLC22A3	rs509707	rs4709426							0.18	Aoyama, 2006
1	C	G					0.51	0.57	–	
2	T	C					0.32	0.26	–	
3	C	C					0.15	0.14	–	
4	T	G					0.03	0.03	–	
SOD2[2]	rs2758357	rs4880	rs2855116 & rs2758332[3]	rs2758330	rs2842980					Nakamura, 2006
1	C	V	A	G	A		0.46	0.48	0.72	
							0.44	0.44	0.98	

Table 2.5 (Continued) Haplotype frequencies among published studies of METH use disorders

Gene Haplotype	Haplotype Markers					Frequency		p-values	Ref
						METH	Control		
2	C	V	A	T	T	0.39	0.38	0.79	
						0.36	0.39	0.49	
3	T	A	B	T	T	0.12	0.10	0.52	
						0.16	0.10	**0.05**	
4	T	V	A	T	T	0.03	0.03	0.75	
						0.02	0.05	0.09	
5	C	V	A	T	A	0.00	0.01	0.08	
						0.02	0.03	0.94	

1 = Haplotypes with frequencies < 5.0% not reported; 2 = For each haplotype the first row represents Japanese and second row are Han-Chinese
3 = A/A: rs2855116 T/T and rs2758332 G/G; A/B:rs2855116 T/G and rs2758332 G/T; B/B: rs2855116 G/G and rs2758332 T/T
Note: Insufficient data was available for DRD4 and PLG haplotypes to be included in this table.

Table 2.6 Top gene networks of the 39 reviewed genes for METH use disorders in the literature

Network	Molecules in Network[1]	Molecules Reviewed	Top Functions
1	ADCY, Angiotensin II receptor type 1, **ARRB2**, **BDNF**, Calmodulin, **CART**, **DRD1, DRD2, DRD3, DRD4**, **DTNBP1**, ERK, Fibrin, G alphai, G protein beta gamma, GABAR-A, **GABRA1, GABRA6, GABRB2, GABRG2, GHRL**, Gi-coupled receptor, hCG, **OPRD1, OPRM1**, p70 S6k, **PAI1**, **PDYN, PICK1**, Pkc(s), Plasminogen Activator, **PLAT**, PP2A, **SLC6A3, SLC6A4**	21	Nutritional Disease, Psychological Disorders, Neurological Disease
2	**ACE**, Actin, **AGT**, Akt, **AKT1**, Ap1, Ck2, **COMT**, Creb, ERK1/2, **FAAH**, FSH, GST, **GSTM1**, **GSTP1**, Hsp70, Hsp90, IKK, IL1, Insulin, Jnk, LDL, Mapk, Mmp, Nfat, NFkB, **NQO1**, P38 MAPK, PDGF BB, PI3K, **PI4KA, PLG, SNCA, SOD2**, Tgf beta	12	Cell Cycle, Skeletal and Muscular System Development and Function, Drug Metabolism
3	APOD, beta-estradiol, BLZF1, cholesterol, CXCL12, CXCR7, CYP2A6, CYP2C18, **CYP2D6**, CYP2J5, CYP3A5, CYP4B1, GORASP2, HMGCS1, HSPA13, IRF6, KDELR3, **NQO2**, NSDHL, **OPRS1**, PRPS1, RAB2A, RAB9A, RGS18, RRBP1, RYR3, SC4MOL, **SIGMAR1**, SPCS2, SQLE, SSR1, SSR2, SSR3, TSPO, **XBP1**	5	Small Molecule Biochemistry, Drug Metabolism, Nucleic Acid Metabolism
4	HNF4A, **SLC22A3**	1	Cellular Development, Lipid Metabolism, Molecular Transport

[1] Genes included in the current review are in bold. Data derived from Ingenuity Pathways Analysis version 6.3-1402

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CHAPTER 3

ASSOCIATIONS OF PUTATIVE AND NOVEL GENETIC VARIANTS FOR METHAMPHETAMINE DEPENDNECE

ABSTRACT

Research into the biological processes that increase susceptibility to methamphetamine dependence has been conducted primarily in Asian populations. This study's purpose was to explore, among a population of methamphetamine-dependent Caucasians, African-Americans, and Hispanics, six putative single nucleotide polymorphisms previously found to be associated with methamphetamine dependence in Asians as well as three novel variants for methamphetamine dependence. 278 non-psychotic males (159 methamphetamine-dependent and 119 controls) were genotyped for nine variants located in eight genes [i.e. V-akt murine thymoma viral oncogene homolog 1 (*AKT1*); arrestin beta 2 (*ARRB2*); brain-derived neurotrophic factor (*BDNF*); dopamine receptor 1 (*DRD1*); catechol-o-methyltransferese (*COMT*); gamma-aminobutyric acid receptor G2 (*GABRG2*); glutathione S-transferase P1 (*GSTP1*); mu-opioid receptor 1 (*OPRM1*)]. All analyses were stratified by ethnicity prior to calculation of genotypic and allelic frequencies, odds ratios (ORs), and 95% confidence intervals. Of the six variants putatively associated with methamphetamine dependence in Asians, *COMT* was associated with methamphetamine dependence at the genotypic ($\chi^2 = 7.25$, $p = .03$) level in African-Americans and the *OPRM1* gene was associated with methamphetamine dependence at the genotypic level among Hispanics ($\chi^2 = 8.55$, $p = .01$). Minor allele effect size (ORs) comparisons suggested convergence between Caucasians and Asians for *BDNF* and divergence between African-Americans and Asians for *AKT1* and *COMT*. Results provide support for further validation of candidate SNPs for METH dependence reported among Asian populations across other ethnic/ancestral groups.

Keywords: methamphetamine, gene association, ethnicity

INTRODUCTION

Methamphetamine (METH) is a powerful illicit psychostimulant that has become increasingly popular throughout North America (Maxwell & Rutkowski, 2008). As a result, initiation of METH use and the progression to abuse and subsequent dependence has received increased attention in both research and clinical settings. However, unlike other substances of abuse (*e.g.*, alcohol, cocaine), efforts to understand the genetic factors that may increase susceptibility to METH dependence have been limited. Twin studies have shown significantly high heritability of stimulant use disorders (Tsuang et al., 1996; Tsuang et al., 1998); thus, the search for risk genes underlying these disorders is warranted. Some progress in identifying risk genes for METH dependence has been made, but almost exclusively in Asian populations. In these studies, several genes have been implicated across several biological pathways, ranging from dopamine-metabolism and signaling to neuronal survival factors. However, replication of these initial genotypic and allelic associations has not been attempted among ethnically diverse populations.

It is generally accepted, as a result of efforts by the International Haplotype Mapping Project (HapMap) (International HapMap Consortium, 2005), that genotypic and allelic variations can differ greatly from one ancestral group to the next. In fact, in a recent genome-wide association study of METH dependence (Uhl et al., 2008), it was reported that ethnic allelic divergence is probable. Thus, it is necessary to verify genetic associations not only within but also across populations. Since the completion of the human genome, a tremendous amount of studies have purported gene-disorder associations that have not been replicated in similar and/or different populations. It is this particular propensity toward type-I errors that requires repeated investigations of genetic

associations within and across populations. Thus, the purpose of this study was to explore for the first time, to our knowledge, putative single nucleotide polymorphisms (SNPs) for METH dependence in an ethnically diverse sample. We selected and examined, among a population of METH-dependent Caucasians, African-Americans and Hispanics, six putative SNPs recently found to be associated with METH dependence in Asian populations. In addition, three novel SNPs for METH dependence were selected for exploration.

METHODS

Participants

Participants were 278 non-psychotic males (159 METH-dependent and 119 control) evaluated at the HIV Neurobehavioral Research Center (HNRC) at the University of California, San Diego, as part of a cohort study focused on central nervous system effects of HIV and methamphetamine. METH dependence was determined by the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders Version IV (SCID), in which all included METH users met dependence criteria in their lifetime and abuse criteria within the previous 18 months. Participants were not actively using other substances, with the exception of cannabis and alcohol. Potential participants were excluded if they met lifetime dependence criteria for other drugs, unless the dependence was judged to be remote (greater than 5 years ago) and episodic in nature. Alcohol dependence within the last year was also an exclusion criterion. Participants with a history of METH dependence were primarily recruited from residential drug treatment programs in the San Diego area, while those participants without a history of METH

abuse were recruited from the larger San Diego community through the use of flyers and appearances at community events.

Ancestry was approximated by use of a single interview question asking participants which ethnic group they identify with. For the current study, participants identifying as Latin-American, Mexican-American or Hispanic were coded as Hispanic (HISP: METH *n*=30, control *n*=16) while those identifying as African-American or black were coded as African-American (AA: METH *n*=12, control *n*=27). Participants identifying as Caucasian or white were coded as Caucasian (CEU: METH *n*=117, control *n*=76). A small proportion of participants who identified as Asian/Pacific Islander (*n*=8) or American Indian (*n*=3) were not included in the current study. All participants gave written consent prior to enrollment and all procedures were approved by the Human Research Protection Program of the University of California, San Diego and San Diego State University.

SNP Identification and Selection

Literature searches were conducted utilizing Medline (January, 2002 to March, 2008) to identify putative and novel genes for METH dependence (see Chapter 2 Search Strategy for more details). From these searches, two lists were created: one with variants reported to be associated with METH dependence and one with variants reported to be associated with other psychoactive substances. These lists were shortened to include only single nucleotide polymorphisms. Variable number tandem repeat (VNTR), deletion, and haplotypic associations were removed due to the limitations of the genotyping technology available for this study. From the remaining SNPs on the list, we selected six putative SNPs from six independent published studies among METH-dependent Asians [i.e. V-akt

murine thymoma viral oncogene homolog 1 (AKT1) (Ikeda et al., 2006); arrestin beta 2, ARRB2 (Ikeda et al., 2007); brain-derived neurotrophic factor (BDNF) (Cheng et al., 2005); catechol-o-methyltransferese (COMT) (Li et al., 2004); glutathione S-transferase P1 (GSTP1) (Hashimoto et al., 2005); mu-opioid receptor 1 (OPRM1) (Zhang et al., 2006)] and three novel SNPs from two genes [i.e. dopamine receptor 1 (*DRD1)* and gamma-aminobutyric acid receptor G2 (*GABRG2*)] linked to other substance-dependent (Batel et al., 2008) and METH populations (Nishiyama et al., 2005), respectively.

DNA Extraction and Genotyping

DNA was extracted from peripheral blood mononuclear cells stored (three to five years) at –70ºC using the QIAamp DNA Mini kit (Qiagen, Valencia, CA; Catalog #51185). Six putative (*AKT1*: rs3730358; *ARRB2*: rs4790694; *BDNF*: rs6265; *COMT*: rs4680; *GSTP1*: rs1695; *OPRM1*: rs2075572) and three novel (*DRD1*: rs265981 & rs4532; *GABRG2*: rs3219203) SNPs for METH dependence were assayed. A Multiplex PCR technique designed using Sequenom SpectroDESIGNER software (version 3.0.0.3) was used by inputting sequence containing each SNP site and 100 bp of flanking sequence on either side of the SNP. The SNPs were then grouped into multiplexes so that the extended product would not overlap in mass with any other oligonucleotide present in the reaction mix, and where no primer-primer, primer-product, or non-specific interactions would occur. The PCR was carried out in 384-well reaction plates in a volume of 5 μl using 10 ng genomic or whole-genome amplified DNA. All subsequent steps, up until the reaction, were spotted onto the SpectroCHIP and carried out in the same reaction plate. After PCR, any unincorporated dNTPs from the PCR were removed from the reaction by digestion with Shrimp alkaline phosphatase. dNTPs were removed

so that they could not play any role in the extension of the oligonucleotide at the SNP site. The extension reaction was then carried out in the presence of the extension oligonucleotide and a termination mix containing mass-modified dideoxynucleotides which extended the oligonucleotide over the SNP site with one base. Before spotting onto the SpectroCHIP, the reaction was cleaned by incubation with a cation-exchange resin which removed any salts present. The extension product was then spotted onto a 384-well spectroCHIP before being flown in the MALDI-TOF mass spectrometer. Data were collected, in real time, using SpectroTYPER Analyzer 3.3.0.15, SpectraAQUIRE 3.3.1.1 and SpectroCALLER 3.3.0.14 (Sequenom) algorithms.

Statistical Analysis

Genotype and allele frequencies for the nine selected SNPs were determined for METH-dependent and control participants classified in each of the three ethnic groups (Caucasian, African-American, and Hispanic) in this study. Odds ratios (ORs) and 95% confidence intervals (CIs) were then calculated using chi-square analysis to determine if alleles for any of the selected variants conferred a susceptibility to METH dependence by ethnicity. For comparison purposes, genotype and allele frequencies, odds ratios, and 95% confidence intervals were also calculated for each of the six studies among Asian METH-dependent participants in the literature corresponding to the six putative SNPs examined in this study. Due to the small sample sizes for each of the ethnic groups and subsequent lower than adequate power, statistical analysis and interpretation was treated as exploratory and preliminary, respectively. All statistical tests and procedures were conducted using STATA® (StataCorp LP, 2005).

RESULTS

Table 3.1 displays genotype frequencies of the six SNPs (*AKT1*, *ARRB2*, *BDNF*, *COMT*, *GSTP1*, *OPRM1*) previously implicated in Asian samples and the three novel SNPs (*DRD1, GABRG2*) selected for this study. Only *COMT* and *OPRM1* showed significant genotypic associations with METH dependence. *COMT* was associated with METH dependence among African-Americans (χ^2=7.25, *df*=2, *p*=0.03) whereas *OPRM1* was associated with the disorder among Hispanics (χ^2=8.55, *df*=2, *p*=0.01).

As it is probable that these statistically significant results represent Type 1 errors due to multiple testing and insufficient power, Table 3.2 provides minor allele counts, frequencies and odds ratios for each variant by ethnicity from which effect size comparisons can be made. Although, no significant allele associations for any of the SNPs under investigation were uncovered, examination of effect size estimates (ORs) revealed both convergent and divergent trends among several of the SNPs when compared to Asian samples. Among Caucasians, a trend toward convergence with Asians was observed for *BDNF* in that ORs for the minor allele frequencies for both ethnic groups were similar (OR=0.59, 95% CI=0.34-1.04 vs. OR=0.64, 95% CI=0.43-.95). Divergent trends were observed when comparing African-Americans and Asians for *AKT1* (OR=0.37, 95% CI=0.06-1.72 vs. OR=1.59, 95% CI=1.06-2.35) and *COMT* (OR=2.41, 95% CI=0.77-7.48 vs. OR=0.77, 95% CI=0.61-.97) minor allele variants.

DISCUSSION

To our knowledge this study is the first to explore previously reported gene-associations for METH dependence in an ethnically diverse population and provide evidence that genotypic susceptibility to METH dependence may differ by ethnicity.

Among the six putative SNPs previously found to be associated with METH dependence among Asians, we replicated associations for the *COMT* Val158Met polymorphism among African-Americans and the *OPRM1* non-coding polymorphism (rs2075572G>C) among Hispanics subjects. Putative associations with variants in *AKT1*, *ARRB2*, *BDNF* and *GSTP1* as well as, novel variants located on *DDR1* and *GABRG2* were not replicated among Caucasian, African-American, or Hispanic subjects. However, when examining minor allele effect size estimates between our diverse sample and previously published Asian samples, divergent (*AKT1*, *COMT*) and convergent (*BDNF*) observations were made. Our relatively low success in statistically replicating other associations between putative variants and METH dependence is likely a result of our relatively small sample sizes and consequent insufficient power. In fact, post-hoc power analysis by ethnicity using minor allelic frequencies (Table 3.3) revealed relatively low statistical power within the current study compared to previous Asian studies. On the other hand, to our knowledge none of the variants examined in this study have been replicated within the population it originally was reported in. Thus, it is also probable that our study, even if adequately powered, would not have replicated the putative associations.

Collectively, the replication of both the *COMT* and *OPRM1* variants as well as a trend towards minor allele frequency convergence for *BDNF* and divergence for *AKT1* and *COMT* variants suggest both a generalized as well as unique genotypic susceptibility to METH dependence given a particular ethnicity, respectively. However, this conclusion should be viewed as preliminary and also weighed against several limitations. First and foremost, ethnicity was used as an approximation of ancestry and thus the potential for admixture within each of the groups examined is of possible concern. In fact, it has been

established that modern Hispanic populations, specifically, Mexican-Americans are highly admixed (Collins-Schramm et al. 2004). Thus, further validation at these loci is required among ethnically/ancestrally diverse groups ideally utilizing available ancestral identification markers (AIMs) for ancestral classification. Second, as aforementioned, sample size and power were less than optimal for the current study and neither of the significant replications in the present study withstood correction for multiple testing. Thus, our findings potentially represent a type-I error, further advocating for substantially larger sample sizes for future genotypic investigations of METH dependence. Finally, the phenotype of interest was METH dependence; however, our control subjects as well as those in the literature report no significant involvement with METH. Thus, it could be that the selected genes in this study are markers for METH initiation and/or abuse, rather than dependence.

Despite these limitations, it is probable that the current as well as previously reported genotype and allele frequency differences may in part be explained by ethnicity and may confer differential susceptibility to METH dependence. These findings are in concordance with a recent genome-wide association study of METH dependence that also concluded potential genetic divergence by ethnicity (Uhl et al., 2008) and provide preliminary support for further validation of candidate SNPs for METH dependence reported among Asian populations across other ethnic/ancestral groups.

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J.H., Grant, I., Tsuang, M.T., Everall, I.P. The dissertation author was the primary investigator and author of this paper.

Table 3.1 Genotype frequencies of putative and novel SNPs in controls and METH dependence by ethnicity

Gene (SNP) Count (frequency)	**Ethnicity**							
	Current Study						**Other Studies**	
	CEU		**AA**		**HISP**		**Asian**	
	METH (n = 117)	**Control (n = 76)**	**METH (n = 12)**	**Control (n = 27)**	**METH (n = 30)**	**Control (n = 16)**	**METH**	**Control**
Putative SNPs								
AKT1 (rs3730358C>T)							*n = 182*	*n = 437*
CC	86 (.74)	50 (.66)	9 (.75)	13 (.48)	19 (.64)	12 (.75)	136 (.74)	364 (.83)
CT	26 (.22)	24 (.32)	3 (.25)	13 (.48)	11 (.36)	3 (.19)	43 (.24)	68 (.16)
TT	5 (.04)	2 (.03)	0 (.00)	1 (.04)	0 (.00)	1 (.06)	3 (.02)	5 (.01)
χ^2 (p-value)	2.29 (p = 0.32)		2.59 (p = 0.27)		3.19 (p = 0.20)		6.08 (p = 0.04)	
ARRB2 (rs4790694C>A)							*n = 177*	*n = 546*
CC	84 (.72)	48 (.63)	5 (.42)	7 (.26)	19 (.64)	11 (.69)	138 (.78)	470 (.86)
CA	31 (.26)	25 (.33)	5 (.42)	16 (.59)	10 (.33)	5 (.31)	39 (.22)	74 (.14)
AA	2 (.02)	3 (.04)	2 (.16)	4 (.15)	1 (.03)	0 (.00)	0 (.00)	2 (.003)
χ^2 (p-value)	2.04 (p = 0.36)		1.17 (p = 0.56)		0.59 (p = 0.74)		7.85 (p = .02)	
BDNF (rs6265G>A)							*n = 103*	*n = 122*
GG	86 (.73)	45 (.59)	10 (.83)	22 (.82)	18 (.60)	13 (.81)	31 (.30)	23 (.19)
GA	29 (.25)	29 (.38)	2 (.17)	5 (.18)	12 (.40)	3 (.19)	57 (.55)	68 (.56)
AA	2 (.02)	2 (.03)	0 (.00)	0 (.00)	0 (.00)	0 (.00)	15 (.15)	31 (.25)
χ^2 (p-value)	4.32 (p = 0.12)		0.02 (p = 0.89)		2.14 (p = 0.14)		6.16 (p = 0.05)	
COMT (rs4680G>A)							*n = 410*	*n = 390*
GG	23 (.20)	18 (.24)	2 (.17)	16 (.59)	9 (.30)	4 (.25)	228 (.56)	181 (.46)
GA	65 (.55)	45 (.59)	9 (.75)	8 (.30)	16 (.53)	9 (.56)	150 (.37)	172 (.44)
AA	29 (.25)	13 (.17)	1 (.08)	3 (.11)	5 (.17)	3 (.18)	32 (.07)	37 (.10)
χ^2 (p-value)	1.71 (p = 0.43)		7.25 (p = 0.03)		0.14 (p = 0.94)		6.77 (p = 0.04)	
GSTP1 (rs1695A>G)							*n = 189*	*n = 199*
AA	15 (.13)	9 (.12)	5 (.42)	10 (.37)	8 (.27)	5 (.31)	144 (.76)	167 (.84)
AG	58 (.50)	29 (.38)	5 (.42)	13 (.48)	18 (.60)	5 (.31)	41 (.22)	32 (.16)

Table 3.1 (Continued) Genotype frequencies of putative and novel SNPs in controls and METH dependence by ethnicity

Gene (SNP) Count (frequency)	**Ethnicity**							
	Current Study						**Other Studies**	
	CEU		**AA**		**HISP**		**Asian**	
	METH (n = 117)	**Control (n = 76)**	**METH (n = 12)**	**Control (n = 27)**	**METH (n = 30)**	**Control (n = 16)**	**METH**	**Control**
GG	44 (.37)	38 (.50)	2 (.16)	4 (.15)	4 (.13)	6 (.38)	4 (.02)	0 (.00)
χ^2 (p-value)	3.03 (p = 0.22)		0.14 (p = 0.93)		4.61 (p = 0.10)		6.56 (p = 0.03)	
OPRM1 (rs2075572G>C)							*n = 128*	*n = 232*
GG	20 (.17)	23 (.30)	3 (.25)	5 (.19)	5 (.17)	0 (.00)	12 (.09)	6 (.03)
GC	57 (.49)	26 (.34)	6 (.50)	16 (.59)	8 (.27)	11 (.69)	43 (.34)	72 (.31)
CC	40 (.34)	27 (.36)	3 (.25)	6 (.22)	17 (.56)	5 (.31)	73 (.57)	154 (.66)
χ^2 (p-value)	5.87 (p = 0.05)		0.32 (p = 0.85)		8.55 (p = 0.01)		8.92 (p = 0.01)	
Novel SNPs								
DRD1 (rs265981G>A)								
GG	51 (.44)	30 (.40)	8 (.66)	20 (.74)	18 (.60)	9 (.56)		
GA	51 (.44)	33 (.43)	4 (.33)	5 (.19)	10 (.33)	5 (.31)	NA	
AA	15 (.12)	13 (.17)	0 (.00)	2 (.07)	2 (.07)	2 (.13)		
χ^2 (p-value)	0.77 (p = 0.68)		1.74 (p = 0.42)		0.45 (p = 0.80)			
DRD1 (rs4532T>C)								
TT	51 (.44)	30 (.40)	7 (.58)	18 (.67)	17 (.57)	9 (.56)		
TC	51 (.44)	33 (.43)	5 (.42)	6 (.22)	11 (.36)	5 (.31)	NA	
CC	15 (.12)	13 (.17)	0 (.00)	3 (.11)	2 (.07)	2 (.13)		
χ^2 (p-value)	0.77 (p = 0.68)		2.54 (p = 0.28)		0.50 (p = 0.78)			
GABRG2 (rs3219203C>T)								
CC	104 (.89)	66 (.87)	11 (.92)	25 (.93)	28 (.93)	16 (1.0)		
CT	12 (10)	10 (.13)	1 (.08)	2 (.07)	2 (.07)	0 (.00)	NA	
TT	1 (.01)	0 (.00)	0 (.00)	0 (.00)	0 (.00)	0 (.00)		
χ^2 (p-value)	1.01 (p = 0.60)		0.01 (p = 0.92)		1.15 (p = 0.29)			

Table 3.2 Minor allele counts and frequencies of putative and novel SNPs in controls and METH dependence by ethnicity

Gene (SNP) Allele Count (frequency)	**Ethnicity**							
	Current Study						**Other Studies**	
	CEU		**AA**		**HISP**		**Asian**	
	METH (n = 117)	**Control (n = 76)**	**METH (n = 12)**	**Control (n = 27)**	**METH (n = 30)**	**Control (n = 16)**	**METH**	**Control**
Putative SNPs								
AKT1 (rs3730358C>T)							*n = 182*	*n = 437*
T	36 (.15)	28 (.18)	3 (.13)	15 (.28)	11 (.18)	5 (.16)	49 (.13)	78 (.10)
OR	0.81		0.37		1.21		1.59	
95% CI (p-value)	0.45 - 1.44 (0.43)		0.06 - 1.55 (0.13)		0.34 - 4.92 (0.74)		1.06 - 2.35 (0.02)	
ARRB2 (rs4790694C>A)							*n = 177*	*n = 546*
A	35 (.15)	31 (.20)	9 (.38)	24 (.44)	12 (.20)	5 (.16)	39 (.11)	78 (.07)
OR	0.69		0.75		1.35		1.61	
95% CI (p-value)	0.39 - 1.22 (0.17)		0.24 - 2.22 (0.57)		0.39 - 5.41 (0.61)		1.04 - 2.41 (0.02)	
BDNF (rs6265G>A)							*n = 103*	*n = 122*
A	33 (.14)	33 (.22)	2 (.08)	5 (.09)	12 (.20)	3 (.09)	87 (.42)	130 (.53)
OR	0.59		0.89		2.42		0.64	
95% CI (p-value)	0.34 - 1.04 (0.05)		0.08 - 5.98 (0.89)		0.58 - 14.33 (0.19)		0.43 - 0.95 (0.02)	
COMT (rs4680G>A)							*n = 410*	*n = 390*
A	123 (.52)	71 (.47)	11 (.46)	14 (.26)	26 (.43)	15 (.47)	214 (.26)	246 (.32)
OR	1.26		2.41		0.87		0.77	
95% CI (p-value)	0.82 - 1.94 (0.26)		0.77 - 7.41 (0.08)		0.34 - 2.25 (0.75)		0.61 - 0.96 (0.02)	
GSTP1 (rs1695A>G)							*n = 189*	*n = 199*
G	88 (.38)	47 (.31)	9 (.38)	21 (.39)	26 (.43)	17 (.53)	49 (.13)	32 (.08)
OR	1.35		0.94		0.68		1.70	
95% CI (p-value)	0.85 - 2.13 (0.17)		0.30 - 2.81 (0.91)		0.26 - 1.74 (0.37)		1.04 - 2.81 (0.03)	
OPRM1 (rs2075572G>C)							*n = 128*	*n = 232*
C	137 (.59)	80 (.53)	12 (.50)	28 (.52)	42 (.70)	21 (.66)	189 (.74)	380 (.82)
OR	1.27		0.93		1.22		0.62	
95% CI (p-value)	0.82 - 1.96 (0.25)		0.34 - 2.71 (0.88)		0.44 - 3.33 (0.66)		0.42 – 0.92 (0.01)	

Table 3.2 (Continued) Minor allele counts and frequencies of putative and novel SNPs in controls and METH dependence by ethnicity

Gene (SNP) Allele Count (frequency)	**Ethnicity**							
	Current Study						**Other Studies**	
	CEU		**AA**		**HISP**		**Asian**	
	METH (n = 117)	**Control (n = 76)**	**METH (n = 12)**	**Control (n = 27)**	**METH (n = 30)**	**Control (n = 16)**	**METH**	**Control**
Novel SNPs								
DRD1 (rs265981G>A)								
A	81 (.35)	59 (.39)	4 (.17)	9 (.17)	14 (.23)	9 (.28)		
OR	0.83		1.00		0.78		NA	
95% CI (p-value)	0.53 - 1.30 (0.40)		0.20 - 4.13 (1.00)		0.27 - 2.37 (0.61)			
DRD1 (rs4532T>C)								
C	81 (.35)	59 (.39)	5 (.21)	12 (.22)	15 (.25)	9 (.28)		
OR	0.83		0.92		0.85		NA	
95% CI (p-value)	0.53 - 1.30 (0.40)		0.22 - 3.33 (0.89)		0.29 - 2.57 (0.69)			
GABRG2 (rs3219203C>T)								
T	14 (.06)	10 (.07)	1 (.04)	2 (.04)	2 (.03)	0 (.00)		
OR	0.90		1.13		6.33		NA	
95% CI (p-value)	0.36 - 2.34 (0.81)		0.02 - 22.71 (0.92)		0.00 - 12.7 (.30)			

OR = Odds ratio (METH vs. Control); 95% CI = 95% confidence interval;

Table 3.3 Post-hoc power analysis and required sample size based on minor allele frequencies by ethnicity

Gene	CEU	AA	HISP	Asian
Putative:				
AKT1 (rs3730358C>T)				
Power	0.10	0.18	0.03	0.53
N (per group)	1235	63	2818	920
ARRB2 (rs4790694C>A)				
Power	0.21	0.04	0.03	0.62
N (per group)	473	544	749	429
BDNF (rs6265G>A)				
Power	0.48	0.04	0.16	0.61
N (per group)	193	6204	89	171
COMT (rs4680G>A)				
Power	0.14	0.32	0.04	0.74
N (per group)	805	50	1239	465
GSTP1 (rs1695A>G)				
Power	0.25	0.04	0.10	0.58
N (per group)	376	18684	206	315
OPRM1 (rs2075572G>C)				
Power	0.18	0.03	0.04	0.68
N (per group)	554	4953	1092	223
Novel				
DRD1 (rs265981G>A)				
Power	0.10	–	0.05	–
N (per group)	1168	–	616	–
DRD1 (rs4532T>C)				
Power	0.10	0.04	0.03	–
N (per group)	1168	12891	1732	–
GABRG2 (rs3219203C>T)				
Power	0.04	–	0.01	–
N (per group)	1156	–	160	–

Note: Estimated sample size calculated using the assumptions: β=.20, α=.05

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CHAPTER 4

IMPACT OF COMT GENOTYPE ON EXECUTIVE FUNCTIONING IN THE CONTEXT OF HIV-INFECTION AND METHAMPHETAMINE DEPENDNECE

ABSTRACT

Putative relationships have been reported between the catechol-O-methyltransferease (COMT) Val allele and impaired executive functioning among healthy individuals. The purpose of this study was to: (1) determine whether this relationship could be observed among individuals with and/or without HIV-infection and/or methamphetamine (METH) dependence, two conditions that can alter dopaminergic system functioning; and (2) explore the specificity of this relationship by examining other cognitive ability domains. 229 men were recruited and classified into one of four groups based on their HIV and METH status: those with HIV-infection and METH dependence (HIV+/METH+; n=58), HIV-infection only (HIV+/METH-; n=72), METH dependence only (HIV-/METH+; n=50), or neither HIV-infection nor METH dependence (HIV-/METH-; n=49). All participants were hepatitis C negative. An objective deficit score of executive functioning was derived from a neuropsychological battery consisting of the Wisconsin Card Sort Test, Trail Making Test Part B and Halstead Category Test. COMT Val158Met polymorphism was assayed from blood-derived DNA. In the METH- groups, those with Met/Met genotype displayed better executive functioning compared to Val/Val carriers, but this effect was attenuated in the METH+ groups. Analysis of other neurocognitive domains did not replicate effects found for executive functioning. Results support the presumed neuroprotective effect of Met/Met genotype on executive functioning among METH- groups. Among METH+ groups, the slower rate of dopamine clearance conferred by Met/Met genotype may increase the risk of adverse effects of METH, making impairment comparable to that of Val allele carriers, and this observed effect may be specific to executive functioning.

INTRODUCTION

Executive functions are a cluster of high-order cognitive capacities largely dependent on the integrity of the prefrontal cortex (PFC), which include decision-making, planning, and self-monitoring, as well as behavior initiation, organization, and inhibition(Anderson & Tranel, 2002). Both HIV infection and methamphetamine (METH) dependence can have adverse effects on cognitive functioning (Gonzalez et al., 2004; Reger, Welsh, Razani, Martin, & Boone, 2002). In fact, Rippeth et al. (Rippeth et al., 2004) has shown both independent and combined effects of HIV and METH on cognitive abilities, including executive functioning. Additionally, a recent meta-analysis illustrated that METH use was associated with deficits in executive and other cognitive functioning (Scott et al., 2007). Furthermore, HIV has been associated with executive functioning deficits and is estimated to affect 60% of AIDS and 35% of asymptomatic patients(Grant, Heaton, & Atkinson, 1995; Heaton et al., 1995; White, Heaton, & Monsch, 1995). These cognitive deficits among METH users and/or HIV-infected individuals may ultimately result in poor decision-making and subsequently may increase the probability of HIV transmission or adversely affect disease management. Thus, understanding the underlying biological (*e.g.* genetic) mechanisms of cognitive impairment, specifically executive functioning, in different environmental contexts may provide avenues for future early identification and preventative treatments of cognitive impairment related to HIV and METH and potentially inform interventions aimed at reducing the current rise in HIV-infection rates.

Dopamine (DA) is a neurotransmitter required for proper cognitive functioning to occur and is a key component in the pleasure/reward pathway. Metabolism of DA is an

important biological mechanism critical to PFC mediated functions (*e.g.* executive functioning). Dopaminergic neurotransmission in the PFC is known to contribute to individual cognitive differences in humans (Starr, Fox, Harris, Deary, & Whalley, 2007)m and high or low levels of DA in the synapse can have negative effects on cognitive functioning (Tunbridge, Harrison, & Weinberger, 2006) Thus, mechanisms that assist in removing dopamine from the synapse (such as catechol-O-methyltransferease (COMT)) play an important role in regulating dopamine levels in the brain and subsequently may moderate cognitive functioning.

COMT is a major mammalian enzyme involved in the metabolic degradation of released DA. Although, noradrenaline transporters (Carboni & Silvagni, 2004) and monoamine oxidase (MAO) (Westerink & Spaan, 1982) contribute to elimination of DA, *COMT* accounts for more than 60% of the metabolism of DA in the PFC (Li et al., 2004). *COMT*'s specificity to the PFC is likely a result of very low expression of dopamine transporters (*DAT*) in the PFC, which when expressed in other regions of the brain has a 1000 times higher affinity for DA (Lewis et al., 2001).

Our primary interest in this study was a prevalent single nucleotide polymorphism (SNP) of *COMT* involving a Valine (Val) to Methionine (Met) amino acid substitution at codon 158 in the membrane-bound *COMT* (MB-*COMT*) or position 108 in the soluble-*COMT* (S-*COMT*). In human post-mortem PFC, the Val allele at this locus is 40% more enzymatically active than the Met allele (Chen et al., 2004). Thus, carriers of the Val allele metabolize dopamine at a more efficient rate, resulting in lower levels of DA in the synapse and ultimately a reduction in DA receptor activation. A recent meta-analysis has provided evidence of a putative relationship between the Val allele and impaired

executive functioning among healthy participants (Barnett, Jones, Robbins, & Muller, 2007). Conversely, the Met allele has been shown to enhance executive functioning (*i.e.* fewer perseverative errors on the Wisconsin Card Sort Test) (Egan et al., 2001) and be more pronounced in males (Barnett et al., 2007). However, Mattay and colleagues (Mattay et al., 2003) proposed and tested a theoretical inverted U-shaped model of the relationship between DA activity in the PFC and cognitive performance in the context of laboratory controlled amphetamine administration in healthy volunteers. Results of that study suggested that the relationship between *COMT* and PFC function is likely to be context-dependent and more complex than a simple dichotomy in which a Val allele is harmful and a Met allele is protective. To our knowledge, the putative context-dependent effect of COMT has not yet been examined in the natural (i.e. non-laboratory) context of methamphetamine and HIV.

The purpose of this study was to examine the presumed protective effect of the *COMT* Met/Met genotype on executive functioning in the context of METH dependence and/or HIV-infection in an ethnically diverse sample. We hypothesized that the contexts of both METH dependence and HIV-infection, two conditions that can alter dopaminergic systems, would modulate the relationship between *COMT* genotype and executive functioning. Secondarily, we explored the specificity of the hypothesized relationship by examining other cognitive functioning domains (*i.e.* learning, recall, speed of information processing, motor speed, verbal fluency, and working memory).

METHODS

Participants

Participants were volunteers evaluated at the HIV Neurobehavioral Research Center (HNRC) at the University of California in San Diego as part of a cohort study focused on central nervous system effects of HIV and methamphetamine. The current study comprised 229 men of Caucasian (n = 158), African-American (n = 31), and non-white Hispanic (n = 40) ethnicity. Participants were classified into one of the following four groups: methamphetamine dependent/HIV seropositive [METH+/HIV+; *n*=58]; methamphetamine dependent/HIV seronegative [METH-only; *n*=50]; methamphetamine non-users/HIV seropositive [HIV-only; *n*=72); and methamphetamine non-users/ HIV seronegative [controls; *n* = 49]. All participants were seronegative for hepatitis C infection.

HIV serological status was determined by enzyme linked immunosorbent assays (ELISA) plus a confirmatory test. METH+ participants met dependence criteria in their lifetime and abuse criteria within the previous 18 months, as determined by the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders Version IV (SCID). However, participants were not actively using other substances, with the exception of cannabis and alcohol. Potential participants were excluded if they met lifetime dependence criteria for other drugs, unless the dependence was judged by a doctoral-level clinician to be remote (greater than 5 years ago) and episodic in nature. Alcohol dependence within the last year was also an exclusion criterion. Participants with a history of METH dependence were primarily recruited from residential drug-treatment programs in the San Diego area, while those participants

without a history of METH abuse were recruited from the larger San Diego community through the use of flyers and appearances at community events. All participants gave written consent prior to enrollment and all procedures were approved by the Human Research Protection Program of the University of California, San Diego and San Diego State University.

Executive Functioning, METH Use, and HIV Characterization

Executive functioning was determined as part of a comprehensive battery of tests covering seven ability domains (Learning, Memory, Attention/Working Memory, Verbal Fluency, Processing Speed, Abstraction/Problem Solving, and Motor Speed), shown in Table 4.1. Raw scores for the component tests were converted to demographically-adjusted T-scores (M = 50, SD = 10), including adjustments for age, education, gender, and ethnicity as available for each test. The demographically-adjusted T-scores for each test were then converted into deficit scores, which reflect degree of impairment by setting performances within the normal range at zero with a range from 0 (T-score > 39; no impairment) to 5 (T-score < 20; severe impairment). Finally, the individual deficit scores were averaged within each domain to derive the domain deficit score, which reflects the severity of deficit within each particular domain. Previous work has demonstrated that deficit scores achieve good diagnostic agreement with classifications made by blind clinical ratings, with a cut point for impairment set at ≥ 0.50 (Carey et al., 2004; Heaton et al., 1995). The executive functioning domain deficit score, of particular focus in this study, was made up of (1) perseverative responses on the Wisconsin Card Sorting Test; (2) errors on the Halstead Category Test, which measures abstraction and cognitive flexibility; and (3) time to complete the Trail Making Test part B, reflecting ability to

switch and maintain attention between ongoing sequences. All neurocognitive testing and scoring was performed by trained psychometrists blinded to participants' genotypes.

Additional information for each participant was collected as it relates to a lifetime diagnosis of Major Depression Disorder (MDD), Bipolar Disorder I or II as well as abuse and/or dependence for sedatives, cannabis, opioids, cocaine, hallucinogens, and alcohol, using the SCID-IV. For METH+ participants, additional information was collected regarding age at first use, years and quantity of cumulative use, and days since last use of METH; whereas for HIV-infected participants, HIV RNA plasma copies were ascertained as part of a larger neuromedical evaluation.

DNA Extraction and Genotyping

DNA was extracted from peripheral blood mononuclear cells stored (three to five years) at –70ºC using the QIAamp DNA Mini kit (Qiagen, Valencia, CA; Catalog #51185). The *COMT* Val158Met polymorphism (rs4680) was assayed along with eight other SNPs as part of a concurrent genetic association project at the HNRC employing a Multiplex PCR technique designed using Sequenom SpectroDESIGNER software (version 3.0.0.3) by inputting a sequence containing 100 bp of flanking sequence on either side of the *COMT* Val158Met polymorphism. The SNP was then grouped into multiplexes so that the extended product would not overlap in mass with any other oligonucleotide present in the reaction mix, and where no primer-primer, primer-product, or non-specific interactions would occur. The PCR was carried out in 384-well reaction plates in a volume of 5 μl using 10 ng genomic or whole-genome amplified (WGA) DNA. All subsequent steps, up until the reaction, were spotted onto the SpectroCHIP and carried out in the same reaction plate. After PCR, any unincorporated dNTPs from

the PCR were removed from the reaction by digestion with Shrimp alkaline phosphatase. dNTPs were removed so that they could not play any role in the extension of the oligonucleotide at the SNP site. The extension reaction was then carried out in the presence of the extension oligonucleotide and a termination mix containing mass-modified dideoxynucleotides which extended the oligonucleotide over the SNP site with one base. Before spotting onto the SpectroCHIP, the reaction was cleaned by incubation with a cation-exchange resin which removed any salts present. The extension product was then spotted onto a 384-well spectroCHIP before being flown in the MALDI-TOF mass spectrometer. Data were collected, in real time, using SpectroTYPER Analyzer 3.3.0.15, SpectraAQUIRE 3.3.1.1 and SpectroCALLER 3.3.0.14 (Sequenom) algorithms. All genotyping was performed by an accredited commercial laboratory (Harvard Medical School-Partners Healthcare Center for Genetics and Genomics, Cambridge, MA CLIA No. 22D1005307).

Statistical Analysis

All statistical tests and procedures were conducted using SPSS 10.0 (SPSS, 2000). Group comparisons on demographic data were performed using analysis of variance (ANOVA). For this study, *COMT* genotype was dichotomized (Met/Met vs. Val/Val or Val/Met) for hypothesis testing based on previous findings (Joober et al., 2002) and preliminary analysis of this sample (not presented) showing that the Met/Met genotype confers a protective effect (M=0.34, sd=0.47) on executive functioning whereas those with a Val/Met (M=0.61, sd=0.73) or Val/Val (M=0.60, sd=0.77) demonstrate similar deficits (p=.989) in executive functioning and did not differ demographically. Mean group differences in executive functioning and other neurocognitive domain performance

were analyzed with Student's t-tests. Group differences in the proportions of impaired participants on the executive functioning domain and background variables were analyzed with a cross-tabs design and Chi-squared tests.

RESULTS

Participant Characteristics

Participant characteristics by group membership are shown in Table 4.2. All four groups were comparable with regard to age and education. However, African-Americans were significantly less represented in the METH-only group compared to controls ($\chi^2_{(1)}$=11.35; p=0.001), HIV-only ($\chi^2_{(1)}$=5.48; p=0.02), and METH+/HIV+ ($\chi^2_{(1)}$=11.86; p=0.001) groups. Lifetime diagnosis of Major Depression was significantly greater among those in the HIV-only ($\chi^2_{(1)}$=7.43; p=0.006) and METH+/HIV+ ($\chi^2_{(1)}$=9.45; p=0.002) groups compared to controls. As expected, METH+ groups had higher rates of lifetime alcohol abuse ($\chi^2_{(1)}$=8.16; p=0.004) and dependence ($\chi^2_{(1)}$=18.20; p=0.001), cannabis abuse ($\chi^2_{(1)}$=11.29; p=0.001) and dependence ($\chi^2_{(1)}$=9.92; p=0.002), cocaine abuse ($\chi^2_{(1)}$=11.58; p=0.001) and dependence ($\chi^2_{(1)}$=17.52; p=0.0001), as well as hallucinogen abuse ($\chi^2_{(1)}$=5.35; p=0.02) compared to METH- groups. METH+ groups did not differ on cumulative days of METH use or length of abstinence; however, the METH-only group did report significantly more total years of METH use compared to the METH+/HIV+ group ($F_{(1,106)}$=9.26; p=0.003). HIV(+) groups did not differ on mean plasma HIV RNA levels (p=0.795).

Genotype and allele distributions in the four groups revealed no significant differences (Table 4.3). Furthermore, when examining differences between genotype and

demographic variables the four groups were also comparable (Table 4.4). However, significant differences were observed in the control group for education ($t_{(47)}$=2.44; p=0.02) and lifetime cannabis abuse ($\chi^2_{(1)}$=3.76; p=0.05), in which carriers of the Met/Met genotype had one more year of education on average and no (0% vs. 27%) history of cannabis abuse, compared to Val allele carriers. In addition, among the METH-only group, 78% of Met/Met homozygotes versus 40% of Val allele carriers had a history of alcohol abuse ($\chi^2_{(1)}$=4.29; p=0.04).

COMT and Executive Functioning

Executive functioning deficit scores (Figure 4.1) were significantly lower among Met/Met homozygotes in both the control ($t_{(47)}$=-3.44; p=0.001) and HIV-only ($t_{(70)}$=-2.90; p=0.007) groups compared to Val allele carriers. However, the Met/Met genotype did not confer better executive functioning among METH-only (p=0.458) or METH+/HIV+ (p=0.733) groups. Similar results were found when examining rates of impairment among the four groups by genotype (Figure 4.2). The rate of impairment among Met/Met homozygotes was lowest for control participants (9%) and greatest for METH+/HIV+ participants (58%), while rates of impairment were generally similar among Val allele carriers across all groups (range 42% - 54%). Analysis stratified by each of the three ethnic groups in our sample also produced similar results at the trend level (Figure 4.3). As expected, Caucasians, our largest group, clearly represented the monotonic trend illustrated in the full sample; whereas, the trend seen in the Hispanic and African-American groups, although similar to the full sample, was less pronounced, which may be a result of reduced power with these smaller subgroups.

COMT and Other Neurocognitive Domains

COMT's association with speed of information processing (SIP), motor speed, learning, recall, verbal fluency, and working memory domains by each group are illustrated in Figure 4.4. Other than a statistically significant deficit score difference for verbal fluency, which can be considered to have an executive component, among those in the METH-only group ($t_{(48)}$=-3.24; p=0.002), no other significant results were found within any of these other neurocognitive domains. In addition, deficit score trends for Met/Met carriers across the four groups were not in accord with those found for executive functioning. The recall domain is a possible exception in that the monotonic trend observed for executive functioning among Met/Met homozygotes is present; however unlike executive functioning, the recall domain shows this same trend for Val allele carriers and thus provides little evidence of a genotype effect.

DISCUSSION

To our knowledge, these findings are the first to demonstrate a context-dependent neuroprotective effect of the *COMT Val*158*Met* polymorphism on executive functioning in a sample of individuals with and/or without METH dependence and/or HIV-infection. Our results support previous work (Bruder et al., 2005; Egan et al., 2001; Joober et al., 2002; Malhotra et al., 2002; Rosa et al., 2004) suggesting a neuroprotective effect, specifically in executive functioning, among relatively healthy individuals with the *COMT* Met/Met genotype. However, in this study we also demonstrate that this neuroprotective effect of the Met/Met genotype on executive functioning is attenuated among METH-dependent individuals, irrespective of their HIV-status. This finding is consistent with Mattay et al. (Mattay et al., 2003) who reported *COMT* interacts with

acute amphetamine exposure in an inverted U-shaped fashion to produce harmful effects on cognitive performance among healthy individuals with a Met allele by disrupting dopamine levels as well as exceeding the critical threshold of dopamine signaling and associated processing load.

Our findings extend this previous work by demonstrating the putative context-dependent effect of the Met/Met genotype on executive functioning in a chronic methamphetamine-exposed sample with and without co-morbid HIV-infection. Among METH+ groups, it may be that the slower rate of dopamine clearance conferred by Met/Met genotype may increase the risk of adverse effects of METH consumption thereby making executive functioning impairment comparable to that of Val allele carriers in the control group. However, our hypothesis that METH-dependent Val-carriers would show improved executive functioning compared to controls as a result of more efficient clearance of DA was not supported. In fact, across all groups within this study, executive functioning deficit scores and rates of impairment were comparable for Val-allele carriers. One potential explanation for this finding is that other polymorphisms on the *COMT* gene or elsewhere in the genome are confounding the presumed effect of the Val allele on executive functioning. In fact, previous work has shown that other loci within the *COMT* gene may affect the efficiency of the PFC (Meyer-Lindenberg et al., 2005); thereby suggesting research into gene-gene (or locus-locus) interaction as well as haplotype effects on executive functioning among METH-dependent populations may be warranted. Thus, in partial accordance with Mattay et al. (2003), the effect of *COMT* Met/Met activity on executive functioning may be dependent on where an individual lands on the inverted U-shaped curve given a particular environmental (*e.g*. METH

dependence) and/or genetic (*e.g*. *COMT* genotype) context. Therefore, future research should also examine other groups and contexts to better clarify the role of *COMT* on executive functioning. In particular, studies are needed which examine females and those of Asian ancestry, as well as other stimulants such as cocaine and other viral contexts such as Hepatitis C that were not examined in the current study.

For our secondary aim, we sought to estimate the extent of the specificity of the effect of the *Val*158*Met* polymorphism on executive functioning by examining other neurocognitive domains among the same sample. We found little evidence to support a similar relationship among speed of information processing, motor speed, learning, recall, verbal fluency, or working memory domains. Although Met/Met homozygotes, as well as carriers of a Val allele within the recall domain, appeared to show a similar monotonic trend to that shown in the executive functioning domain, it is likely that this parallel trend for both genotypes in the recall domain is a result of an environmental insult (*e.g*. METH dependence, HIV-infection) rather than the *Val*158*Met* polymorphism. In fact, the deficit score trend for the recall domain among both genotypes across the four groups replicates previous findings of global neurocognitive impairment among METH-dependent and/or HIV-infected individuals (Rippeth et al., 2004). Surprisingly, we did not observe the full presumed relationship between *COMT* and working memory which is also largely dependent on the integrity of the PFC and has been demonstrated in previous work (Mattay et al., 2003). One possible explanation for this discordance is the difference in tests used to estimate working memory. Mattay et al. (2003) employed the N-back test whereas in the current study we used a battery of three tests (Table 4.1) that did not include the N-back test. Thus, it may be that the relationship between *COMT* and

working memory is test-specific. On the other hand, when we examined the relationship between *COMT* and our executive functioning domain consisting of three tests [including the Wisconsin Card Sort Test used by Mattay et al. (2003)] we observed concordance with Mattay and colleagues (2003). In fact, examination of the three individual tests included in the executive functioning domain (Figure 4.5) revealed all three tests demonstrated the trend in which the Met/Met genotype has a neuroprotective effect among control and HIV-only groups but is attenuated among METH+ groups. Thus, our results provide preliminary evidence suggesting relatively strong specificity of the *Val*158*Met* polymorphism on tests of executive functioning.

Although these findings provide further insight into the relationship between *COMT* and neurocognitive functioning, specifically executive functioning, several limitations should be considered. The sample for our study was relatively small for a genotype-phenotype investigation. Although, in light of previous work (Barnett et al., 2007) examining *COMT* and neurocognition as well as the high minor allele (Met) frequency of the *COMT* Val158Met polymorphism with the populations under investigation (>40%), our total sample size for the HIV-only group provided adequate power (1-β=0.82) albeit power for the other groups was slightly below optimal (Control: 1-β=0.67; METH-only: 1-β=0.68; HIV+/METH+: 1-β=0.74). In addition, stratified analysis by the three ethnic groups resulted in a loss of power and subsequently inhibited statistical replication of the *COMT*-executive functioning relationship demonstrated in the full sample, albeit results were supportive in terms of the magnitude and direction of observed effects. On a similar note, ethnicity was used as an approximation of ancestry; thus the potential for admixture within each of the groups examined is of potential

concern. In fact, it has been established that allele frequencies for the *COMT* Val158Met polymorphism differ across populations (Palmatier, Kang, & Kidd, 1999) and among modern Hispanic populations, specifically, Mexican-Americans admixture is relatively high (Collins-Schramm et al., 2004). Therefore, further validation of the relationship between the Val158Met loci and executive functioning is required among larger ethnically/ancestrally diverse groups ideally utilizing available ancestral identification markers (AIMs) for ancestral classification.

Despite these limitations, our findings suggest a specific context-dependent relationship between *COMT* and executive functioning. Furthermore, it is clear from previous work as well as the current study that the link between *COMT* and neurocognitive functioning is not fully understood. However, continued work in this area could potentially lead to relevant public health innovations such as personalized health promotion interventions that may assist in curbing the transmission of HIV, other sexually transmitted infections, and non-adherence to treatment regimens as well as pharmacological treatments for executive dysfunction in both healthy and vulnerable populations.

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Table 4.1 Neurocognitive Test Battery

Domain	**Tests**
Learning	BVMT-R Learning, HVLT-R Learning, Story Memory Test-Learning, Figure Memory Test-Learning
Memory	BVMT-R Delayed Recall, HVLT-R Delayed Recall, Story Memory Test Retention, Figure Memory Test Retention
Attention/Working Memory	PASAT Total Correct, WAIS-III Letter-Number Sequencing
Processing Speed	Trail Making Test A, WAIS-III Symbol Search, WAIS-III Digit Symbol, Stroop Color-Word Incongruent
Executive Functioning	Trail Making Test B, Halstead Category Test Errors, WCST-64 Perseveration
Motor Speed	Grooved Pegboard Test
Verbal Fluency	Letter Fluency (FAS)

Table 4.2 Participant characteristics (N = 229)

Characteristic	Control (n=49) 1	HIV only (n=72) 2	METH only (n=50) 3	METH & HIV (n=58) 4	
All Groups					
Age (years) *M* (sd)	37 (12)	40 (9)	36 (10)	37 (7)	
Education (years) *M* (sd)	13 (2)	13 (2)	13 (2)	13 (3)	
WRAT4 *M* (sd)	103 (11)	101 (10)	96 (12)	99 (11)	1,2 > 3**
Ethnicity (%)	25	33	30	30	
Caucasian	21	29	26	25	
African-American	32	48	0	19	1,2 > 3,4*
Hispanic	15	30	23	33	2,3,4 > 1*
WCST perseverative errors (T-score) *M* (sd)	46 (12)	47 (15)	41 (13)	46 (15)	
Category Test errors (T-score) *M* (sd)	49 (10)	45 (9)	46 (10)	43 (10)	1 > 4*
Trails Making Test Part B (time) *M* (sd)	52 (11)	50 (10)	47 (10)	47 (11)	1 > 4*
Executive Functioning (deficit score) *M* (sd)	0.38 (0.61)	0.55 (0.67)	0.64 (0.74)	0.65 (0.79)	
Executive Functioning (% impaired)	17	43	50	52	
MDD (% lifetime)	20	44	33	49	2,3,4 > 1*
Bipolar I or II (% lifetime)	0	4	4	7	
Sedative (% lifetime)					
Abuse	2	0	4	3	
Dependence	0	0	0	0	
Cannabis (% lifetime)					
Abuse	21	11	36	33	3,4 > 1,2**
Dependence	4	3	24	10	3,4 > 1,2**
Stimulant (% lifetime)					
Abuse	4	2	-	-	
Dependence	0	0	100	100	3,4 > 1,2**

Table 4.2 (Continued) Participant characteristics (N = 229)

Characteristic	Control (n=49) 1	HIV only (n=72) 2	METH only (n=50) 3	METH & HIV (n=58) 4	
Opiod (% lifetime)					
Abuse	0	0	6	5	
Dependence	0	0	0	0	
Cocaine (% lifetime)					
Abuse	2	4	19	15	3,4 > 1,2*
Dependence	0	0	16	16	3,4 > 1,2**
Hallucinogen (% lifetime)					
Abuse	4	3	16	7	3 > 1,2,4*
Dependence	0	0	0	2	
Alcohol (% lifetime)					
Abuse	15	30	47	38	2,3,4 > 1*
Dependence	10	6	30	28	3,4 > 1,2**
Poly-drug (% lifetime)					
Abuse	0	0	0	0	
Dependence	0	0	0	0	
METH+ Groups					
Age at first METH use (mean yrs) *M* (sd)	-	-	23 (9)	25 (7)	
Total METH use (mean yrs) *M* (sd)	-	-	10 (6)	7 (6)	3 > 4**
Last use of METH (mean days) *M* (sd)	-	-	125 (106)	176 (330)	
HIV+ Groups					
HIV RNA, plasma (log copies/ml) *M* (sd)	-	3.5 (1.1)	-	3.5 (1.2)	

* = $p < .05$; ** = $p < .005$

Table 4.3 Distribution of genotypes and alleles by group

Genotype N (%)	Control (n=49)	HIV only (n=72)	METH only (n=50)	METH(+)/HIV(+) (n=58)
Val/Val	15 (31)	25 (35)	7 (14)	18 (31)
Val/Met	23 (47)	38 (53)	34 (68)	28 (48)
Met/Met	11 (22)	9 (12)	9 (18)	12 (21)
Val	53 (54)	88 (61)	48 (48)	64 (55)
Met	45 (46)	56 (39)	52 (52)	52 (45)

There were no significant genotype or allele differences

Table 4.4 Participant characteristics by genotype and group (N = 229)

	Control		HIV only		METH only		HIV & METH	
Characteristic	Val/Val or Val/Met (N = 38)	Met/Met (N = 11)	Val/Val or Val/Met (N = 63)	Met/Met (N = 9)	Val/Val or Val/Met (N = 41)	Met/Met (N = 9)	Val/Val or Val/Met (N = 46)	Met/Met (N = 12)
Age (years) *M* (sd)	35 (11)	40 (15)	39 (9)	40 (8)	36 (9)	35 (11)	37 (7)	36 (7)
Education (years) *M* (sd)	13 (1)	14 (2)*	13 (2)	13 (2)	13 (2)	13 (2)	13 (2)	14 (3)
WRAT4 *M* (sd)	102 (11)	108 (8)	101 (10)	107 (6)	95 (13)	102 (8)	99 (10)	98 (15)
Ethnicity (%) column								
Caucasian	66	73	67	67	81	89	65	75
African-American	21	18	22	11	0	0	11	8
Hispanic	13	9	16	22	19	11	24	17
MDD (% lifetime)	16	36	43	56	33	33	51	41
Bipolar I or II (% lifetime)	0	0	5	0	5	0	7	8
Sedative (% lifetime)								
Abuse	3	0	0	0	5	0	4	0
Dependence	0	0	0	0	0	0	0	0
Cannabis (% lifetime)								
Abuse	27	0*	11	11	41	14	33	33
Dependence	5	0	5	0	20	44	13	0
Opioid (% lifetime)								
Abuse	0	0	0	0	7	0	7	0
Dependence	0	0	0	0	0	0	0	0
Cocaine (% lifetime)								
Abuse	3	0	3	11	18	25	16	9
Dependence	0	0	0	0	15	22	11	33
Hallucinogen (% lifetime)								
Abuse	5	0	3	0	20	0	4	17
Dependence	0	0	0	0	0	0	2	0

Table 4.4 (Continued) Participant characteristics by genotype and group (N = 229)

	Control		HIV only		METH only		HIV & METH	
Characteristic	Val/Val or Val/Met (N = 38)	Met/Met (N=11)	Val/Val or Val/Met (N=63)	Met/Met (N = 9)	Val/Val or Val/Met (N = 41)	Met/Met (N = 9)	Val/Val or Val/Met (N = 46)	Met/Met (N = 12)
Alcohol (% lifetime)								
Abuse	17	9	32	11	40	78*	37	42
Dependence	13	0	6	0	29	33	31	17
METH+ Groups								
Age at first METH use (mean yrs) *M* (sd)	-	-	-	-	22 (8)	24 (12)	24 (7)	27 (9)*
Total METH use (mean yrs) *M* (sd)	-	-	-	-	12 (5)	9 (4)	11 (6)	6 (4)
Last use of METH (mean days) *M* (sd)	-	-	-	-	129 (115)	110 (59)	195 (364)	104 (121)
HIV+ Groups								
HIV RNA, plasma (log copies/ml) *M* (sd)	-	-	3.5 (1.1)	3.2 (1.5)	-	-	3.5 (1.1)	3.7 (1.0)

* $p < .05$

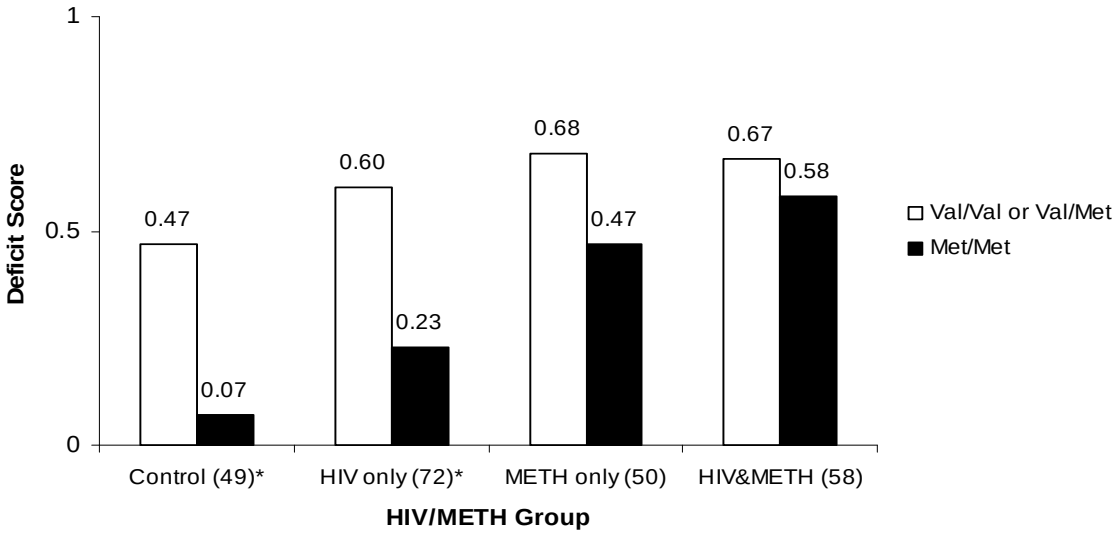

Figure 4.1 Executive Functioning Deficit Scores by HIV/METH Group and COMT Genotype. Note. Bars represent mean deficit scores. * Statistically significant at $p < .01$.

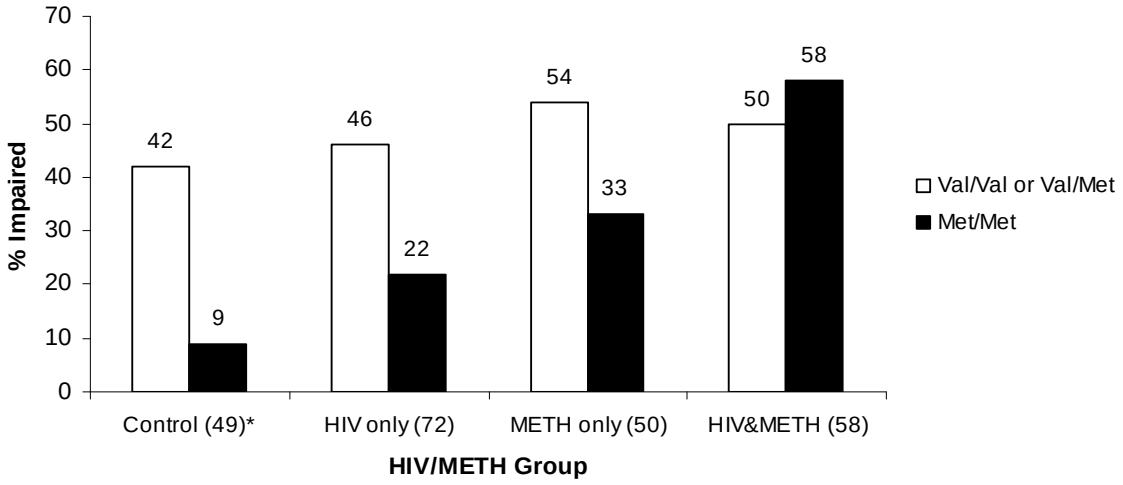

Figure 4.2 Executive Functioning Impairment by HIV/METH Group and COMT Genotype. Note. Impairment defined as an executive functioning domain deficit score > .50. * Statistically significant at $p < .05$.

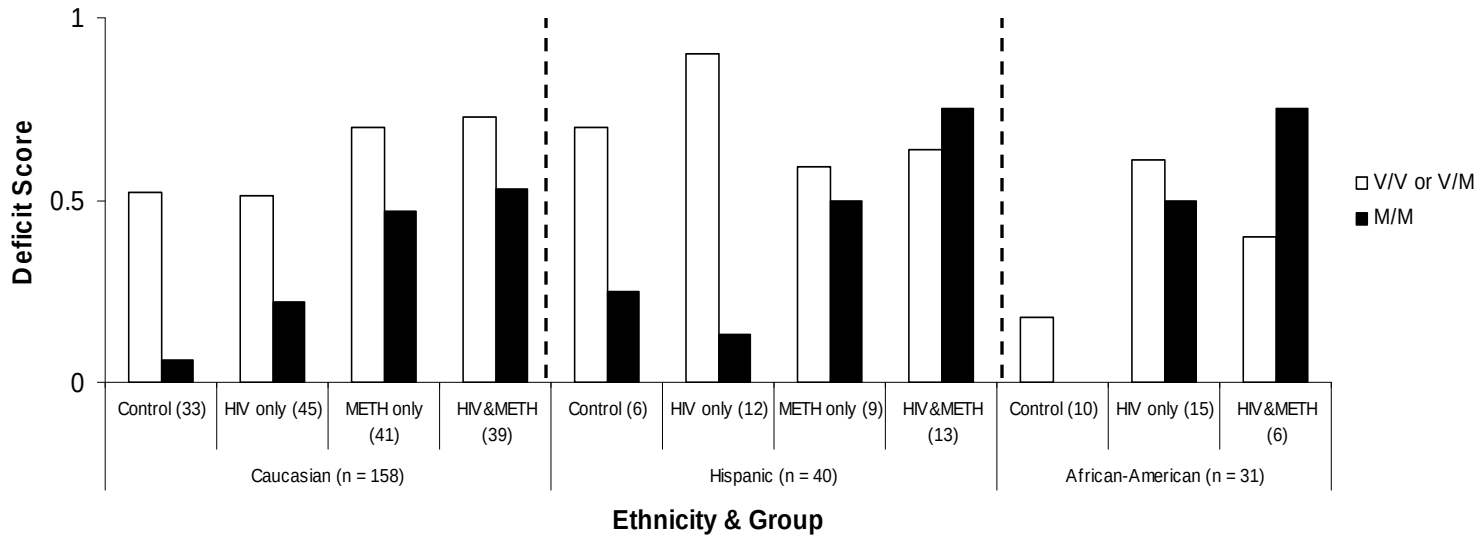

Figure 4.3 Executive Functioning Deficit by HIV/METH Group and COMT Genotype Stratified by Ethnicity. No statistically significant differences were found.

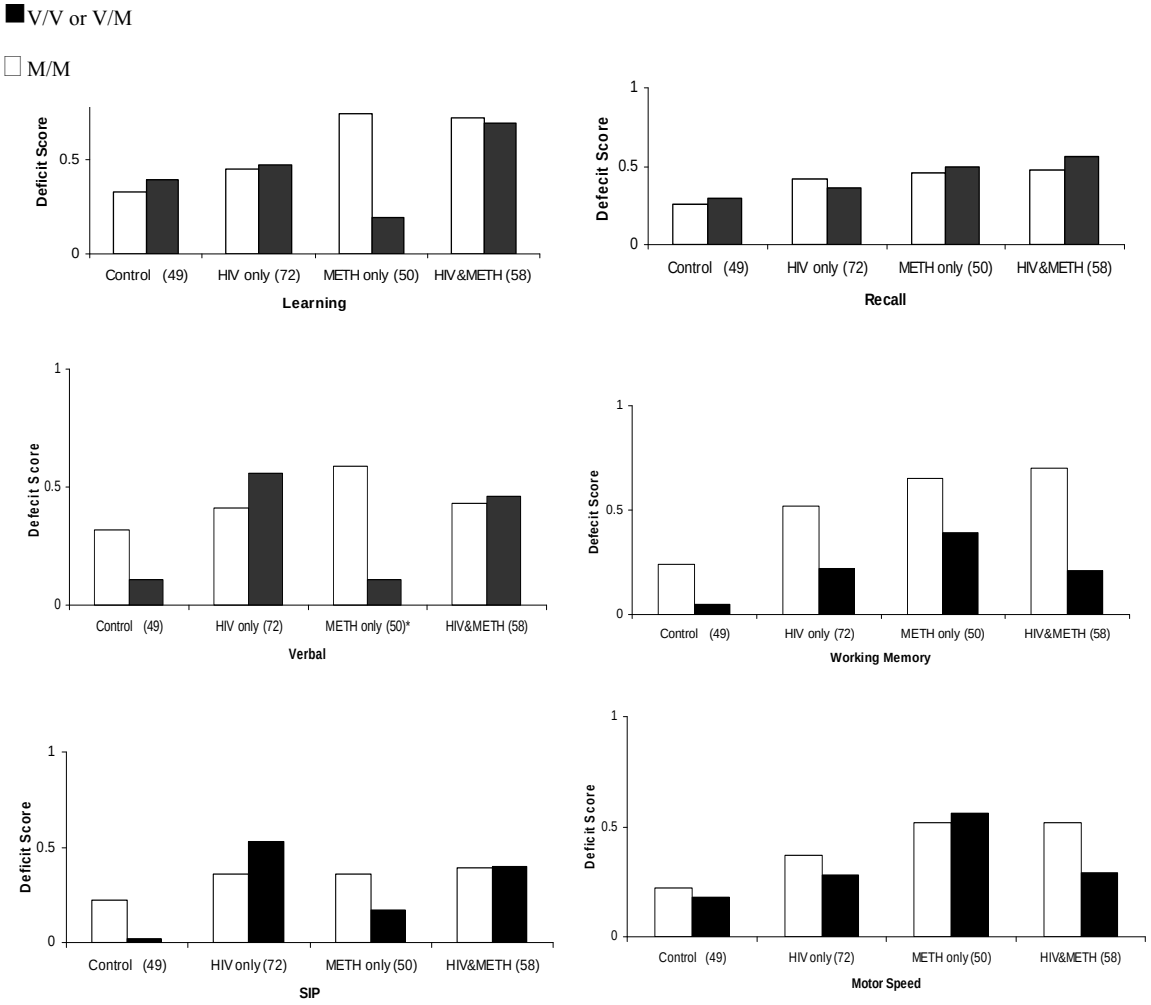

Figure 4.4 Deficit Scores for Learning, Recall, Verbal Fluency, Working Memory, Speed of Information Processing, and Motor Speed by HIV/METH Group and COMT Genotype. Note. Dark bars represent Met/Met genotype and light bars represent Val/Val and Val/Met genotype. * Statistically significant at $p < .05$. SIP = speed of information processing.

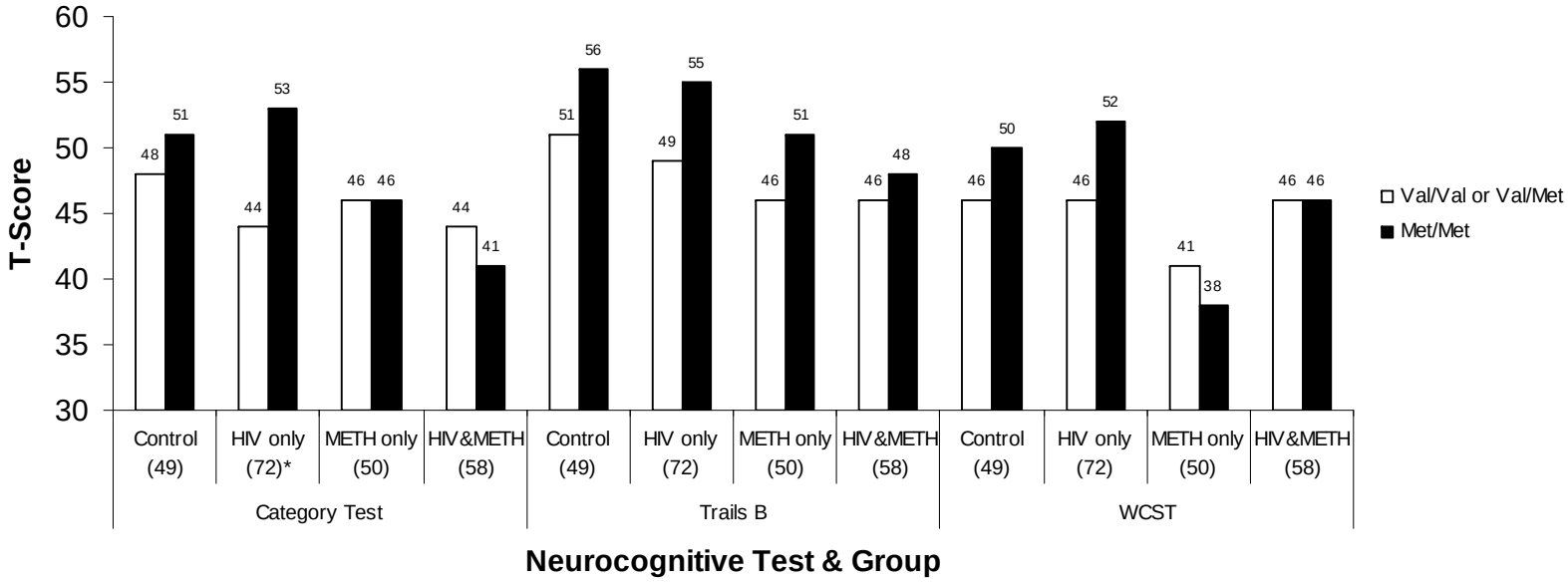

Figure 4.5 Average T-scores for Individual Executive Functioning Tests by HIV/METH Group and COMT Genotype. * Statistically significant at $p < .05$.

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CHAPTER 5

SEXUAL BEHAVIOR AND NEGATIVE MOOD IN THE CONTEXT OF HIV-INFECTION AND METHAMPHETAMINE DEPENDENCE

ABSTRACT

Research comparing the independent and combined contextual effects of methamphetamine dependence (METH) and HIV-infection (HIV) on mood and sexual behavior among men who have sex with men (MSM) has been sparse and inconsistent. This study examined the contextual influence of METH, HIV-infection and their combination on mood states and sexual behavior. 175 non-monogamous MSM concordant or discordant for METH and HIV were included. Multivariate analysis was conducted to examine mood and sexual behavior differences between groups, as well as to elucidate the relationship between mood and sexual risk behavior and explore the potential moderator (i.e. contextual) effects of METH and/or HIV on this relationship. METH+/HIV+ participants reported condom use less than 25% of the time whereas METH-/HIV+ participants reported condom use 51-75% of the time. METH+ and HIV+ status were associated with higher depression and confusion scores. Univariate regressions revealed negative relationships between mood states (depression, tension, anger, fatigue and confusion) and condom use. Neither METH nor HIV status moderated the relationships between negative mood and condom use. Results are derived from cross-sectional data, sample sizes for each of the four groups were relatively small, and condom use could not be linked to specific sexual practices and/or partner types. METH dependence, HIV seropositivity, and negative moods are associated with reduced condom use among non-monogamous MSM. Independent effects of METH dependence and negative mood on condom use suggest that sexual risk reduction interventions for MSM should incorporate multi-faceted approaches, including substance abuse and mental health treatment.

INTRODUCTION

Despite numerous studies investigating the link between negative mood and sexual behavior among men who have sex with men (MSM) within the context of methamphetamine (METH) and human immunodeficiency virus-infection (HIV) (Bancroft et al., 2003; Bancroft, Janssen, Strong, & Vukadinovic, 2003; S. J. Semple, Zians, Grant, & Patterson, 2005b; S. J. Semple, Zians, Grant, & Patterson, 2006a; S. J. Semple, Patterson, & Grant, 2005; Shoptaw, Peck, Reback, & Rotheram-Fuller, 2003), little research compares the independent and combined contextual (i.e. moderating) effects of METH and HIV on negative mood and sexual behavior among MSM. This is unfortunate, since understanding mood states and sexual practices of MSM within these independent and combined contexts, and estimating the effect of these contexts, have important implications for HIV prevention and public health.

It has been well established that MSM who use meth engage in sexual practices at an increased rate, duration, and risk compared to when meth is not used (Halkitis, Fischgrund, & Parsons, 2005; Halkitis, Green, & Mourgues, 2005; S. J. Semple, Zians, Grant, & Patterson, 2005a; Shoptaw, 2006). Likewise, it has been reported that a substantial proportion of HIV-positive individuals continue to engage in sexual risk behavior for at least a year after diagnosis (Gorbach, Drumright, Daar, & Little, 2006; Kalichman, Kelly, & Rompa, 1997) and that the frequency of sexual risk behavior among those HIV-positive is greater than HIV-negative MSM (Halkitis, Shrem, & Martin, 2005).

Negative mood states, particularly depression have also been demonstrated to be highly prevalent among users of methamphetamine (Peck, Reback, Yang, Rotheram-Fuller, & Shoptaw, 2005) and HIV- infected individuals (Dew et al., 1997). However, research examining the link between negative mood and sexual behavior has revealed inconsistent findings. A meta-analysis by Crepaz and Marks (Crepaz & Marks, 2001)(2001) reported a "null relationship" after review of 25 studies in which the relationship between negative mood and sexual risk behavior was assessed. This finding may be a result of differential effects of negative mood on sexual behavior in which, for some, negative mood will reduce, and for others will increase sexual risk behavior (Bancroft, Janssen, Strong, & Vukadinovic, 2003). Furthermore, it is believed that among MSM the link between mood and sexual behavior is more complex than it is for heterosexual men (Bancroft et al., 2003). Contributing to this complexity are increases in the rates of HIV-infection (Center for Disease Control, 2003, Center for Disease Control, 2005) and METH use (Center for Disease Control, 2007) among MSM. Thus, it is apparent that when examining the relationship between negative mood and sexual risk behavior it is imperative to also examine the contextual effects of METH and HIV status on this relationship. Contextual effects can be viewed as a third variable and are often denoted as a moderating variable (i.e., METH, HIV). Moderating variables can strengthen or weaken the effect observed between two factors (i.e. negative mood, sexual behavior). Examination of potential moderators is important in that, if identified, they suggest the possibility that different causal mechanisms are in operation in distinct subpopulations (Kraemer, Stice, Kazdin, Offord, & Kupfer, 2001).

Unfortunately, research to date has primarily examined the METH/HIV context relevant to mood and sexual behavior without inclusion of comparison or control groups. In addition, a majority of the research has focused on comorbid METH and HIV but has not explored the moderating effect of METH or HIV. Thus, the purpose of this study was to address these limitations in the current literature by examining both mood states and sexual behavior among non-monogamous MSM concordant and discordant for HIV-infection and meth dependence and exploring the moderating effects of meth dependence and HIV-status on the relationship between mood and sexual risk behavior. We hypothesized that participants in the METH+/HIV+ group would report greater frequency of negative mood states and sexual risk behavior (i.e. lower condom use) compared to comparison participants (i.e. METH-/HIV-). We also hypothesized that a significant negative association between negative mood and sexual risk behavior would be detected, and that this association would be moderated by METH and/or HIV status.

METHODS

Participants

Participants were volunteers evaluated at the HIV Neurobehavioral Research Center (HNRC) at the University of California in San Diego as part of a cohort study focused on central nervous system effects of HIV and methamphetamine. The current study comprised 175 sexually active non-monogamous men who have sex with men (MSM). In this study, men were classified as non-monogamous if they stated they had "no current partner" at time of assessment. Monogamous MSM were excluded because unsafe sexual behavior within a monogamous relationship can be considered less risky than in non-monogamous relationships (McKusick, Coates, Morin, Pollack, & Hoff,

1990). Participants were further classified into one of the following four groups: Methamphetamine dependent/HIV seropositive (METH+/HIV+; *n*=71); Methamphetamine dependent/HIV seronegative (METH+/ HIV-; *n*=20); Methamphetamine non-users/ HIV seropositive (METH-/HIV+; *n*=64); and Methamphetamine non-users/ HIV seronegative (HIV-/METH-; *n* = 20).

HIV serological status was determined by enzyme linked immunosorbent assays (ELISA) plus a confirmatory test. METH+ participants met dependence criteria in their lifetime and abuse criteria within the previous 18 months, as determined by the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders Version IV (SCID). However, participants were not actively using other substances, with the exception of cannabis and alcohol. Potential participants were excluded if they met lifetime dependence criteria for other drugs, unless the dependence was judged to be remote (greater than 5 years ago) and episodic in nature by a doctoral level clinician. Alcohol dependence within the last year was also an exclusion criterion. Participants with a history of methamphetamine dependence were primarily recruited from residential drug treatment programs in the San Diego area, while those participants without a history of methamphetamine abuse were recruited from the larger San Diego community through the use of flyers and appearances at community events. All participants gave written consent prior to enrollment and all procedures were approved by the Human Research Protection Program of the University of California, San Diego and San Diego State University.

Background Characteristics

Data were collected on the participants' age, ethnicity, education and partner preference. Age and education were coded in years. Ethnicity was coded as 0 (*ethnic minority*) or 1 (*Caucasian*) and partner preference was coded as 0 (*males only*) or 1 (*both males and females*). In addition, lifetime occurrence of mood (i.e. Major Depression, Bipolar) and substance abuse (i.e. cannabis, alcohol, cocaine, etc.) disorders was ascertained utilizing the SCID-IV. Further information was gathered regarding age at first use and years of cumulative use of methamphetamine, as well as HIV RNA plasma copies among HIV seropositive groups.

Sexual Behavior Questionnaire

Sexual behavior was assessed through an HNRC-developed self-report measure covering the preceding year. Information was gathered with regard to age at first intercourse, number of different sex partners and number of injection drug user (IDU) sex partners. Age at time of first intercourse was coded in years for both male and female partners. However, when two different ages were given for first intercourse, the younger of the two ages was used. In addition, participants were asked to indicate the percentage of time that they used a condom as well as engaged in mutual masturbation, oral, vaginal, anal (receptive & insertive) and/or intoxicated sex. Responses were recorded on a 6-item, Likert-type scale with a value of 0 = 0%, 1 = 1-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100%.

Mood Questionnaires

Current mood was assessed utilizing the Beck Depression Inventory-I (BDI-I) (Beck, 1972) and the Profile of Moods States (POMS) (McNair PM, Lorr M, &

Drappelman L, 1971) questionnaires. The BDI-I is a twenty-one question multiple choice self-report inventory asking participants how they have felt on average in the last week. It is composed of items relating to depression symptoms such as hopelessness and irritability, cognitions such as guilt or feelings of being punished, as well as physical symptoms such as fatigue, weight loss, and lack of interest in sex. Scores for the BDI-I range from 0-63 with greater scores indicative of more severe depression.

The POMS is a self-report questionnaire measuring mood states over the past 7 days. The measure consists of 65 adjectives (such as 'hopeless', 'annoyed', 'sluggish') or short phrases ('sorry for things done', 'ready to fight'), which the participant rates on a five-point Likert-type scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, 4 = extremely). Utilizing scoring guidelines (McNair PM et al., 1971), 6 subscales were calculated that included depression-dejection, anger-hostility, tension-anxiety, fatigue-inertia, vigor-activity and confusion-bewilderment. Each subscale was interpreted utilizing each participant's raw score. Raw scores for depression-dejection, anger-hostility, tension-anxiety, fatigue-inertia, vigor-activity and confusion-bewilderment subscales ranged from 0-60, 0-48, 0-36, 0-28, 0-32 and 0-28, respectively. A Total Mood Disturbance (TMD) score was calculated by adding the raw scores from depression-dejection, anger-hostility, tension-anxiety, fatigue-inertia and confusion-bewilderment and then subtracting the vigor-activity score, which resulted in a value between -32 and 200, with higher scores indicative of people with less stable mood profiles.

Statistical Analysis

All statistical tests and procedures were conducted using SPSS 10.0. Analysis of variance (ANOVA) was conducted to determine mean differences in mood states and sexual behavior between participants concordant and discordant for METH and HIV. In addition, pairwise comparisons utilizing a Tukey adjustment for multiple tests were conducted to examine *post hoc* differences between specific groups. Effect sizes were also calculated utilizing the Hedges' $\hat{g}$ bias-corrected method (Hedges & Olkin, 1985) to examine potential clinically significant differences between each group and controls while taking into account differences in sample sizes. Finally, to assess the contextual effects of METH and HIV on the association between negative mood and condom use, a moderator analysis using a hierarchical multiple linear regression was run for METH and HIV status according to Barron and Kenny's approach (Baron & Kenny, 1986) for establishing moderation. Prior to running each analysis, all predictors (i.e., mood scales) were centered and the moderator variables (METH or HIV) contrast coded to reduce problems resulting from multicollinearity (Kraemer & Blasey, 2004). In addition, interaction terms were created by multiplying METH or HIV status by the centered mood scales. The centered scale and METH or HIV status as well as the new interaction term were entered as independent variables into a hierarchical multiple regression equation (Figure 5.1). Moderation was considered present if path *c* was found to be statistically significant ($p < .05$).

RESULTS

Participant Characteristics

Participant characteristics are summarized in Table 5.1. All four groups were similar in regard to age, ethnicity, education and partner preference. Groups also had similar frequencies of lifetime Major Depression (MDD) and Bipolar (both I and II) Disorder. Methamphetamine dependent groups (METH+) were significantly more likely to have had lifetime cannabis or opioid abuse diagnosis, as well as a lifetime cannabis dependence and remote episodic cocaine dependence. Among METH+ participants, those seronegative (HIV-) reported significantly more cumulative years of methamphetamine use than seropositive (HIV+) participants.

Sexual Behavior

Sexual behavior data for the four participant groups are listed in Table 5.2. Analysis for condom use [$F(3,171) = 4.02$; $p<.01$], intoxicated sex [$F(3,171) = 43.84$; $p<.005$] and number of IDU partners [$F(3,171) = 6.38$; $p<.005$] showed significant differences between groups. *Post-hoc* Tukey tests indicated that METH+/HIV+ participants reported greater engagement in intoxicated sex and reported a greater number of IDU partners compared to both METH-/HIV+ and METH-/HIV- participants. Among HIV+ participants, METH+ status was significantly associated with decreased condom use (95% CI 2.3 ± 0.5 vs. 3.5 ± 0.4; $p < .005$). However, this was not the case among HIV- participants (95% CI 2.7 ± 1.0 vs. 2.6 ± 1.0; $p = .737$). (Figure 5.2).

Table 5.2 also provides effect size estimates utilizing the Hedges ($\hat{g}$) bias-corrected method (with the METH-/HIV- group as the reference group). The METH+/HIV+ ($\hat{g}$ =.71) and METH+/HIV- ($\hat{g}$ =.80) groups reported younger sexual

débuts compared to the METH-/HIV- group. Furthermore, receptive anal sex was reported more frequently by the METH+/HIV+ ($\hat{g}$ =.63), METH+/HIV- ($\hat{g}$ =.55) and METH-/HIV+ ($\hat{g}$ =.51) groups compared to METH-/HIV-. In addition, the METH+/HIV+ group reported greater total number of sexual partners ($\hat{g}$ =.42) compared to the METH-/HIV- group.

Mood

A significant difference between groups was found for BDI depressed mood [$F(3,171) = 4.51$; $p<.005$] as well as the POMS confusion-bewilderment [$F(3,171) = 3.12$; $p<.05$] (Table 5.3). *Post hoc* Tukey tests indicated that the METH+/HIV+ group reported significantly higher depression and confusion-bewilderment scores than those in the METH-/HIV- group. Group differences related to other mood states measured by the POMS did not reach statistical significance. However, examination of the effect size estimates indicated moderate differences between the METH+/HIV+ group ($\hat{g}$ =.62) and the METH-/HIV- group with regard to reported tension-anxiety. In addition, METH+/HIV+ ($\hat{g}$ =.55) and METH-/HIV+ ($\hat{g}$ =.52) groups reported greater fatigue-inertia than the METH-/HIV- group. Furthermore, METH+/HIV+ ($\hat{g}$ =.63) and METH+/HIV- ($\hat{g}$ =.56) groups had greater TMD scores than the METH-/HIV- group.

Negative Mood and Condom Use

Table 5.4 provides results of the univariate regression analysis between all mood scales and condom use as well as a moderator analysis for all mood scales with METH or HIV as the potential moderator. Significant unadjusted relationships were found between all mood scales and condom use. When adjusting each model for METH or HIV-status, significant independent main effects for mood on condom use were found for tension-

anxiety (METH: t = -2.67, df = 172, p = .008; HIV: t = -3.15, df = 172, p = .002), vigor-activity (METH:t = 2.80, df = 172, p = .006; HIV: t = 3.03, df = 172, p = .003), fatigue-inertia (METH: t = -2.38, df = 172, p = .019; HIV: t = -2.62, df = 172, p = .010) and TMD (METH: t = -2.56, df = 172, p = .011; HIV: t = -3.01, df = 172, p = .003), whereas BDI depression (t = -2.22, df = 172, p = .028) and confusion-bewilderment (t = -2.30, df = 172, p = .023) main effects remained significant only in the context of HIV. In addition, METH status but not HIV status had significant main effects on condom use regardless of which mood scale was in the model. However, interaction effects between METH and mood or HIV and mood were not observed for condom use; thus, neither METH nor HIV was found to moderate the relationship between negative mood and condom use (Table 5.4; Step 2*, c*).

DISCUSSION

We found that the independent and combined contexts of METH and HIV play an influential role in negative mood states as well as sexual behavior patterns among non-monogamous MSM. We also found a significant negative association between negative mood states and condom use. However, neither the METH nor the HIV context was found to have a moderating effect on the association between negative mood and condom use.

More specifically, in terms of sexual behavior, participants in the METH+/HIV+ group reported that 6-25% of their sexual encounters included receptive anal and/or insertive anal sex and 51-75% of encounters included oral sex. Compared to recent reports of METH+/HIV+ MSM sexual practices (Halkitis, Shrem et al., 2005; S. J. Semple, Zians, Grant, & Patterson, 2006b), these rates of sexual behavior are not

uncharacteristically high. However, upon examination of condom use frequencies among METH+/HIV+ participants, it is clear that these rates of sexual behavior could be of substantial concern in relation to the spread of HIV and other sexual transmitted infections.

Approximately 75% or more of sex among METH+/HIV+ participants was unprotected. Interestingly, the METH-/HIV+ group reported significantly greater use of condoms. Thus, it appears that among those in HIV+ groups, METH use is a critical factor in the frequency of condom use: among METH+ individuals, frequency of condom use is 6-25% and among METH- individuals it is at 51-75%. However, recent work (S. J. Semple, Zians, Grant, & Patterson, 2006b) found that although unprotected sex among METH+/HIV+ individuals was widespread, fewer unprotected sex acts were performed with HIV- and unknown partners compared to HIV+ partners. This said, the current study examined non-monogamous MSM only, and thus, although we did not capture this information specifically, the potential for sex with HIV- and unknown partners may be greater. However, even if all of the HIV+ participants in this study had sex with seroconcordant partners, this still may contribute to an increased risk of reinfection or superinfection with HIV variants as well as transmission of other sexually transmitted infections (STIs). Thus, interventions to address condom use and potentially other protective behaviors among HIV-infected MSM METH users are warranted.

The METH+/HIV+ group not only had a greater likelihood of unprotected sex but also reported more than twice the number of partners in the previous year than the other groups. Previous studies (Halkitis, Shrem et al., 2005; S. J. Semple, Zians, Grant, & Patterson, 2006b) have attributed greater number of partners to METH use, which is

known to increase sexual arousal and thus sexual partner seeking. However, in this study, although participants in both METH+ groups reported much higher rates of sex while intoxicated than did the METH- groups, only the METH+/HIV+ group reported a significantly greater number of partners than the METH- groups. In fact, the METH-/HIV+ group reported a higher, albeit not significant, number of partners than the METH+/HIV- group.

In addition to unprotected sex and number of partners, injection drug use and sexual encounters with IDUs can increase risk for reinfection and transmission of HIV and other STIs. In this study, the METH+/HIV+ group, and to a lesser extent the METH+/HIV- group, reported greater number of IDU partners in the past year than the METH- groups. Although this finding is not surprising given the likely close proximity of IDU behavior to METH use behavior, it supports a further need for prevention efforts among IDUs and their partners.

This study also examined other sexual behaviors such as mutual masturbation, vaginal sex as well as sexual debut. Due to the nature of the study population, vaginal sex was rarely reported across all groups. Conversely, mutual masturbation was reported relatively frequently among all groups. However, effect size estimations for age at first sexual intercourse revealed moderate differences between the METH+ groups and the control group. METH+ groups reported sexual debuts on average 2-3 years earlier than METH- groups. Although these findings did not meet statistical significance, a *post hoc* correlation analysis between METH use onset and sexual debut did reveal a significant positive association (*rho* = .22; p =.035), suggesting a potential value of both drug abuse and HIV prevention interventions in at-risk adolescents.

In terms of depressed mood, many studies have reported higher rates among METH+ (Peck et al., 2005; S. J. Semple, Patterson, & Rant, 2005) and HIV+ (Dew et al., 1997; Evans et al., 1999) individuals. In this study, levels of depression based on established criteria for the BDI ((American Psychiatric Association. Task Force for the Handbook of Psychiatric Measures & Rush, 2000), across all groups fell within the range of mild symptomatology, except among the METH-/HIV- group, which was classified as minimal. Yet, we observed that participants in the METH+/HIV+ group reported depression scores that were greater than those in either of the single-risk groups. The METH+ only and HIV+ only groups had similarly elevated depression scores, suggesting an additive effect of the combined risk factors on mood disturbance.

In addition to depression, we also found that the METH+/HIV+ group reported significantly more confusion-bewilderment than the control group. Confusion-bewilderment may be indicative of cognitive difficulties as a result of METH dependence and/or the known central nervous system consequences of HIV-infection. This is supported by recent work (Rippeth et al., 2004) with a similar sample of MSM concordant and discordant for METH and HIV that identified a monotonic relationship between number of risk factors and cognitive impairment as determined by detailed neuropsychological assessment.

Of the remaining individual mood scales examined in this study it is worth noting that clinically useful (determined by effect size, $\hat{g}$) findings, although not statistically significant, were observed for tension-anxiety, anger-hostility, fatigue-inertia and the TMD composite scales. Among METH+ groups, tension-anxiety and anger-hostility were

greater compared to METH- groups. Although elevated tension and anger are characteristic of withdrawal symptoms, *post hoc* correlation analysis between days abstinent from METH and both tension-anxiety and anger-hostility scales revealed null associations (tension-anxiety: *rho* = -.01; p =.961; anger-hostility: *rho* = -.06; p =.595), suggesting that such elevations may reflect longstanding mood disturbance in the METH+ group. Among METH+/HIV+, METH+/HIV- and METH-/HIV+ groups, effect sizes for fatigue-inertia were of moderate magnitude ($\hat{g}$ = .45 - .55) when compared to controls. Thus, reports of fatigue appear to be similar in METH+ only and HIV+ only groups and increased within the combined context of METH and HIV.

Finally, we examined overall mood disturbance using the POMS TMD composite scale. Individuals in METH+ groups tended to report greater TMD scores compared to METH- groups. However, when applying a cut-off of TMD > 42, which has been indicative of significant mood disturbance (Lorr, Sonn, & Katz, 1967), we found similar frequencies of mood disturbances among METH+/HIV+ (42%), METH+/HIV- (45%) and METH-/HIV+ (39%) groups. Although not empirically significant, effect sizes reveal that all three groups had moderately elevated mood disturbance ($\hat{g}$ = .39 - .63) compared to the 20% rate in the control group. Thus, participant mood is an important factor within the context of METH and HIV and future work should include measurements of mood among these unique groups to better inform intervention development.

A relationship between mood and sexual risk behavior, although inconsistent in the literature, was found in this study between all measured mood scales and condom use. After adjusting for METH or HIV-status, significant main effects of tension-activity,

vigor-activity, fatigue-inertia and TMD were found for condom use within the context of both METH and HIV, whereas main effects of depression and confusion-bewilderment were only significant within the context of HIV. This supports the notion that a relationship does exist between mood and sexual risk behavior and that this relationship is potentially context dependent. However, results from the moderator analysis do not suggest a moderating effect of either METH or HIV on the relationship between mood and condom use. This finding is perhaps related to our relatively small sample and homogeneity on the mood scales in which detection of a moderating effect is weakened as a result of not having a full range of values for the independent variables (i.e. mood scales) (Aguinis, 2004; Bennett, 2000). Thus, larger and more heterogeneous samples are required to address the moderating effects of these contexts further.

There are several limitations that must be considered. First, the study is cross-sectional and thus temporal order of the relationships examined cannot be established. For example, it is possible that a subset of the METH using population who has a propensity for risk behaviors through some mechanism not measured in this study is the subset that ends up contracting HIV, and therefore their risky sex profiles obtained in this study reflect longstanding characteristics. Certainly, METH and HIV status were determined prior to the current mood assessment; thus, the temporal order of the variables is not completely unknown. Nevertheless, mood that was assessed, although prefaced in the "past 7 days", may actually represent a longstanding mood state pre-dating the participants' current METH and/or HIV status. Second, sample size for each of the four groups was relatively small and therefore the study may lack sufficient power to detect effects that otherwise are present, thus having a greater probability of Type II errors. In

addition, the measure utilized to capture sexual behavior asked respondents to select an answer within a range of frequencies and thus the estimates of the frequencies of sexual behavior are imprecise and introduce statistical "noise." Finally, we were unable to link condom use to specific sexual practices and/or to specific partner types. Thus, it is unknown to what extent unprotected sex within this study occurred within a specific sexual practice and with whom this sexual practice was performed. Therefore, these results are preliminary and require replication in prospective investigations.

In summary, the present findings suggest that mood and sexual behavior of non-monogamous MSM differ depending on the context in which they are examined. As hypothesized, participants in the METH+/HIV+ group reported significantly greater negative mood and sexual risk behavior when compared to controls. Further, this study suggests a complex relationship between negative mood and condom use in the context of HIV and METH. Although a consistent relationship between negative mood and condom use was found, of potentially greater importance is that METH and to a lesser extent HIV-status, potentially modifies these negative mood effects on condom use. Thus, our data support sexual risk reduction interventions among non-monogamous MSM that incorporate multi-faceted approaches, including both substance abuse and mental health treatment.

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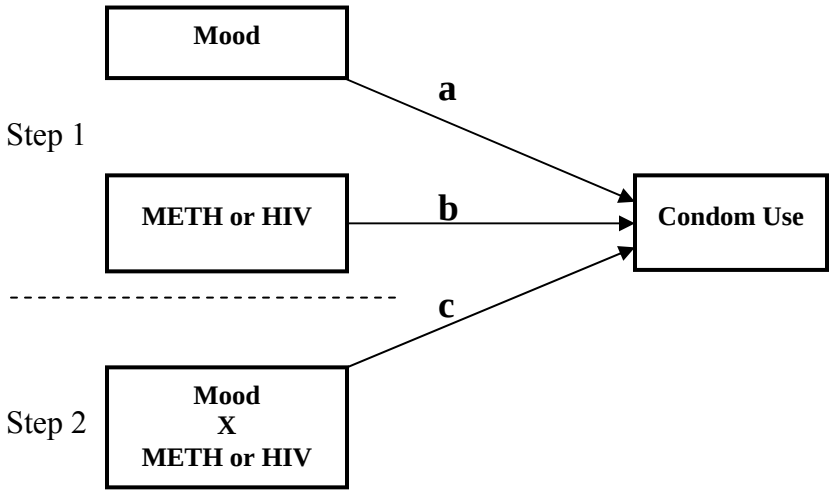

Figure 5.1 Moderator model. *Note*: *a* represents the impact of mood as a predictor; *b* represents the impact of METH or HIV as a moderator; and *c* represents the impact of the interaction of mood and METH or HIV on condom use.

Table 5.1 Participant characteristics

	METH+		METH-		
	HIV+ (n=71)	HIV- (n=20)	HIV+ (n=64)	HIV- (n=20)	
Characteristic	1	2	3	4	
All Groups					
Age (years) *M* (sd)	37 (7)	40 (8)	40 (8)	40 (13)	
Ethnicity (% ethnic minority)	30	30	33	25	
Education (years) *M* (sd)	13 (3)	14 (2)	14 (2)	13 (2)	
Partner preference (% males only)	90	80	97	95	
MDD (% lifetime)	52	26	45	30	
Bipolar (% lifetime)	9	5	3	5	
Sedative (% lifetime)					
Abuse	10	5	0	5	
Dependence	0	0	0	0	
Cannabis (% lifetime)					
Abuse	34	33	8	5	1,2 > 3,4**
Dependence	17	16	3	0	1,2 > 3,4*
Stimulant (% lifetime)					
Abuse	-	-	-	-	
Dependence	100	100	0	0	1,2 > 3,4**
Opioid (% lifetime)					
Abuse	10	5	0	0	1,2 > 3,4*
Dependence	0	0	0	5	
Cocaine (% lifetime)					
Abuse	19	11	5	5	
Dependence	17	5	0	0	1,2 > 3,4**
Hallucinogen (% lifetime)					
Abuse	14	5	3	5	
Dependence	1	0	0	0	
Alcohol (% lifetime)					
Abuse	44	61	31	6	
Dependence	30	42	3	5	
METH+ Groups					
Age first METH use (mean yrs) *M* (sd)	26 (7)	24 (10)	-	-	
Total METH use (mean yrs) *M* (sd)	5 (5)	11 (6)	-	-	2 > 1**
Last use of METH (mean days) *M* (sd)	93 (121)	81 (83)	-	-	
HIV+ Groups					
HIV RNA, plasma (log copies/ml) *M* (sd)	3.6 (1.1)	-	3.6 (1.1)	-	

* = $p < .05$; ** = $p < .005$

Table 5.2 Sexual behavior differences among MSM concordant & discordant for Methamphetamine and HIV[a]

	METH+						METH-					
	HIV+ (n=71) 1			HIV- (n=20) 2			HIV+ (n=64) 3			HIV- (Control) (n=20) 4		
N=175	Median	M (sd)	$\hat{g}$[b]	Median	M (sd)	$\hat{g}$[b]	Median	M (sd)	$\hat{g}$[b]	Median	M (sd)	*post hoc*[c]
Sexual Behaviors												
Condom use*	2.0	2.3 (1.8)	0.16	4.0	2.7 (2.3)	0.04	4.0	3.5 (1.8)	0.64	3.0	2.6 (2.3)	1 < 3
Intoxicated sex**	4.0	3.4 (1.6)	1.75	4.0	3.2 (2.1)	1.37	0.0	0.8 (1.3)	0.08	0.0	0.7 (1.2)	3,4 < 1
Oral sex	5.0	4.1 (1.2)	0.33	5.0	4.0 (1.8)	0.19	4.0	3.6 (1.3)	0.08	4.0	3.7 (1.2)	
Receptive anal	2.0	2.6 (1.8)	0.63	3.5	2.7 (2.3)	0.55	2.0	2.3 (1.6)	0.51	0.5	1.5 (1.8)	
Insertive anal	2.0	2.3 (1.6)	0.24	1.5	2.2 (1.9)	0.15	2.0	2.5 (1.8)	0.35	1.0	1.9 (2.0)	
Mutual masturbation	4.0	3.8 (1.8)	0.06	5.0	4.7 (1.7)	0.47	4.5	4.1 (1.8)	0.12	4.0	3.9 (1.5)	
Vaginal sex	0.0	0.4 (1.2)	0.23	0.0	0.6 (1.4)	0.07	0.0	0.1 (0.6)	0.61	0.0	0.7 (1.7)	
Other Sexual Variables												
Total partners	10.0	21.6 (34.9)	0.42	4.0	9.0 (15.4)	0.07	3.0	11.0 (23.2)	0.14	2.0	7.9 (16.8)	
Total IDU partners**	1.0	2.8 (4.9)	0.62	2.0	2.8 (4.5)	0.81	1.0	0.4 (1.7)	0.23	1.0	0.05 (0.24)	3,4 < 1
Age at 1st intercourse	15.0	15.1 (4.4)	0.71	15.0	14.5 (4.0)	0.80	16.0	15.9 (5.3)	0.41	17.5	18.0 (4.2)	

* = $p < .05$; ** = $p < .005$

a = Medians & means are of frequencies of the behavior in the current year; 0=0%, 1=1-5%, 2=6-25%, 3=26-50%, 4=51-75%, 5=76-100%

b = Hedges' $\hat{g} = (mean_1 - mean_2)/SD_{pooled} \times (1-[3/4(n_1+n_2)-9)]$

c = Multiple pairwise comparisons using a Tukey adjustment

Table 5.3 Depression and other mood state differences among MSM concordant & discordant for Methamphetamine and HIV

	METH+						METH-					
	HIV+ (n=71) 1			HIV- (n=20) 2			HIV+ (n=64) 3			HIV- (Control) (n=20) 4		
N=175	Median	M (sd)	$\hat{g}^a$	Median	M (sd)	$\hat{g}^a$	Median	M (sd)	$\hat{g}^a$	Median	M (sd)	*post hoc*[b]
Depression Scales												
BDI**	15.0	15.1 (9.1)	0.98	12.0	13.0 (10.9)	0.69	11.0	12.9 (9.3)	0.71	7.0	6.7 (5.5)	4 < 1,3
Other Mood States												
Tension-Anxiety	11.0	12.5 (7.3)	0.62	10.0	11.5 (7.1)	0.47	8.0	10.3 (8.2)	0.27	5.5	8.2 (6.1)	
Vigor-Activity	15.0	14.8 (7.3)	0.47	16.0	15.8 (6.3)	0.40	15.0	15.4 (7.4)	0.41	18.5	18.3 (5.3)	
Anger-Hostility	7.0	9.7 (9.1)	0.37	6.0	9.3 (9.4)	0.34	5.0	7.7 (8.7)	0.14	3.0	6.4 (6.8)	
Fatigue-Inertia	8.0	10.1 (6.7)	0.55	9.0	9.7 (7.8)	0.45	8.0	9.9 (6.9)	0.52	5.0	6.4 (5.5)	
Confusion-Bewilderment*	10.0	9.7 (6.3)	0.77	7.0	8.8 (6.9)	0.60	10.0	8.3 (6.2)	0.57	3.0	4.9 (5.2)	4 < 1
Total mood disturbance	36.0	42.7 (41.5)	0.63	29.5	39.5 (45.9)	0.56	36.0	33.3 (43.0)	0.39	7.0	16.9 (34.2)	
TMD score > 42[c]		42%			45%			39%			20%	

* = $p < .05$; ** = $p < .005$

a = Hedges' $\hat{g} = (mean_1 - mean_2)/SD_{pooled} \times (1-[3/4(n_1+n_2)-9)]$

b = Multiple pairwise comparisons using a Tukey adjustment

c = Total mood disturbance score > 42 indicative of significant psychological stress

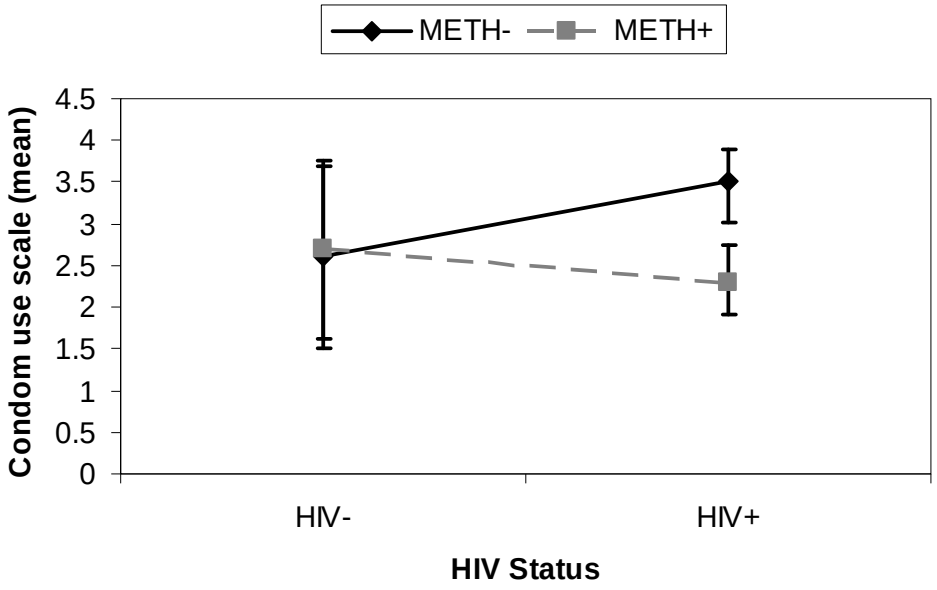

Figure 5.2 Condom use for METH+ and METH- participants in the context of HIV-infection. *Note:* METH groups differed for HIV+ ($p = .005$) but not HIV- ($p = .737$).

Table 5.4 METH and HIV as Moderators of the relationship between mood and condom use using hierarchical multiple linear regression, *N*=175

	Standardized regression coefficients[1]			
		Step 1 (Main Effects)		Step 2 (Interaction)
Predictor Moderator	Univariate Model	*a*	*b*	*c*
Depression	–0.16*			
METH		–0.12	–0.19*	–0.110
HIV		–0.17*	0.08	0.090
Tension-Anxiety	–0.23*			
METH		–0.19*	–0.18*	–0.040
HIV		–0.23*	0.07	0.040
Vigor-Activity	0.22*			
METH		0.20*	–0.20*	0.100
HIV		0.23*	0.07	–0.110
Anger-Hostility	–0.05*			
METH		–0.09	–0.20*	–0.002
HIV		–0.12	0.05	–0.150
Fatigue-Inertia	–0.19*			
METH		–0.18*	–0.20*	–0.010
HIV		–0.20*	0.07	0.020
Confusion-Bewilderment	–0.16*			
METH		–0.13	–0.19*	–0.090
HIV		–0.17*	0.07	–0.030
Total mood disturbance	–0.22*			
METH		–0.19*	–0.19*	–0.080
HIV		–0.22*	0.07	–0.020

[1] = regression coefficients based on a 0 - 5 condom use scale; 0=0%, 1=1-5%, 2=6-25%, 3=26-50%, 4=51-75%, 5=76-100%

a = impact of predictor (mood)

b = impact of moderator (METH or HIV)

c = impact of interaction (mood x METH or mood x HIV)

* = $p < .05$

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CHAPTER 6

COMT GENOTYPE, EXECUTIVE DYSFUNCTION, AND SEXUAL RISK BEHAVIOR IN THE CONTEXT OF HIV-INFECTION AND METHAMPHETAMINE DEPENDNECE

ABSTRACT

Catechol-O-methyltransferease (*COMT*) metabolizes prefrontal cortex dopamine (DA), a neurotransmitter involved in executive behavior; the Val158Met genotype has been linked to executive dysfunction, which might increase sexual risk behaviors favoring HIV transmission. We examined the main and interaction effects of *COMT* genotype and executive functioning on sexual risk behavior among participants with or without HIV infection and methamphetamine dependence; both, conditions linked to DA disturbance and risk behavior. 192 sexually active non-monogamous men received a self-administered sexual behavior questionnaire that asked about the percent time they used a condom, engaged in oral, vaginal, anal and/or intoxicated sex, as well as the number of different sexual partners in the past year. All subjects were hepatitis C negative. An executive deficit score was derived from the Wisconsin Card Sorting Test, Trail Making Test Part B, and Halstead Category Test. *COMT* Val158Met polymorphism was assayed from blood-derived DNA. Linear regressions revealed a significant main effect of executive dysfunction but not *COMT* on number of sexual partners. However, a significant *COMT* x executive dysfunction interaction was found for number of sexual partners and insertive anal sex. Regressions stratified by *COMT* genotype revealed that the relationship between executive dysfunction and number of sexual partners was statistically significant for carriers of the Met/Met ($p < .001$) and to a lesser extent Val/Met ($p < .048$) genotypes but not Val/Val carriers. *COMT* genotypic differences may moderate the influence of executive functioning on sexual risk-taking, supporting a role of DA metabolism in these behaviors. In the context of HIV and methamphetamine dependence, dopaminergic overactivity in prefrontal cortex conferred by the Met/Met

genotype appears to result in a liability for executive dysfunction and potentially associated risky sexual behavior.

INTRODUCTION

HIV infection is a global pandemic and the population is growing due to successful treatment with highly active antiretroviral therapy (HAART) (2006). Although rates of HIV have been reduced in the United States among most groups as a result of successful public health efforts (*e.g.* condom accessibility, education programs, media campaigns), sexual risk behavior and subsequent acquisition and/or spread of HIV and other sexually transmitted infections are still of concern among men who have sex with men as well as drug using populations (2006). Thus, it is evident that despite research and efforts to understand and curb sexual risk behavior within these vulnerable populations, additional work employing novel approaches are needed.

Sexual risk behaviors can be viewed as a composite of numerous behaviors that collectively make-up a complex behavioral phenotype. As with most complex phenotypes, sexual risk behavior is heterogeneous and several factors contribute to the variance that can be observed from one individual to another. To date, a majority of work examining risk factors for sexual risk behavior phenotypes have primarily focused on psychosocial factors (reviewed in (DiClemente et al., 2008) and/or other complex/heterogeneous behavioral phenotypes such as substance use behaviors (Fortenberry, 1995; Leigh & Stall, 1993) as indicators for current or future sexual risk behavior. Ultimately these indicators, upon sufficient replication, become candidates for public health interventions that aim to prevent and reduce sexual risk behaviors. However, the trouble with many of these candidates is that they are too proximal to sexual risk behaviors and often co-occur, making it difficult to disentangle temporal precedence and ultimately limit prevention efforts. One relatively novel approach is to

examine intermediate phenotypes or endophenotypes (Gottesman & Gould, 2003) such as neurocognitive factors as well as biological (*i.e.* genetic) factors that are more distal to the onset of sexual risk behavior and thus are potentially more advantageous candidates for identifying vulnerable individuals and informing prevention efforts for sexual risk behavior.

Studies in the literature examining neurocognitive and biological factors as indicators for sexual risk behaviors are limited. In fact, only two studies to date have examined neurocognitive factors (Gonzalez et al., 2005; Stacy, Newcomb, & Ames, 2000) and none to our knowledge have examined biological factors as potential indicators. Although, this paucity of research is surprising given previous work linking both neurocognitive (Barclay et al., 2007; Barker et al., 2007; Hall, Elias, & Crossley, 2006) and genetic (Rankinen & Bouchard, 2006; Tafti, Maret, & Dauvilliers, 2005; Triche, Hossain, & Paidas, 2008) indicators to other health related behaviors, research has established the dopminergic system as a common link between neurocognitive functioning and sexual behavior.

The dopminergic system has been shown to be involved in sexual arousal, motivation and the subsequent rewarding effect of sexual behavior (for detailed review see (Melis & Argiolas, 1995)). Furthermore, DA in the human brain, specifically in the prefrontal cortex (PFC), has been shown to be necessary for proper cognitive functioning to occur and high or low levels of DA in this brain region are known to contribute to individual cognitive differences in humans (Nieoullon, 2002; Starr, Fox, Harris, Deary, & Whalley, 2007). The PFC is of particular importance when examining risk behavior in that executive functions such as decision-making, planning, self-monitoring as well as

behavior initiation, organization, and inhibition are largely dependent on PFC integrity (Anderson & Tranel, 2002). Impairment in executive functioning may result in difficulties in assessing relationships between a person's current behavior and future outcomes; thereby resulting in choices and/or responses on the premise of immediate rewards versus long-term consequences and an ultimate potential increase in the likelihood for participation in sexual risk behaviors (Bechara, 2003; Gonzalez et al., 2005). Thus, mechanisms responsible for maintaining a dopamine balance within the brain and in particular the PFC would appear to be good biological candidates for further exploration of an association between executive dysfunction and sexual risk behavior.

On such candidate is catechol-O-methyltransferease (*COMT*) which is a mammalian enzyme involved in the metabolic degradation of released dopamine, particularly in the PFC (Lewis et al., 2001). Of particular interest to this study is a common polymorphism involving a Val to Met substitution at codon 158. The Val allele of the *COMT* Val158Met polymorphism is 40% more enzymatically active than the Met allele (Chen et al., 2004). Thus, carriers of the Met allele metabolize dopamine at a less efficient rate, resulting in higher levels of dopamine in the synapse and ultimately an escalation in dopamine receptor activation. This differentiation of dopamine receptor activity dependent on *COMT* genotype has led to several investigations into the relationship between *COMT* and executive dysfunction. In fact, a recent meta-analysis has provided evidence that the Met allele may enhance executive functioning among healthy participants (Barnett, Jones, Robbins, & Muller, 2007) and be more pronounced in males {Barnett, 2007 #1490}. However, to our knowledge no work has examined the relationship between *COMT* and sexual risk behavior; albeit studies of similar behaviors

such as novelty seeking (Hosak, Libiger, Cizek, Beranek, & Cermakova, 2006; Reuter & Hennig, 2005; Tsai, Hong, Yu, & Chen, 2004), reward dependence (Tsai et al., 2004), as well as affective arousal and regulation (Drabant et al., 2006) have demonstrated significant relationships.

Given the aforementioned paucity of research in the current literature addressing the contribution of genetic and neurocognitive factors on sexual risk behavior, the primary aim of this study was to examine the main effects of executive functioning as well as the main effects of the *COMT* Val158Met polymorphism on sexual risk behavior among a ethnically diverse population of men with and without METH dependence and/or HIV-infection. Within this aim, we hypothesized that the highly active *COMT* Val/Val genotype and associated deficits in executive functioning would be independently associated with sexual risk behaviors. In addition, as a result of previously mentioned research that has demonstrated an association between *COMT* genotype and executive functioning we also explored the potential interaction effects of *COMT* and executive functioning on sexual risk behavior.

METHODS

Participants

Participants were volunteers evaluated at the HIV Neurobehavioral Research Center (HNRC) at the University of California in San Diego as part of a cohort study focused on central nervous system effects of HIV and methamphetamine. The current study comprised 192 sexually active non-monogamous men with and without methamphetamine dependence (METH+/-) and/or HIV-infection (HIV+/-). Men were classified as non-monogamous if they stated they had “no current partner” at time of

assessment. Monogamous men were excluded because unsafe sexual behavior within a monogamous relationship is less risky than in non-monogamous relationships (McKusick, Coates, Morin, Pollack, & Hoff, 1990).

All participants underwent a comprehensive characterization procedure that included collection of demographic, neuromedical, psychiatric as well as neuropsychiatric information. HIV serological status was determined by enzyme linked immunosorbent assays (ELISA) plus a confirmatory test. Lifetime METH dependence was determined by the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders Version IV (SCID-IV). However, participants were not actively using other substances, with the exception of cannabis and alcohol. Potential participants were excluded if they met lifetime dependence criteria for other drugs, unless the dependence was judged to be remote (greater than 5 years ago) and episodic in nature by a doctoral level clinician. Alcohol dependence within the last year was also an exclusion criterion. All participants were seronegative for hepatitis C infection.

Additional information for each participant was collected as it relates to current depressed mood as well as lifetime diagnosis of Major Depression Disorder (MDD) and/or Bipolar Disorder I or II. Current depressed mood was assessed utilizing the Beck Depression Inventory-I (BDI-I) (Beck, 1972) and MDD and Bipolar Disorder were ascertained using the SCID-IV. Information was also collected to determine lifetime dependence on sedatives, cannabis, opioids, cocaine, hallucinogens, and alcohol, using the SCID-IV. For METH+ participants, additional information was collected regarding age at first use, years of use, and days since last use of METH; whereas for HIV+ participants, HIV RNA plasma copies was ascertained as part of a larger neuromedical

evaluation. All participants gave written consent prior to enrollment and all procedures were approved by the Human Research Protection Program of the University of California, San Diego and San Diego State University.

Executive Functioning

Executive functioning was determined as part of a larger comprehensive battery of tests covering seven ability domains (Learning, Memory, Attention/Working Memory, Verbal Fluency, Processing Speed, Abstraction/Problem Solving, and Motor Speed). The executive functioning domain deficit score, of particular focus in this study, was made up of (1) perseverative responses on the Wisconsin Card Sorting Test (R. K. Heaton & Staff, 1993); (2) errors on the Halstead Category Test (DeFilippis & Staff, 1993), which measures abstraction and cognitive flexibility; and (3) time to complete the Trail Making Test part B (Trails B) (Army Individual Test Battery, 1944), reflecting ability to switch and maintain attention between ongoing sequences. Raw scores for each of these component tests were converted to demographically-adjusted T-scores (M = 50, SD = 10), including adjustments for age, education, gender, and ethnicity as available for each test. The demographically-adjusted T-scores for each test were then converted into deficit scores, which reflect degree of impairment by setting performances within the normal range at zero with a range from 0 (T-score > 39; no impairment) to 5 (T-score < 20; severe impairment). Finally, the individual deficit scores were averaged to derive the domain deficit score, which reflects the severity of executive functioning deficit. Previous work has demonstrated that deficit scores achieve good diagnostic agreement with classifications made by blind clinical ratings (Carey et al., 2004; R. K. Heaton et al.,

1995). All neurocognitive testing and scoring was performed by trained psychometrists blinded to participants' genotypes.

Sexual Risk Behavior

Sexual risk behavior was assessed through an HNRC-developed self-report measure covering the preceding year. Information was gathered with regard to age at first intercourse as well as number of different sex partners. Age at time of first intercourse was coded in years for both male and female partners. However, when two different ages were given for first intercourse, the younger of the two ages was used. In addition, participants were asked to indicate the percentage of time that they used a condom as well as engaged in oral, vaginal, anal (receptive & insertive) and/or intoxicated sex. Responses were recorded on a 6-item, Likert-type scale with a value of 0 = 0%, 1 = 1-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100%.

DNA Extraction and Genotyping

DNA was extracted from peripheral blood mononuclear cells stored (three to five years) at –70ºC using the QIAamp DNA Mini kit (Qiagen, Valencia, CA; Catalog #51185). The *COMT* Val158Met polymorphism (rs4680) was assayed along with eight other SNPs as part of a concurrent genetic association project at the HNRC. A multiplex PCR technique designed using Sequenom SpectroDESIGNER software (version 3.0.0.3) was employed by inputting a sequence containing 100 bp of flanking sequence on either side of the *COMT* Val158Met polymorphism. The SNP was then grouped into multiplexes so that the extended product would not overlap in mass with any other oligonucleotide present in the reaction mix, and where no primer-primer, primer-product, or non-specific interactions would occur. The PCR was carried out in 384-well reaction

plates in a volume of 5 μl using 10 ng genomic or whole-genome amplified (WGA) DNA. All subsequent steps, up until the reaction, were spotted onto the SpectroCHIP and carried out in the same reaction plate. After PCR, any unincorporated dNTPs from the PCR were removed from the reaction by digestion with Shrimp alkaline phosphatase. dNTPs were removed so that they could not play any role in the extension of the oligonucleotide at the SNP site. The extension reaction was then carried out in the presence of the extension oligonucleotide and a termination mix containing mass-modified dideoxynucleotides which extended the oligonucleotide over the SNP site with one base. Before spotting onto the SpectroCHIP, the reaction was cleaned by incubation with a cation-exchange resin which removed any salts present. The extension product was then spotted onto a 384-well spectroCHIP before being flown in the MALDI-TOF mass spectrometer. Data were collected, in real time, using SpectroTYPER Analyzer 3.3.0.15, SpectraAQUIRE 3.3.1.1 and SpectroCALLER 3.3.0.14 (Sequenom) algorithms. All genotyping was performed by an accredited commercial laboratory (Harvard Medical School-Partners Healthcare Center for Genetics and Genomics, Cambridge, MA CLIA No. 22D1005307).

Statistical Analysis

All statistical tests and procedures were conducted using SPSS 10.0 (SPSS, 2000). Univariate comparisons across the three *COMT* genotypes (*i.e.* Val/Val, Val/Met, Met/Met) were performed using one-way analysis of variance (ANOVA) for continuous and chi-squared tests for categorical variables. In cases, where data violated normality assumptions medians were calculated and non-parametric tests (*i.e.* Kruskal-Wallis) performed. To examine the main and explore the interaction effects of executive

functioning and *COMT* on sexual risk behaviors, hierarchical multiple linear regressions in accord with Barron and Kenny's approach (Baron & Kenny, 1986) were conducted for each of the seven sexual risk behaviors (see Sexual Risk Behavior section) under study. Prior to running each analysis, the executive functioning variable was centered and the *COMT* genotype contrast coded to reduce problems resulting from multicollinearity (Kraemer and Blasey, 2004). In addition, interaction terms were created by multiplying *COMT* genotype by the centered executive functioning variable. Next, multiple linear regressions were used to examine potential confounders based on univariate genotype comparisons described above. These confounders included: ethnicity, METH status, HIV status and age at first intercourse. We also included BDI scores based on inclusion of this measure in recent work testing a similar hypothesis (Gonzalez, 2005). Results showed that METH status, HIV status, and age at first intercourse accounted for a significant unique variance for all sexual behaviors under investigation (R^2 range: 0.06 – 0.39, ps < 0.02). Thus to control for these potential confounding effects, the residuals derived from each of the sexual behavior models were used as the dependent variables for all subsequent regression models. The centered executive functioning variable and *COMT* genotype as well as the new interaction term were then entered as independent variables into seven individual hierarchical multiple regression models using the residuals described above as the dependent variable. For models in which a significant interaction was observed, a final round of regressions were conducted stratified by *COMT* genotype to determine the nature of the interaction between executive functioning and *COMT* on the particular sexual risk behavior. Due to the exploratory nature of the interaction

analysis we selected a relaxed *p*-value ($p < 0.10$) to reduce Type II errors, albeit the traditional *p*-value of 0.05 was used for all other analyses.

RESULTS

Participant Characteristics

Characteristics of the full sample by each of the three *COMT* genotypes are summarized in Table 6.1. All three genotype groups were comparable in age, education, sexual behavior, executive functioning, as well as psychiatric and substance dependence histories. However, Val/Val carriers were significantly more likely to identify as African-American ($\chi^2 = 17.67$, $p = 0.001$), report an earlier age of first intercourse ($F_{(2,189)} = 3.51$, $p = 0.032$), and be seropositive for HIV ($\chi^2 = 6.57$, $p = 0.038$). Whereas, Met-carriers (*i.e.* Met/Met or Val/Met) were significantly more likely to identify as Caucasian ($\chi^2 = 14.32$, $p = 0.001$). Additionally, among METH+ participants Val/Val carriers reported significantly greater total years of METH use ($F_{(2,87)} = 3.12$, $p = 0.050$) compared to their Met-carrying counterparts.

Main Effects of Executive Functioning and COMT

Table 6.2 provides standardized multiple linear regression coefficient estimates for main and interaction effects of executive functioning and *COMT* genotype for each of the seven sexual risk behaviors adjusting for METH status, HIV status, and age at first intercourse. A significant main effect for the executive functioning domain score was observed for number of partners ($\beta = 0.21$, $p = 0.005$). Additionally, results from the individual executive functioning tests showed a significant main effect for the Wisconsin Card Sort and Halstead Category tests in adjusted models of oral sex ($\beta = 0.20$, $p =$

0.009) and condom use (β = -0.16, p = 0.030), respectively. Main effects were not observed for *COMT* genotype in any of the regression models.

Interaction Effects of Executive Functioning and COMT

Applying an exploratory cut-off of $p < 0.10$, significant interactions between the executive functioning domain score and *COMT* were observed for number of sexual partners (β = 0.50, p = 0.038), insertive anal sex (β = 0.50, p = 0.046), and receptive anal sex (β = 0.50, p = 0.081) (Table 6.2). Subsequent stratified analysis by *COMT* genotype, revealed that among carriers of the Met/Met (β = 0.52, p = 0.001) and to a lesser extent Val/Met (β = 0.20, p = 0.048) genotype, the executive functioning domain score was significantly associated with greater number of sexual partners in the past 12 months. Stratified analysis for insertive and receptive anal sex revealed similar results in that among Met/Met and Val/Met carriers the domain score was associated with greater percentage of insertive (Met/Met: β = 0.18; Val/Met: β = 0.11) and receptive (Met/Met: β = 0.18; Val/Met: β = 0.11) anal sex in the past 12 months, albeit not statistically significant.

Results of regression analyses to examine interactions between each of the three individual executive functioning tests and *COMT* genotype are also shown in Table 6.2. For the Wisconsin Card Sort Test no interactions were observed. However, for Trails B, significant interactions with *COMT* were observed for insertive (β = -0.99, p = 0.015) and receptive (β = -0.75, p = 0.066) anal sex, as well as oral sex (β = -0.68, p = 0.096). Stratified regression analysis showed that among carriers of the Met/Met genotype, Trails B was significantly associated with greater percentage of insertive (β = -0.38, p = 0.028) but not receptive (β = -0.22, p = 0.225) anal sex. Interestingly, among carriers of the

Val/Val genotype, *T*-scores on Trails B had a significant positive association with oral sex (β = 0.35, p = 0.013). Finally, for the Halstead Category Test, a single interaction with *COMT* was observed for condom use (β = -1.13, p = 0.006). Among carriers of the Met/Met (β = -0.49, p = 0.004) and to a lesser extent Val/Met (β = -0.19, p = 0.064) genotype, *T*-scores were negatively associated with condom use.

DISCUSSION

To our knowledge this study is the first to examine main effects as well as explore the interaction effects of *COMT* genotype and executive functioning on sexual risk behavior. Our main findings suggest significant executive functioning main effects for number of sexual partners as well as frequency of oral sex and condom use. In addition, results of our exploratory interaction analyses provide evidence that *COMT* genotype and executive functioning interact in models of number of sexual partners, condom use, insertive and receptive anal sex, as well as oral sex. Stratified analyses further suggest that the strength of these associations are dependent on the number of Met alleles the individual was carrying, with the exception of oral sex in which Val/Val was the informative genotype.

Our significant executive functioning main effects for sexual risk behaviors are discordant with the only other study, to our knowledge, that has examined the association between executive functioning and sexual risk behavior (Gonzalez et al., 2005). In that study, no association was found between executive functioning and sexual risk behavior among an African-American sample of men and women poly-substance abusers with and without HIV-infection. However, three major methodological differences may explain our discordant findings. First, Gonzalez and colleagues (2005) estimated sexual risk

behavior in the past 6 months compared to our window of 12 months and also utilized a composite score rather than individual sexual risk behaviors as their dependent variable. Second, executive functioning was assessed using the Iowa Gambling Task, delayed non-matching to sample paradigm, and Stroop task-reaction time version which respectively measure decision-making, working memory, and response inhibition. Although these tests are well justified, other components of executive functioning such as perseveration, cognitive sequencing, and concept formation which were assessed in the current study, were not examined. Third and finally, regression models were adjusted for sensation seeking, a factor shown in previous research to be associated with sexual risk behavior (Hendershot, Stoner, George, & Norris, 2007; Kalichman et al., 1994; Kalichman, Heckman, & Kelly, 1996; Parsons & Halkitis, 2002); however, in the current study sensation seeking data was not available and was not adjusted for. Thus, future work examining the association between executive functioning and sexual risk behaviors are warranted; particularly research utilizing larger samples with diverse measures of executive functioning and models adjusting for sensation seeking and other personality covariates.

Novel to the current study, we demonstrated several genotype *(i.e. COMT*) by endophenotype (*i.e.* executive functioning) interactions for sexual risk behaviors. A relaxed significance criterion ($p < 0.10$) produced significant interactions for number of sexual partners, condom use, insertive and receptive anal sex, as well as oral sex. These interactions collectively advocate for further investigation of genotype-endophenotype interactions for sexual risk behavior. However, due to the exploratory nature of these interactions our discussion will be confined to interactions observed for number of sexual

partners, frequency of insertive anal sex and condom use, as interactions observed in these models met the traditional significance criterion ($p < 0.05$).

We observed both a main and interaction effect for number of sexual partners, albeit only within the model including the composite executive functioning deficit score. In this model we found that among carriers of the Met allele (*i.e.* Met/Met or Val/Met), a positive association between executive functioning deficit and number of sexual partners was present. Thus, among Met allele carriers those with greater deficit scores reported greater number of sexual partners; whereas among Val/Val carriers this association was not significant. Similar to results for number of sexual partners, stratified analysis showed that among carriers of the Met/Met but not Val/Met or Val/Val genotype an association between executive functioning and frequency of insertive anal sex was present, alhtough only statistically significant for models including Trails B. Thus, individuals with lower *T*-scores (*i.e.* greater impairment) on Trails B reported greater frequency of insertive anal sex only if they were carriers of the Met/Met genotype. Finally, the strongest interaction observed was between *COMT* and the Halstead Category Test for frequency of condom use. Contrary to the expected association, results suggest a negative association among carriers of the Met/Met genotype in which lower *T*-scores on the Category Test resulted in greater reported frequency of condom use. This unexpected finding may be a result of successful harm reduction campaigns aimed at both HIV-infected and METH using populations, although this is pure speculation.

Collectively, these findings provide preliminary evidence of differential susceptibility to sexual risk behavior via executive functioning, dependent on *COMT* genotype, particularly the Met/Met genotype (Figure 6.1). Although the role of the

Met/Met genotype is contrary to our hypothesis, our findings, when placed in the context of previous research are informative. Recent research has linked the *COMT* Met/Met genotype to novelty seeking behavior in healthy (Golimbet, Alfimova, Gritsenko, & Ebstein, 2007) and methamphetamine using (Hosak et al., 2006) populations. In addition, work by Gonzalez and colleagues (2005) on executive functioning and sexual risk behavior demonstrated that sensation seeking was independently associated with sexual risk, particularly among HIV-seropositive individuals. Thus, it appears that individuals with the Met/Met genotype may have a lower tolerance for monotony and may seek and participate in higher risk behaviors such as METH use or unprotected sex. Furthermore, work by our group and others (Mattay et al., 2003) have suggested that possession of the Met allele enhances executive functioning in healthy controls; however, this neuroprotective effect is significantly reduced among individuals with methamphetamine dependence. Thus, it is probable that in our sample, of which approximately half were methamphetamine dependent, the putative protective effect of the Met/Met genotype is diminished and propensity to sexual risk behavior enhanced.

It is apparent that the associations between *COMT*, executive functioning, and sexual risk behavior are highly complex and context dependent. The current study provides preliminary evidence of these complex relationships and advocates for larger investigations that improve upon and consider several of the limitations that have been presented. Future work should also attempt to address independent and interaction effects of other putative polymorphisms particularly those involved in dopamine synthesis (*e.g.* Tyrosine Hydroxylase), metabolism (*e.g.* Monoamine Oxidase A), and reception (*e.g.* Dopamine Receptors D1-4). Completion of such work in combination with the current

work as well as others previous work will further our understanding of the genotypic and endophenotypic factors involved in the phenotypic expression of sexual risk behaviors and potentially assist with risk identification, prevention, and treatment efforts in the future.

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Table 6.1 Characteristics of full sample by COMT genotype

	Full Sample (n=192)	COMT genotype[b] Val/Val (n=54)	Val/Met (n=103)	Met/Met (n=35)	
Age (years) *M* (sd)	37 (9)	35 (9)	38 (9)	39 (11)	
Education (years) *M* (sd)	13 (2)	13 (2)	13 (2)	14 (2)	
WRAT4 *M* (sd)	100 (12)	99 (11)	100 (12)	104 (11)	
Ethnicity (row %)					
Caucasian	71	52	78	83	v/v < v/m, m/m**
African-American	15	32	7	11	v/v > v/m, m/m**
Hispanic	14	17	16	6	
Executive Functioning Battery					
Wisconsin Card Sort Test *T* (sd)	45 (14)	47 (16)	44 (13)	46 (13)	
Trials Part B *T* (sd)	49 (11)	51 (12)	47 (10)	52 (11)	
Halstead Category Test *T* (sd)	46 (10)	47 (10)	44 (10)	47 (9)	
Domain Deficit Score *M* (sd)	.55 (.69)	.56 (.68)	.62 (.74)	.35 (.47)	
Executive Impairment (%)	45	46	50	31	
Sexual Characteristics/Behavior					
Age at first intercourse M (sd)	15 (4)	14 (4)	16 (4)	17 (4)	v/v < m/m*
Sexual Preference (% heterosexual)	33	35	31	38	
Number partners in past 12mo *Median* (IQR)	3 (1,10)	4 (1, 11)	3 (1, 10)	2 (1, 5)	
Condom use (>0% in past 12mo)	72	74	71	70	
Insertive anal (>0% in past 12mo)	62	60	67	52	
Receptive anal (>0% in past 12mo)	58	60	62	46	
Oral sex (>0% in past 12mo)	93	94	93	94	
Intoxicated Sex (>0% in past 12mo)	64	63	66	61	
Vaginal Sex (>0% in past 12mo)	37	35	35	44	
DSM-IV Psychiatric Disorder (% lifetime)					
Major Depression	36	36	35	40	
Bipolar I or II	4	8	3	3	
Beck Depression Inventory *M* (sd)	12 (9)	11 (8)	13 (10)	10 (9)	

Table 6.1 (Continued) Characteristics of full sample by COMT genotype

	Full Sample (n=192)	COMT genotype[b] Val/Val (n=54)	Val/Met (n=103)	Met/Met (n=35)	
DSM-IV Substance Dependence (% lifetime)					
Sedative	0	0	0	0	
Cannabis	9	9	11	6	
Opioid	0	0	0	0	
Cocaine	7	7	5	14	
Hallucinogen	0	0	0	0	
Alcohol	17	15	20	14	
Methamphetamine Parameters					
Methamphetamine Dependent (%)	47	37	52	49	
Age at first METH use, yrs *M* (sd)	24 (9)	23 (9)	25 (8)	27 (10)	
Total METH use, yrs *M* (sd)	11 (6)	13 (7)	11 (6)	8 (4)	v/v > m/m*
Last use of METH, days *Median* (IQR)	91 (36, 274)	122 (45, 731)	91 (32, 236)	91 (30, 244)	
HIV Parameters					
HIV seropositive (%)	56	70	51	49	v/v > v/m, m/m*
HIV RNA, plasma (log copies/ml) *M* (sd)	2.1 (1.9)	2.4 (1.7)	2.0 (2.0)	1.7 (1.9)	

Table 6.2 Multivariate linear regression coefficients for main, interaction, and stratified effects of executive functioning and COMT in seven sexual risk behavior models

	Standardized Beta[a]					
	Main Effect		Interaction	Stratified[b]		
EF Measure	EF	COMT	EF x COMT	Val/Val	Val/Met	Met/Met
Sexual Risk Behavior Model	(n=192)	(n=192)	(n=192)	(n=54)	(n=103)	(n=35)
Domain Deficit Score						
1. Partners (# past 12mo)	0.21**	0.10	0.50**	0.03	0.20**	0.52***
2. Condom use (% past 12mo)	0.03	0.13	0.24	–	–	–
3. Insertive anal (% past 12mo)	0.06	0.07	0.50**	-0.18	0.11	0.18
4. Receptive anal (% past 12mo)	0.05	0.05	0.44*	-0.17	0.11	0.13
5. Oral sex (% past 12mo)	-0.10	0.07	0.40	–	–	–
6. Intoxicated Sex (% past 12mo)	0.07	-0.06	0.08	–	–	–
7. Vaginal Sex (% past 12mo)	-0.03	-0.04	-0.28	–	–	–
Wisconsin Card Sort Test (T-score)						
1. Partners (# past 12mo)	-0.09	0.08	-0.18	–	–	–
2. Condom use (% past 12mo)	-0.10	0.12	-0.25	–	–	–
3. Insertive anal (% past 12mo)	0.01	0.07	-0.31	–	–	–
4. Receptive anal (% past 12mo)	-0.02	0.04	-0.38	–	–	–
5. Oral sex (% past 12mo)	0.20**	0.09	0.13	–	–	–
6. Intoxicated Sex (% past 12mo)	-0.04	-0.07	0.32	–	–	–
7. Vaginal Sex (% past 12mo)	0.04	-0.04	-0.04	–	–	–
Trails B (T-score)						
1. Partners (# past 12mo)	-0.01	0.08	–0.54	–	–	–
2. Condom use (% past 12mo)	-0.07	0.11	0.03	–	–	–
3. Insertive anal (% past 12mo)	-0.06	0.06	–0.99**	0.18	-0.03	–0.38**
4. Receptive anal (% past 12mo)	-0.04	0.03	–0.75*	0.17	-0.06	-0.22
5. Oral sex (% past 12mo)	0.10	0.06	–0.68*	0.35**	0.01	0.01
6. Intoxicated Sex (% past 12mo)	-0.11	-0.07	0.13	–	–	–
7. Vaginal Sex (% past 12mo)	0.07	-0.04	0.37	–	–	–
Halstead Category Test (T-score)						
1. Partners (# past 12mo)	-0.11	0.08	-0.16	–	–	–
2. Condom use (% past 12mo)	–0.16**	0.11	–1.13**	0.08	–0.19*	–0.49***
3. Insertive anal (% past 12mo)	0.01	0.05	-0.22	–	–	–
4. Receptive anal (% past 12mo)	-0.01	0.03	-0.59	–	–	–
5. Oral sex (% past 12mo)	0.06	0.06	-0.50	–	–	–
6. Intoxicated Sex (% past 12mo)	-0.04	-0.06	-0.44	–	–	–
7. Vaginal Sex (% past 12mo)	-0.05	-0.04	0.25	–	–	–

a = All regression models adjusted for METH status, HIV status, Age at first intercourse.

b = Stratified analysis of EF effects by genotype was conducted if a significant ($p < 0.10$) interaction was observed.

EF = executive functioning; COMT = catechol-o-methyltransferease (0=Val/Val; 1=Val/Met; 2=Met/Met)

* $p < 0.10$; ** $p < 0.05$, *** $p < 0.005$

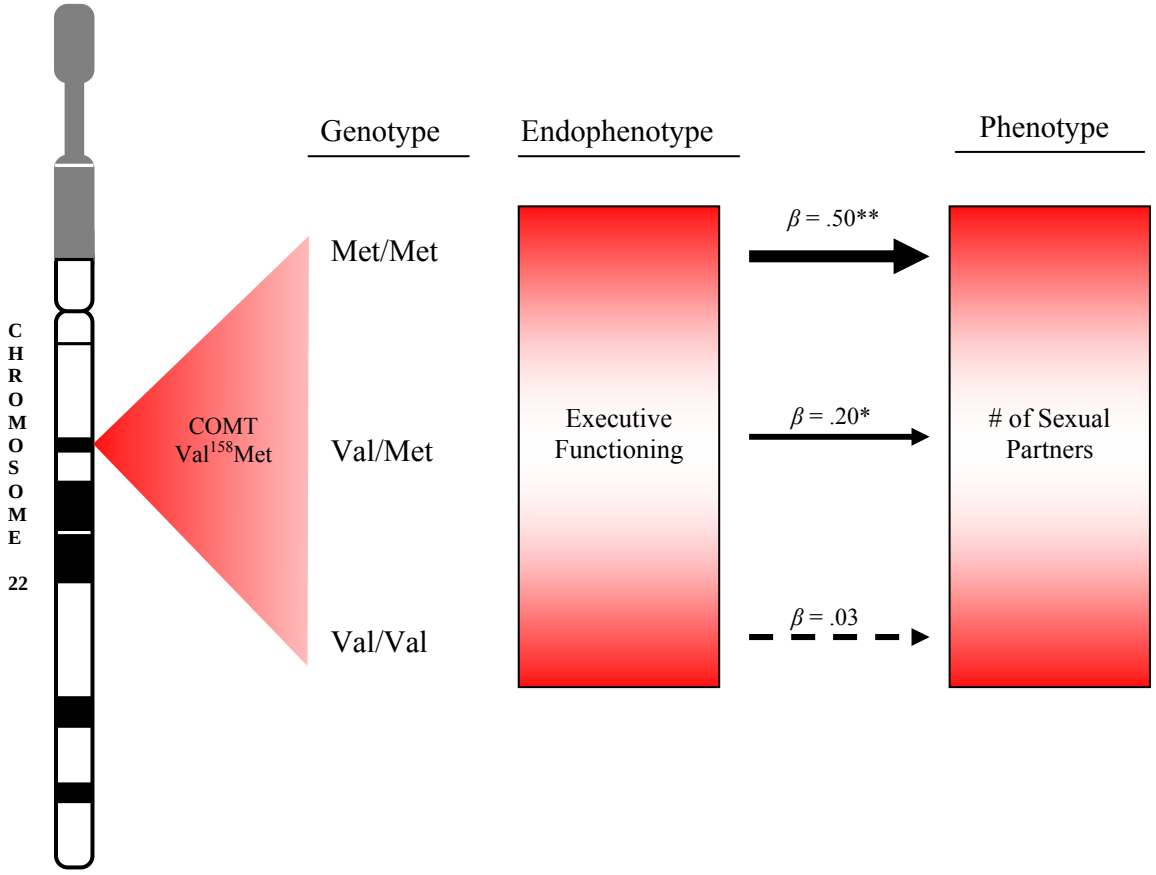

Figure 6.1 Theoretical model illustrating the interaction effect of a single genotype (i.e. *COMT* Val158Met polymorphism) and endophenotype (i.e. executive functioning) on a behavioral phenotype (i.e. number of sexual partners). Among carriers of the *COMT* Met/Met and to a lesser extent the Val/Met genotype the association between executive functioning and number of sexual partners is significantly stronger than among carriers of the Val/Val genotype. * $p < 0.05$; ** $p < 0.01$

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CHAPTER 7

DISCUSSION AND SIGNIFICANCE

DISCUSSION AND SIGNIFICANCE

The preceding chapters of this dissertation, guided by both system biology and ecological frameworks, sought to describe and test hypotheses aimed at obtaining a better understanding of: (1) the genetic epidemiology of METH use disorders and (2) the influence genetic and neurocognitive factors have on HIV risk behavior; while paying particular attention to the context in which these factors operate.

Genetic Epidemiology of METH Use Disorders

This dissertation began by providing the first systematic review and synthesis of the current gene association literature (Chapter 2) as well as the first examination of several putative and novel polymorphisms for METH dependence in an ethnically diverse population (Chapter 3). Results demonstrated that the path to elucidating the genetic factors for METH use disorders and other complex disorders/behaviors will require considerable methodological effort, large collaborations, and continued advances in technology. Furthermore, this work played an important role in constructing a foundation for the genetic component of the dissertation and assisted in the identification of a candidate gene (i.e. catechol-o-methlytransferase, COMT) that could be used to test the conceptual model (Figure 1.1) presented in Chapter 1.

Genes, Neurocognition, and HIV Risk Behaviors

As aforementioned, this dissertation aimed to examine not only the influence of genetic but also neurocognitive factors on HIV risk behavior. Until recently, examination of HIV risk behaviors have been guided predominately by traditional models of individual health behavior such as the theory of reasoned action (Fishbein, 1980) and the transtheoretical model (Prochaska & DiClemente, 1983) which emphasize social-

cognitive variables. However, little attention has been given to models that include biological and neurocognitive (Hall, Elias, & Crossley, 2006) variables. The omission of these variables from current health behavior models may partially explain the limited success of these models in predicting HIV as well as other risk behaviors. Although this dissertation was not comprehensive, in that many of the traditional health behavior variables (e.g. self-efficacy, intention) have been omitted, it does provide preliminary support for inclusion of genetic and neurocognitive variables in models of health behavior.

Specifically, in chapters 4 and 6 the COMT Val158Met polymorphism and executive dysfunction were examined within the context of METH dependence and HIV-infection. In chapter 4 results showed that the previously reported Val158Met polymorphism-executive dysfunction association (Barnett, Jones, Robbins, & Muller, 2007) was present in HIV-infected and control groups but was not observed for those within the METH dependence groups. Furthermore, in chapter 6 results showed a moderating effect of the Val158Met polymorphism on the association between executive dysfunction and HIV risk behavior. These results suggest a complex and context dependent relationship between the Val158Met polymorphism, executive dysfunction, and HIV risk behavior.

Context Matters

Both systems biology and ecological frameworks emphasize the importance of context when observing any phenomenon. A "contextual factor" is a generic term that encompasses any environment or circumstance in which a phenomenon of interest could occur. In this dissertation contextual factors referred to the participant's METH status and

HIV serostatus. In chapter 5, the influence of these contextual factors on HIV risk behaviors were explicitly investigated among a sample of men who have sex with men. Results showed significant differences in HIV risk behaviors such as condom use, intoxicated sex, and number of IDU partners across four independent contexts (i.e. HIV-METH-; HIV+/METH-; HIV-/METH+; HIV+/METH+). Furthermore, in a sub-analysis presented in chapter 5, it was also shown that context influenced mood, particularly depression. Thus it would appear, as both system biology and ecological frameworks would suggest, context is an important factor to consider when examining HIV risk behavior as well as mood and failure to do so could result in erroneous conclusions.

Beyond these main contextual factors (*i.e.* METH and HIV status), it is also important to note the genotype could also be viewed as a context in that every individual's genetic environment is present when a behavior is emitted. In fact, a contextual factor is often viewed as a moderating variable by which the strength of an association can be attenuated or exacerbated. Thus, COMT genotype in this dissertation could also be viewed as a contextual factor, specifically in chapter 6 were COMT was shown to moderate the association between executive dysfunction and HIV risk behavior.

Future Directions

This dissertation has provided a foundation for further clarification of the genetic factors of METH dependence as well as the underlying potential biological and environmental mechanisms of executive dysfunction and HIV risk behavior among HIV-infected and/or METH dependent individuals. However, replication and further testing of the presented hypotheses and methods in a variety of contexts are required before

translation of these findings to innovative clinical interventions and/or prevention protocols can be initiated.

From a genetic perspective, this dissertation is limited by the examination of only one gene polymorphism (*i.e.* COMT Val158Met) and thus future studies with other candidate genes are warranted. Genes involved in dopamine metabolism as well as dopamine synthesis and reception (Figure 7.1) are a good starting point due to dopamine's role in sexual arousal, motivation, neurocognitive functioning, and the subsequent rewarding effect of sexual behavior (Melis & Argiolas, 1995; Nieoullon, 2002; Starr, Fox, Harris, Deary, & Whalley, 2007). Other than COMT, monoamine oxidase A (MAOA) assists in the metabolism of dopamine in the prefrontal cortex (Hotamisligil & Breakefield, 1991) and a variable number tandem repeat mutation in the promoter region of this gene has been linked with impulsive behavior in humans (Caspi et al., 2002; Chen et al., 1991). Beyond dopamine metabolism, genes essential for dopamine synthesis such as tyrosine hydroxylase (TH) and DOPA decarboxylase (DDC) may also play an important role in executive functioning and HIV risk behavior. To date research examining the links between TH variants and executive functioning and/or HIV risk behavior have not been reported. However, Akil et al. (2003) has reported a potential interaction between COMT and TH by which COMT genotype affects TH gene expression. DDC follows TH in the synthesis of dopamine and thus may also be a candidate for further investigation; however little to no DDC research related to neurocognitive or behavioral phenotypes have been conducted to date. Thus, the role of TH and DDC variants in executive functioning and HIV risk behavior are warranted. Unlike dopamine synthesis genes, dopamine receptor genes have been given significant

attention related to neurocognitive impairment as well as novelty seeking which has been associated with HIV risk behavior (Gonzalez et al., 2005). Among the many dopamine receptor genes, D1 and D3 variants have recently been implicated in prefrontal cortex functioning in healthy adults (Lane et al., 2008). Furthermore, dopamine receptor D2 and D4 variants have been linked to novelty seeking behavior (Dalley et al., 2007; Ebstein et al., 1996) although these effects have been inconsistent (Kluger, Siegfried, & Ebstein, 2002).

From a neurocognitive perspective, executive functioning is only one of several defined neurocognitive domains (see Table 4.1) that could be examined for links between genetic and phenotypic (*e.g.* risk behavior) factors. Although, the executive functioning domain is thought to have the most plausible links to risk behavior (Barclay et al., 2007; Barker et al., 2007; Hall et al., 2006) this domain certainly does not act alone and studies of other domains in varying genetic and environmental contexts would further our understanding of the underpinning factors that contribute to HIV and other risk behaviors.

Finally and of great importance, future work that tests and documents the effects of context on putative as well as novel associations for METH use disorders, executive dysfunction, and HIV risk behaviors is required. It is becoming apparent that diseases and behavior do not respond to a "one size fits all" approach. Thus, future work in a variety of contexts will allow for better tailoring of strategies and eventually reduction in personal and social burden associated with failed intervention strategies. These new approaches will be complex and require interdisciplinary collaborations with scientists in

various disciplines. However, these efforts will undoubtedly put us in a position to better understand risk behavior and begin to develop strategies to improve the public's health.

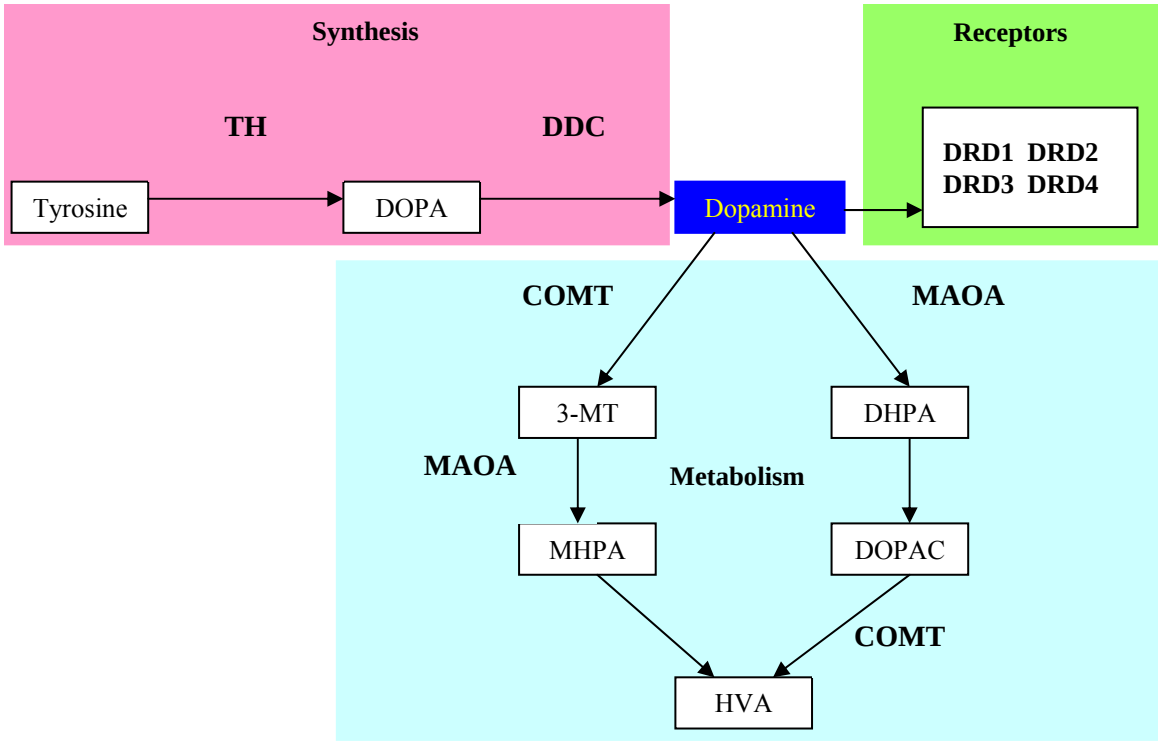

Figure 7.1. Genes involved in Dopamine Synthesis, Metabolism, and Reception. Genes are in bold and the pathways in which they play a role are shown. TH = Tyrosine Hydroxylase; DDC = DOPA Decarboxylase; DRD1-4 = Dopamine Receptors D1-4; COMT = Catechol-O-Methyltransferease; MAOA = Monoamine Oxidase A.

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