Published in final edited form as:

Psychiatry Res. 2010 July 30; 178(2): 295–298. doi:10.1016/j.psychres.2009.07.019.

Preliminary evidence of ethnic divergence in associations of putative genetic variants for methamphetamine dependence

Chad A. Bousman^{a,b,d,*}, Stephen J. Glatt^{c,d}, Mariana Cherner^a, J. Hampton Atkinson^a, Igor Grant^a, Ming T. Tsuang^{a,d}, Ian P. Everall^{a,d}, and the HNRC Group

^aDepartment of Psychiatry, University of California San Diego, La Jolla, California, USA

^bSan Diego State/University of California, San Diego Joint Doctoral Program in Public Health (Health Behavior)

^cDepartment of Psychiatry and Behavioral Sciences, and Medical Genetics Research Center, SUNY Upstate Medical University, Syracuse, NY

^dCenter for Behavioral Genomics, Department of Psychiatry, University of California, San Diego

Abstract

Research into the biological processes that increase susceptibility to methamphetamine dependence has been conducted primarily in Asian populations. Using a case-control design this study's purpose was to explore, among a population of methamphetamine-dependent Caucasians, six putative single nucleotide polymorphisms previously found to be associated with methamphetamine dependence in Asian populations. 193 non-psychotic males (117 methamphetamine-dependent and 76 controls) were genotyped for variants located in six genes (*AKT1*, *ARRB2*, *BDNF*, *COMT*, *GSTP1*, *OPRM1*). Genotypic and allelic frequencies, odds ratios, and 95% confidence intervals were calculated. None of the putative gene associations were significantly replicated in our sample of Caucasian men. Effect size comparisons suggest a trend toward allelic divergence for arrestin beta 2 (*ARRB2*) and glutathione S-transferase P1 (*GSTP1*) and allelic convergence for brain-derived neurotrophic factor (*BDNF*). Results provide preliminary support for further exploration and validation of candidate SNPs for METH dependence reported among Asian populations across other ethnic/ancestral groups.

Keywords

AKT1; COMT; OPRM1; ARRB2; BDNF; GSTP1

1. Introduction

Methamphetamine (METH) is a powerful illicit psychostimulant that has become increasingly popular throughout North America (Maxwell and 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

© 2009 Elsevier Ireland Ltd. All rights reserved.

*Send correspondence to: Chad Bousman, MPH, PhD Department of Psychiatry, University of California San Diego 140 Arbor Dr., San Diego, CA 92103, USA cbousman@ucsd.edu .

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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; Kendler et al., 2003); 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 (for reviews see (Barr et al., 2006; Bousman et al., 2009). 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 non-Asian 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 large 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 a non-Asian sample. We selected and examined, among a population of METH-dependent Caucasians, six putative SNPs (*AKT1*, *ARRB2*, *BDNF*, *COMT*, *GSTP1*, *OPRM1*) recently found to be associated with METH dependence in Asian populations.

2. Methods

2.1 Participants

Participants were 193 non-psychotic unrelated males (117 METH-dependent and 76 controls) 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. The mean age of control ($M=39.6$, $sd=10.2$) and METH-dependent ($M=38.6$, $sd=8.2$) participants were not statistically different ($t=0.73$, $df=191$, $p=0.42$).

Recruitment methods have been described in detail elsewhere (Rippeth et al., 2004). Briefly, METH dependence was determined using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders Version IV (SCID) and was administered by research psychologists, trained postdoctoral fellows or clinical psychology graduate students. 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 or a history of head injury with loss of consciousness greater than thirty minutes was reported. In addition, a history of neurological, seizure disorder, schizophrenia, or bipolar affective disorder with psychotic features also resulted in exclusion from the study. 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 Caucasian or white were selected, as other ethnic group sample sizes were extremely small. 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.

2.2 SNP Identification and Selection

A recently generated list of 18 significant gene associations for METH dependence (Bousman et al., 2009) was 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 (Li et al., 2004; Cheng et al., 2005; Hashimoto et al., 2005; Ikeda et al., 2006; Zhang et al., 2006; Ikeda et al., 2007) among METH-dependent Asians based on their relatively high minor allele frequency ($m > 0.20$) among the European-American population (International HapMap Consortium, 2005). The final lists of selected putative and novel SNPs are presented in Table 1.

2.3 DNA Extraction and Genotyping

DNA was extracted from peripheral blood mononuclear cells stored (three to five years) at -70°C 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) 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 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. To ensure data quality, genotypes for all subjects were also checked manually. All genotyping was performed by an accredited commercial laboratory (Harvard Medical School-Partners Healthcare Center for Genetics and Genomics, Cambridge, MA CLIA No. 22D1005307).

2.4 Statistical analysis

Genotype and allele frequencies for the nine selected SNPs were determined for METH-dependent and control participants. Odds ratios (ORs) and 95% confidence intervals (CIs) were then calculated using chi-square analysis to estimate the effect size for each selected variant for METH dependence. 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. In addition, gene-gene interactions between each of the six variants were explored using univariate logistic regressions. Due to the small sample size 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).

3. Results

Table 2 displays genotype frequencies of the six SNPs (*AKT1*, *ARRB2*, *BDNF*, *COMT*, *GSTP1*, *OPRM1*) previously implicated in Asian samples. Among controls each of the selected genotypes was in Hardy-Weinberg equilibrium (HWE) and allele frequencies were similar to those reported by the National Center for Biotechnology Information SNPs Database with the exception of *OPRM1* (HWE: $\chi^2=7.48$, $df=1$, $p=0.006$). None of the polymorphisms found to be significantly associated with METH dependence among Asian populations were replicated in this sample of Caucasian men, albeit *OPRM1* was nearly significant ($\chi^2=5.87$, $df=2$, $p=0.053$).

Table 3 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. 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, $df=2$, 95% CI=0.34-1.04 vs. OR=0.64, $df=2$, 95% CI=0.43-.95). Divergent trends were observed for *ARRB2* (OR=0.69, $df=2$, 95% CI=0.39-1.22 vs. OR=1.61, $df=2$, 95% CI=1.04-2.41) and *OPRM1* (OR=0.74, $df=2$, 95% CI=0.48-1.14 vs. OR=1.70, $df=2$, 95% CI=1.04-2.81) minor allele variants.

Exploration of all 15 possible gene-gene interactions for METH dependence among the six putative variants revealed one significant interaction between *ARRB2* and *BDNF* ($B = 0.33$, $df = 1$; $p = 0.008$). However, this interaction did not withstand Bonferroni correction ($p < 0.003$) for multiple testing.

4. Discussion

To our knowledge this study is the first to explore previously reported gene-associations for METH dependence in non-Asian (Caucasian) population and provide evidence that genotypic susceptibility to METH dependence may differ by ethnicity, albeit previous research (Sery et al., 2001) has examined genotypic susceptibility in Caucasian METH users. Among the six putative SNPs previously found to be associated with METH dependence among Asians, we did not statistically replicate any association at either the genotypic or allelic level. However, when examining minor allele effect size estimates between our sample and previously published Asian samples, divergent (*ARRB2*, *OPRM1*) 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 using minor allelic frequencies (Table 4) 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.

Although we were unable to statistically replicate putative variants of METH dependence, we did identify trends towards minor allele frequency convergence for *BDNF* and divergence for *ARRB2* and *OPRM1* variants suggesting both a generalized as well as unique genetic 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. Thus, further validation at these loci is required among ethnically/ancestrally diverse groups ideally utilizing available ancestral informative markers (AIMs) for ancestral classification. Second, as aforementioned, sample size and power were less than optimal for the current study and failure to find significant replications in the present study potentially represent a type-II (false-negative) error, further advocating for substantially larger sample sizes for future genotypic investigations of METH dependence. In addition, the small sample size may have also resulted in random disequilibrium of the *OPRM1* polymorphism and thus results for this variant should be interpreted cautiously. 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.

Acknowledgments

The authors wish to acknowledge support from the United States National Institutes of Health (grant numbers P01-DA12065 and P30-MH62512) and the contributions of study participants and staff at the HIV Neurobehavioral Research Center (HNRC), San Diego, CA, USA.

The HNRC Group is affiliated with the University of California, San Diego, the Naval Hospital, San Diego, and the Veterans Affairs San Diego Healthcare System, and includes: Director: Igor Grant, M.D.; Co-Directors: J. Hampton Atkinson, M.D., Ronald J. Ellis, M.D., Ph.D., and J. Allen McCutchan, M.D.; Center Manager: Thomas D. Marcotte, Ph.D.; Business Manager: Melanie Sherman; Naval Hospital San Diego: Braden R. Hale, M.D., M.P.H. (P.I.); Neuromedical Component: Ronald J. Ellis, M.D., Ph.D. (P.I.), J. Allen McCutchan, M.D., Scott Letendre, M.D., Edmund Capparelli, Pharm.D., Rachel Schrier, Ph.D.; Jennifer Marquie-Beck; Terry Alexander, R.N.; Neurobehavioral Component: Robert K. Heaton, Ph.D. (P.I.), Mariana Cherner, Ph.D., Steven Paul Woods, Psy.D., David J. Moore, Ph.D.; Matthew Dawson, Donald Franklin; Neuroimaging Component: Terry Jernigan, Ph.D. (P.I.), Christine Fennema-Notestine, Ph.D., Sarah L. Archibald, M.A., John Hesselink, M.D., Jacopo Annese, Ph.D., Michael J. Taylor, Ph.D., Neurobiology Component: Eliezer Masliah, M.D. (P.I.), Ian Everall, FRCPsych., FRCPsych., Ph.D., Cristian Achim, M.D., Ph.D.; Neurovirology Component: Douglas Richman, M.D., (P.I.), David M. Smith, M.D.; International Component: J. Allen McCutchan, M.D., (P.I.); Developmental Component: Ian Everall, FRCPsych., FRCPsych., Ph.D. (P.I.), Stuart Lipton, M.D., Ph.D.; Clinical Trials Component: J. Allen McCutchan, M.D., J. Hampton Atkinson, M.D., Ronald J. Ellis, M.D., Ph.D., Scott Letendre, M.D.; Participant Accrual and Retention Unit: J. Hampton Atkinson, M.D. (P.I.), Rodney von Jaeger, M.P.H.; Data Management Unit: Anthony C. Gamst, Ph.D. (P.I.), Clint Cushman (Data Systems Manager), Daniel R. Masys, M.D. (Senior Consultant); Statistics Unit: Ian Abramson, Ph.D. (P.I.), Florin Vaida, Ph.D., Christopher Ake, Ph.D.

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, Department of Defense, nor the United States Government.

References

- Barr AM, Panenka WJ, MacEwan GW, Thornton AE, Lang DJ, Honer WG, Lecomte T. The need for speed: an update on methamphetamine addiction. *Journal of Psychiatry & Neuroscience* : JPN. 2006; 31:301–313.
- Bousman, CA.; Glatt, SJ.; Everall, IP.; Tsuang, MT. American Journal of Medical Genetics, part B, Neuropsychiatric Genetics. International Society of Psychiatric Genetics; 2009. Genetic association studies of methamphetamine use disorders: A systematic review and synthesis. The official publication of the
- Cheng CY, Hong CJ, Yu YW, Chen TJ, Wu HC, Tsai SJ. Brain-derived neurotrophic factor (Val66Met) genetic polymorphism is associated with substance abuse in males. *Brain Research. Molecular Brain Research*. 2005; 140:86–90. [PubMed: 16109452]
- Hashimoto, T.; Hashimoto, K.; Matsuzawa, D.; Shimizu, E.; Sekine, Y.; Inada, T.; Ozaki, N.; Iwata, N.; Harano, M.; Komiyama, T.; Yamada, M.; Sora, I.; Ujike, H.; Iyo, M. American Journal of Medical Genetics, part B, Neuropsychiatric Genetics. Vol. 135. International Society of Psychiatric Genetics; 2005. A functional glutathione S-transferase P1 gene polymorphism is associated with methamphetamine-induced psychosis in Japanese population; p. 5-9. The official publication of the
- Ikedo M, Iwata N, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshiya Y, Sekine Y, Iyo M, Harano M, Komiyama T, Yamada M, Sora I, Ujike H, Inada T, Ozaki N. Positive association of AKT1 haplotype to Japanese methamphetamine use disorder. *The International Journal of Neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*. 2006; 9:77–81. [PubMed: 15982448]
- Ikedo M, Ozaki N, Suzuki T, Kitajima T, Yamanouchi Y, Kinoshita Y, Kishi T, Sekine Y, Iyo M, Harano M, Komiyama T, Yamada M, Sora I, Ujike H, Inada T, Iwata N. Possible association of beta-arrestin 2 gene with methamphetamine use disorder, but not schizophrenia. *Genes, Brain, and Behavior*. 2007; 6:107–112.
- International HapMap Consortium. A haplotype map of the human genome. *Nature*. 2005; 437:1299–1320. [PubMed: 16255080]
- Kendler KS, Jacobson KC, Prescott CA, Neale MC. Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. *The American Journal of Psychiatry*. 2003; 160:687–695. [PubMed: 12668357]
- Li, T.; Chen, CK.; Hu, X.; Ball, D.; Lin, SK.; Chen, W.; Sham, PC.; Loh, e.; Murray, RM.; Collier, DA. American Journal of Medical Genetics, part B, Neuropsychiatric Genetics. Vol. 129. International Society of Psychiatric Genetics; 2004. Association analysis of the DRD4 and COMT genes in methamphetamine abuse; p. 120-124. The official publication of the
- Maxwell JC, Rutkowski BA. The prevalence of methamphetamine and amphetamine abuse in North America: a review of the indicators, 1992-2007. *Drug and Alcohol Review*. 2008; 27:229–235. [PubMed: 18368603]
- Rippeth JD, Heaton RK, Carey CL, Marcotte TD, Moore DJ, Gonzalez R, Wolfson T, Grant I, HNRC Group. Methamphetamine dependence increases risk of neuropsychological impairment in HIV infected persons. *Journal of the International Neuropsychological Society* : JINS. 2004; 10:1–14. [PubMed: 14751002]
- Sery O, Vojtova V, Zvolosky P. The association study of DRD2, ACE and AGT gene polymorphisms and metamphetamine dependence. *Physiological Research / Academia Scientiarum Bohemoslovaca*. 2001; 50:43–50. [PubMed: 11300226]
- Tsuang MT, Lyons MJ, Eisen SA, Goldberg J, True W, Lin N, Meyer JM, Toomey R, Faraone SV, Eaves L. Genetic influences on DSM-III-R drug abuse and dependence: a study of 3,372 twin pairs. *American Journal of Medical Genetics*. 1996; 67:473–477. [PubMed: 8886164]
- Tsuang MT, Lyons MJ, Meyer JM, Doyle T, Eisen SA, Goldberg J, True W, Lin N, Toomey R, Eaves L. Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. *Archives of General Psychiatry*. 1998; 55:967–972. [PubMed: 9819064]
- Uhl GR, Drgon T, Liu QR, Johnson C, Walther D, Komiyama T, Harano M, Sekine Y, Inada T, Ozaki N, Iyo M, Iwata N, Yamada M, Sora I, Chen CK, Liu HC, Ujike H, Lin SK. Genome-wide

- association for methamphetamine dependence: convergent results from 2 samples. *Archives of General Psychiatry*. 2008; 65:345–355. [PubMed: 18316681]
- Zhang H, Luo X, Kranzler HR, Lappalainen J, Yang BZ, Krupitsky E, Zvartau E, Gelernter J. Association between two mu-opioid receptor gene (OPRM1) haplotype blocks and drug or alcohol dependence. *Human Molecular Genetics*. 2006; 15:807–819. [PubMed: 16476706]

Table 1

Selected Putative SNPs for Methamphetamine Dependence

Gene	Gene Name	SNP ID	Chr.	Function	Reference
AKT1	V-akt murine thymoma viral oncogene homolog 1	rs3730358	14q	Mediates dopamine-associated behavior	Ikeda et al. 2006
ARRB2	Arrestin, beta 2	rs4790694	17p	Mediates dopamine signaling pathways	Ikeda et al. 2007
BDNF	Brain-derived neurotrophic factor	rs6265	11p	Modulates dopaminergic functions	Cheng et al. 2005
COMT	Catechol-O-methyltransferase	rs4680	22q	Metabolism of catecholamine transmitters	Li et al. 2004
GSTP1	Glutathione S-transferase P1	rs1695	11q	Detoxification of xenobiotics	Hashimoto et al. 2005
OPRM1	Mu-opioid receptor 1	rs2075572	6q	Mediates opiate response	Zhang et al. 2006

Chr = Chromosome; SNP = single nucleotide polymorphism

Table 2

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

Gene (SNP) Count (frequency)	Ethnicity			
	Caucasian		Asian ^I	
	METH (n = 117)	Control (n = 76)	METH	Control
<i>AKT1</i> (rs3730358C>T)			<i>N</i> = 182	<i>n</i> = 437
CC	86 (0.74)	50 (0.66)	136 (0.74)	364 (0.83)
CT	26 (0.22)	24 (0.32)	43 (0.24)	68 (0.16)
TT	5 (0.04)	2 (0.03)	3 (0.02)	5 (0.01)
χ^2 (p-value)	2.29 (p = 0.32)		6.08 (p = 0.04)	
<i>ARRB2</i> (rs4790694C>A)			<i>n</i> = 177	<i>n</i> = 546
CC	84 (0.72)	48 (0.63)	138 (0.78)	470 (0.86)
CA	31 (0.26)	25 (0.33)	39 (0.22)	74 (0.14)
AA	2 (0.02)	3 (0.04)	0 (0.00)	2 (0.003)
χ^2 (p-value)	2.04 (p = 0.36)		7.85 (p = .02)	
<i>BDNF</i> (rs6265G>A)			<i>n</i> = 103	<i>n</i> = 122
GG	86 (0.73)	45 (0.59)	31 (0.30)	23 (0.19)
GA	29 (0.25)	29 (0.38)	57 (0.55)	68 (0.56)
AA	2 (0.02)	2 (0.03)	15 (0.15)	31 (0.25)
χ^2 (p-value)	4.32 (p = 0.12)		6.16 (p = 0.05)	
<i>COMT</i> (rs4680G>A)			<i>n</i> = 410	<i>n</i> = 390
GG	23 (0.20)	18 (0.24)	228 (0.56)	181 (0.46)
GA	65 (0.55)	45 (0.59)	150 (0.37)	172 (0.44)
AA	29 (0.25)	13 (0.17)	32 (0.07)	37 (0.10)
χ^2 (p-value)	1.71 (p = 0.43)		6.77 (p = 0.04)	
<i>GSTP1</i> (rs1695A>G)			<i>n</i> = 189	<i>n</i> = 199
AA	15 (0.13)	9 (0.12)	144 (0.76)	167 (0.84)
AG	58 (0.50)	29 (0.38)	41 (0.22)	32 (0.16)
GG	44 (0.37)	38 (0.50)	4 (0.02)	0 (0.00)
χ^2 (p-value)	3.03 (p = 0.22)		6.56 (p = 0.03)	
<i>OPRM1</i> (rs2075572G>C)			<i>n</i> = 128	<i>n</i> = 232
GG	20 (0.17)	23 (0.30)	73 (0.57)	154 (0.66)
GC	57 (0.49)	26 (0.34)	43 (0.34)	72 (0.31)
CC	40 (0.34)	27 (0.36)	12 (0.09)	6 (0.03)
χ^2 (p-value)	5.87 (p = 0.05)		8.92 (p = 0.01)	

^I = genotypic frequencies extracted from index study listed in Table 1

Table 3

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

Gene (SNP) Allele Count (frequency)	Ethnicity			
	CEU		Asian	
	METH (n = 117)	Control (n = 76)	METH	Control
AKT1 (rs3730358C>T)			n = 182	n = 437
T	36 (0.15)	28 (0.18)	49 (0.13)	78 (0.10)
OR	0.81		1.59	
95% CI (p-value)	0.45 - 1.44 (0.43)		1.06 - 2.35 (0.02)	
ARRB2 (rs4790694C>A)			n = 177	n = 546
A	35 (0.15)	31 (0.20)	39 (0.11)	78 (0.07)
OR	0.69		1.61	
95% CI (p-value)	0.39 - 1.22 (0.17)		1.04 - 2.41 (0.02)	
BDNF (rs6265G>A)			n = 103	n = 122
A	33 (0.14)	33 (0.22)	87 (0.42)	130 (0.53)
OR	0.59		0.64	
95% CI (p-value)	0.34 - 1.04 (0.05)		0.43 - 0.95 (0.02)	
COMT (rs4680G>A)			n = 410	n = 390
A	123 (0.52)	71 (0.47)	214 (0.26)	246 (0.32)
OR	1.26		0.77	
95% CI (p-value)	0.82 - 1.94 (0.26)		0.61 - 0.96 (0.02)	
GSTP1 (rs1695A>G)			n = 189	n = 199
G	146 (0.62)	105 (0.69)	49 (0.13)	32 (0.08)
OR	0.74		1.70	
95% CI (p-value)	0.48 - 1.14 (0.17)		1.04 - 2.81 (0.03)	
OPRM1 (rs2075572G>C)			n = 128	n = 232
C	137 (0.59)	80 (0.53)	67 (0.26)	84 (0.18)
OR	1.27		1.60	
95% CI (p-value)	0.82 - 1.96 (0.25)		1.09 - 2.35 (0.01)	

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

Table 4

Post-hoc power analysis and sample size calculations based on minor allele frequencies by ethnicity

Gene	Caucasian	Asian
AKT1 (rs3730358C>T)		
Power	0.10	0.53
N (per group)	1235	920
ARRB2 (rs4790694C>A)		
Power	0.21	0.62
N (per group)	473	429
BDNF (rs6265G>A)		
Power	0.48	0.61
N (per group)	193	171
COMT (rs4680G>A)		
Power	0.14	0.74
N (per group)	805	465
GSTP1 (rs1695A>G)		
Power	0.25	0.58
N (per group)	376	315
OPRM1 (rs2075572G>C)		
Power	0.18	0.68
N (per group)	554	223

Note: Estimated sample size calculated using the assumptions: $\beta=.20$, $\alpha=.05$