Published in final edited form as:

Am J Med Genet B Neuropsychiatr Genet. 2008 September 5; 147B(6): 793–798. doi:10.1002/ajmg.b.30716.

Catechol-O-Methyltransferase (*COMT*) Gene Variants: Possible Association of the Val158Met Variant With Opiate Addiction in Hispanic Women

Bronson E. Oosterhuis¹, K. Steven LaForge¹, Dmitri Proudnikov¹, Ann Ho¹, David A. Nielsen¹, Robert Gianotti¹, Sandra Barral², Derek Gordon³, Suzanne M. Leal^{4,5}, Jurg Ott^{5,6}, and Mary Jeanne Kreek^{1,*}

¹ Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, New York

² G.H. Sergievsky Center, Columbia University, New York, New York

³ Department of Genetics, Rutgers University, Piscataway, New Jersey

⁴ Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas

⁵ Laboratory of Statistical Genetics, The Rockefeller University, New York, New York

⁶ Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China

Abstract

Catechol-O-methyltransferase (*COMT*) catalyzes the breakdown of catechol neurotransmitters, including dopamine, which plays a prominent role in drug reward. A common single nucleotide polymorphism (SNP), G472A, codes for a Val158Met substitution and results in a fourfold down regulation of enzyme activity. We sequenced exon IV of *COMT* gene in search for novel polymorphisms and then genotyped four out of five identified by direct sequencing, using TaqMan assay on 266 opioid-dependent and 173 control subjects. Genotype frequencies of the G472A SNP varied significantly ($P = 0.029$) among the three main ethnic/cultural groups (Caucasians, Hispanics, and African Americans). Using a genotype test, we found a trend to point-wise association ($P = 0.053$) of the G472A SNP in Hispanic subjects with opiate addiction. Further analysis of G472A genotypes in Hispanic subjects with data stratified by gender identified a point-wise significant ($P = 0.049$) association of G/A and A/A genotypes with opiate addiction in women, but not men. These point-wise significant results are not significant experiment-wise (at $P < 0.05$) after correction for multiple testing. No significant association was found with haplotypes of the three most common SNPs. Linkage disequilibrium patterns were similar for the three ethnic/cultural groups.

Keywords

dopamine metabolism; gene variant; polymorphism; gender; heroin addiction

Drugs of abuse alter levels of neurotransmitters such as dopamine and serotonin, and dopaminergic systems play a prominent role in drug reward. Catechol-O-methyltransferase (*COMT*) is of importance in the biological actions and metabolism of dopamine because it

*Correspondence to: Mary Jeanne Kreek, M.D., Patrick E. and Beatrice M. Haggerty Professor, Head of Laboratory, Laboratory of the Biology of Addictive Diseases, Box 171, 1230 York Avenue, New York, NY 10021. kreek@rockefeller.edu.

catalyzes the biotransformation of catechol neurotransmitters including dopamine. The COMT enzyme is widely distributed in peripheral and central tissues [reviewed in Männistö and Kaakkola, 1999]. There are two forms of COMT in humans and other mammals. The soluble form (S-COMT) is 50 amino acids shorter than the membrane-bound form (MB-COMT). The variant forms are generated through alternative splicing. In most tissues, S-COMT accounts for only a small fraction of overall COMT activity [Rivett et al., 1983]. The highest ratios of MB-COMT to S-COMT are in the brain, and since MB-COMT has a higher substrate affinity for catecholamines than the soluble form, this MB-COMT may be important in regions where substrate levels are low [Rivett and Roth, 1982; Rivett et al., 1983; Roth, 1992; Männistö and Kaakkola, 1999].

The human *COMT* gene is located on chromosome 22q11.21, and MB-COMT is organized into six exons [Grossman et al., 1992; Tenhunen et al., 1994]. A common missense polymorphism (G472A, Val158Met substitution in the membrane-bound form) in exon IV of the *COMT* gene is well recognized to account for heritable differences in enzyme thermolability and activity [Weinshilboum and Dunnette, 1981; Lotta et al., 1995; Lachman et al., 1996]. The Met variant of the enzyme has activity fourfold lower compared to the Val variant. Variants of the *COMT* gene have been associated in some, but not all, studies with schizophrenia [e.g., Matthysse and Baldessarini, 1972; Egan et al., 2001], panic disorder [e.g., Hamilton et al., 2002; Woo et al., 2004], major depression and bipolar disorders [e.g., Fahndrich et al., 1982; Massat et al., 2005], obsessive compulsive disorder [e.g., Karayiorgou et al., 1997, 1999; Niehaus et al., 2001], attention deficit hyperactivity disorder [e.g., Eisenberg et al., 1999], and efficacy of response to L-Dopa in the treatment of Parkinson's disease [e.g., Reilly et al., 1980; Rivera-Calimlim and Reilly, 1984; Bialecka et al., 2004]. Recent studies have also found association of *COMT* gene variants with differences in higher order functioning, including memory and specific cognitive tasks in patients with schizophrenia, other psychiatric disorders, brain injury, as well as in normal control subjects [Diamond et al., 2004; Goldberg and Weinberger, 2004; Weickert et al., 2004; de Frias et al., 2005; Lipsky et al., 2005; Reuter et al., 2005; Bertolino et al., 2006].

The goal of this study was to sequence exon IV of the *COMT* gene in opioid-dependent and control subjects in search for novel polymorphisms and to investigate the possible association of identified variants with opioid dependence.

The 439 unrelated subjects participating in this study were recruited between February 7, 1995, and January 20, 2000. Each subject signed informed consent approved by the Rockefeller University Hospital Institutional Review Board for genetic studies. Subjects provided self-reported ethnic/cultural affiliation. Urine and blood samples were obtained and screened for drugs of abuse. Addiction history was characterized using the Addiction Severity Index (ASI) [McLellan et al., 1992]. All opioid-addicted subjects of the study were former heroin addicts recruited from methadone maintenance treatment programs in New York City; these subjects met U. S. Federal criteria for such treatment (daily self-administration of multiple doses of opiate drugs continuing for one or more years, the acquisition of dependence and tolerance, and demonstration of drug-seeking behavior).

Control subjects were not currently abusing drugs or alcohol. Subjects were excluded from the control group if for any period of 6 months or more, or last 30 days an illicit drug or alcohol to intoxication was used three or more times per week. Previous use of cannabis for three or more times per week for between 6 months and 4 years was not an exclusion criterion.

DNA was extracted from peripheral blood lymphocytes using a salting out procedure and stored at -80°C . Polymerase chain reaction (PCR) forward (5'-

CCAGCGGCCAGGCATTT-3') and reverse (5'-AGGCCCCACTCTGTCCC-3') primers were designed for exon IV of *COMT* gene (GenBank accession Z26491) using Oligo 4.1 program (National Biosciences, Ply-mouth, MN). PCR reactions were performed as previously described using step-down protocol (Yuferov et al., 2004). The samples were then purified and sequenced in both forward and reverse directions with the same primers used for amplification at the Rockefeller University DNA Sequencing Center. The forward and reverse electropherograms were assembled using SeqMan™ software (DNASTAR, Inc., Madison, WI) and were read independently by two researchers who had no knowledge of the subjects' phenotypic classifications.

Four of the five single nucleotide polymorphisms (SNPs) identified by direct sequencing (G304A, C408G, C438T, and G472A) were then genotyped by TaqMan® assays. Oligonucleotide primers and TaqMan® MGB probes (Supplement 1) were designed using Primer Express software (Applied Biosystems, Foster City, CA) and then custom-synthesized by Applied Biosystems. PCR cycling was performed in two replicates using Platinum® quantitative PCR SuperMix-UDG (Invitrogen, Carlsbad, CA) on a GeneAmp® PCR system 9700 and the dual 384-well sample block module (Applied Biosystems) using manufacturer's protocol. Genotype analysis was performed on the ABI Prism® 7900 sequence detection system using SDS 2.2 software (Applied Biosystems).

Demography of study subjects is given in Table I. Data collected from individuals from three major ethnic/cultural groups only (Caucasians, Hispanics or African Americans) were used for statistical analysis. Chi-square tests for deviations from Hardy–Weinberg equilibrium were performed. A low frequency (<0.01) SNP G304A was excluded from the analysis. Tests for differences in allele frequencies in control subjects from the three main ethnic/cultural groups were performed using Fisher's Exact Test [Freeman and Halton, 1951; Fisher, 1960]. Association of genotypes and alleles with opiate addiction was evaluated using Fisher's Exact Test. Results were corrected for multiple testing using the Bonferroni correction [Westfall and Young, 1993]. Association of haplotypes formed by polymorphisms C408G, C438T, G472A with opioid addiction was computed using the methods implemented in the SNPHAP (<http://www-gene.cimr.cam.ac.uk/clayton/software/>) and PHASE softwares [Stephens et al., 2001; Stephens and Donnelly, 2003]. Pairwise linkage disequilibrium between the three most common SNPs was calculated as computed by the Δ^2 measure of disequilibrium [Weir, 1990]. Patterns of LD were graphically plotted using GOLD software [Abecasis and Cookson, 2000].

By direct sequencing of exon IV of the *COMT* gene in DNA from 279 subjects we identified five SNPs: G304A, C408G, A431G, C438T, and G472A. Polymorphism C408G is synonymous (Leu136Leu). Polymorphisms G304A, A431G, C438T, and G472A result in amino acid change in the protein sequence: Ala102Thr, Asp144Gly, Ala146Val, and Val158Met, respectively. In the three predominant ethnic/cultural groups tested there were no significant deviations from Hardy–Weinberg equilibrium in case or control groups for any of the four SNPs at a significance level of $P < 0.05$.

Tests for differences in SNP genotype or allele frequencies among the three control ethnic/cultural groups (evaluated using Fisher's Exact Test) show point-wise significant differences for SNP G472A in the genotype ($P = 0.029$), but not the allele test ($P = 0.064$). Also, SNPs C438T and G472A show differences in allele frequencies that approach point-wise nominal significance at the $P < 0.05$ level ($P = 0.057$ and 0.064 , respectively).

Table II shows tests of genetic association of common SNPs with heroin addiction. We found a trend to nominal point-wise (uncorrected) association ($P = 0.053$) of polymorphism G472A in the Hispanic group with opioid addiction. Association analysis of this SNP with

the data stratified by gender using Chi-square tests is shown in Table III. In Hispanics, we found a point-wise significant association ($P = 0.049$) for genotypes containing the low-activity Met allele (G/A and A/A) with heroin addiction in women, but not in men. This finding was not experiment-wise significant.

The results of association of haplotypes formed by polymorphisms C408G, C438T, G472A with opioid addiction using SNPHAP and PHASE programs are shown in Table IV. No significant association between opiate dependence and haplotypes was found.

Supplements 2 and 3 show the linkage disequilibrium patterns in three control populations (Caucasian, Hispanic, and African American) as computed by the Δ^2 measure of disequilibrium. Interestingly, while the value of Δ^2 varies among populations, the patterns are virtually identical. The SNP pair that shows the most significant evidence for association is the pair C408A-G472A. The P values for testing pairwise linkage disequilibrium for this pair are 3.0×10^{-19} , 8.0×10^{-6} , and 2.0×10^{-3} , for the Caucasian, Hispanic and African American control groups, respectively.

As the result of direct sequencing, we identified five polymorphisms in exon IV of the *COMT* gene (G304A, C408G, A431G, C438T, and G472A). The functional change due to the amino acid substitution resulting from G472A (Val158Met) has been extensively studied. The G304A SNP, which was found in low allelic frequency, codes for an amino acid substitution of alanine to threonine, but there are no reports of its possible impact on function. A novel low frequency SNP A431G codes for an Asp144Gly amino acid change.

Our findings of a point-wise significant association of the genotypes containing the 158Met allele must be considered provisional since the significance was lost after correcting for multiple testing and also, this finding was observed in a small subset of study subjects: only one gender (females) and one ethnic/cultural group (Hispanics) which is known to be a cultural classification with significant admixture. Also, the odds ratio (OR) for opioid dependence for genotypes containing the 158Met allele for Hispanic females was 3.3, but for genetic contributions in complex disorders it is expected to be in the range of 1.2–1.4. Power calculations showed that with an OR of 3.3 the sample size of this group would have had reasonable power ($\alpha = 0.05$, $1 - \beta = 0.80$) to detect association using additive and multiplicative inheritance models, but not autosomal dominant or autosomal recessive inheritance models [Gordon et al., 2005]. The association of the 158Met allele with risk for opioid dependence in Hispanic women deserves to be further studied in additional patient or population samples.

Prolonged administration of drugs of abuse can lead to alterations in dopaminergic functioning, which has been postulated to underlie partially the development and persistence of addictions. For example, chronic administration of cocaine in experimental animal models results in lower striatal dopamine levels, and long-lasting reduction in striatal dopamine receptors, particularly dopamine D2 receptors [Maisonneuve et al., 1995; Tsukada et al., 1996; Maggos et al., 1998; Zhang et al., 2003]. Human studies of brain imaging document reductions in striatal dopamine D2 receptors in subjects addicted to a variety of drugs of abuse, including cocaine, heroin, alcohol, and methamphetamine [Volkow et al., 2004]. In a human positron emission tomography study, cocaine-addicted subjects who were administered methylphenidate (which cocaine-addicted subjects report as being similar to cocaine) showed increased activation of regions in the right medial orbital prefrontal cortex, whereas control subjects had decreased activation in these brain regions. These changes were associated with mood elevation and increased craving for cocaine in the addicted subjects [Volkow et al., 2005]. Evidence that the Val158Met genotype of the *COMT* enzyme can influence the effects of amphetamine on prefrontal cortical function comes from

a functional magnetic resonance imaging study in which the drug enhanced the prefrontal functioning in individuals homozygous for the Val allele during a working memory task. When administered amphetamine, subjects with the homozygous Met genotype showed no enhancement of cortical efficiency at low to moderate working memory load, and a decrease in cortical functioning when performing a task requiring a high working load [Mattay et al., 2003].

Previous studies have reported mixed results when evaluating the Val158Met polymorphism in association with addictive diseases. In a study of alcoholism, the low-activity Met allele was found to be associated with Type 1 (late-onset) alcoholism in two Finnish populations [Tiihonen et al., 1999]. In contrast, the high-activity Val allele and the Val/Val genotype was associated with risk for abuse and dependence on several drugs of abuse in Caucasian subjects recruited in the Baltimore, Maryland metropolitan area [Vandenberg et al., 1997]. The high-activity Val allele was also reported at a higher frequency in methamphetamine-abusing subjects compared to controls in Han Chinese studied in Taiwan [Li et al., 2004]. An association of the Val allele with heroin addiction was also found in a family-based haplotype relative risk study in three Israeli ethnic groups: Ashkenazi Jewish, non-Ashkenazi Jewish, and Palestinian Arab [Horowitz et al., 2000]. Finally, a study conducted in a Chinese population reported no differences in genotype or allele frequencies for the Val158Met polymorphism between opiate-dependent cases and controls [Cao et al., 2003].

The Val158Met substitution has been shown to have gender-specific implications. In vitro cellular studies have shown that physiological concentrations of 17-beta-estradiol can down-regulate *COMT* gene transcription and protein expression [Xie et al., 1999; Jiang et al., 2003]. Another study reported an association of low-activity Met alleles and obsessive-compulsive disorder in males, but not in females [Karayiorgou et al., 1999]. Studies in mice showed that *COMT* homozygous knockout females develop increased anxiety in a light/dark model compared to *COMT* knockout males. The same study found increased aggressive behavior in *COMT* heterozygous knockouts [Gogos et al., 1998] compared to other genotypes in males. In this study we performed association tests with data stratified by both ethnicity and gender for the G472A SNP. Our finding suggests that risk for opiate addiction contributed by this polymorphism may be limited to women and to specific ethnic/cultural group (Hispanics).

Additional studies of association of Val158Met polymorphism and other variants of the *COMT* gene with opioid and other addictions should be done to confirm and extend our findings. In addition, since past studies have found associations of the Val/Met polymorphism with personality traits, it would be worthwhile to study these traits in opioid-dependent individuals. Furthermore, it would be of interest to perform allele and genotype association tests with the amount of heroin or other drug consumption (both in amount and frequency of use) in dependent subjects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank K. Bell, RN, E. Ducat, NP, D. Melia, RN, G. Bart, MD, L. Borg, MD, P. McHugh, MD, and S. Kellogg, PhD for the assessment of subjects and for collecting blood samples. We also thank V. Yuferov, PhD and S. Schlussman, PhD for a critical review of the manuscript. This research was supported by grants NIH-NIMH-R01-44292 (J.O.) NIH-NIMH-R01-79880 (M.J.K.); NIH-NIDA-P60-05130 (M.J.K.); NIH-NIDA-K05-00049 (M.J.K.); and NIH-RR-UL1-RR024143 (Barry Collier).

Grant sponsor: NIH-NIMH; Grant number: R01-44292; Grant sponsor: NIH-NIMH; Grant number: R01-79880;
Grant sponsor: NIH-NIDA; Grant number: P60-05130; Grant sponsor: NIH-NIDA; Grant number: K05-00049;
Grant sponsor: NIH-RR; Grant number: UL1-RR024143.

References

- Abecasis GR, Cookson WO. GOLD-graphical overview of linkage disequilibrium. *Bioinformatics* 2000;16:182–183. [PubMed: 10842743]
- Bertolino A, Blasi G, Latorre V, Rubino V, Rampino A, Sinibaldi L, Caforio G, Petruzzella V, Pizzuti A, Scarabino T, Nardini M, Weinberger DR, Dallapiccola B. Additive effects of genetic variation in dopamine regulating genes on working memory cortical activity in human brain. *J Neurosci* 2006;26:3918–3922. [PubMed: 16611807]
- Bialecka M, Drozdziak M, Klodowska-Duda G, Honczarenko K, Gawronska-Szklarz B, Opala G, Stankiewicz J. The effect of monoamine oxidase B (MAOB) and catechol-*O*-methyltransferase (COMT) polymorphisms on levodopa therapy in patients with sporadic Parkinson's disease. *Acta Neurol Scand* 2004;110:260–266. [PubMed: 15355491]
- Cao L, Li T, Liu X. Association study of heroin dependence and catechol-*O*-methyltransferase gene. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2003;20:127–130. (Translated from Chinese). [PubMed: 12673581]
- de Frias CM, Annerbrink K, Westberg L, Eriksson E, Adolfsson R, Nilsson LG. Catechol *O*-methyltransferase Val158Met polymorphism is associated with cognitive performance in nondemented adults. *J Cogn Neurosci* 2005;17:1018–1025. [PubMed: 16102234]
- Diamond A, Briand L, Fossella J, Gehlbach L. Genetic and neuro-chemical modulation of prefrontal cognitive functions in children. *Am J Psychiatry* 2004;161:125–132. [PubMed: 14702260]
- Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci USA* 2001;98:6917–6922. [PubMed: 11381111]
- Eisenberg J, Mei-Tal G, Steinberg A, Tartakovsky E, Zohar A, Gritsenko I, Nemanov L, Ebstein RP. Haplotype relative risk study of catechol-*O*-methyltransferase (COMT) and attention deficit hyperactivity disorder (ADHD): Association of the high-enzyme activity Val allele with ADHD impulsive-hyperactive phenotype. *Am J Med Genet* 1999;88:497–502. [PubMed: 10490706]
- Fahndrich E, Muller-Oerlinghausen B, Coper H. Longitudinal assessment of MAO-, COMT-, and DBH-activity in patients with bipolar depression. *Int Pharmacopsychiatry* 1982;17:8–17. [PubMed: 6979526]
- Fisher, RA. The design of experiments. Edinburgh: Oliver and Boyd; 1960.
- Freeman GH, Halton J. Note on an exact treatment of contingency, goodness of fit and other problems of significance. *Biometrika* 1951;38:141–149. [PubMed: 14848119]
- Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, Karayiorgou M. Catechol-*O*-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc Natl Acad Sci USA* 1998;95:9991–9996. [PubMed: 9707588]
- Goldberg TE, Weinberger DR. Genes and the parsing of cognitive processes. *Trends Cogn Sci* 2004;8:325–335. [PubMed: 15242692]
- Gordon D, Haynes C, Blumenfeld J, Finch SJ. PAWE-3D: Visualizing power for association with error in case-control genetic studies of complex traits. *Bioinformatics* 2005;21(20):3935–3937. [PubMed: 16123114]
- Grossman MH, Emanuel BS, Budarf ML. Chromosomal mapping of the human catechol-*O*-methyltransferase gene to 22q11.1-q11.2. *Genomics* 1992;12:822–825. [PubMed: 1572656]
- Hamilton SP, Slager SL, Heiman GA, Deng Z, Haghighi F, Klein DF, Hodge SE, Weissman MM, Fyer AJ, Knowles JA. Evidence for a susceptibility locus for panic disorder near the catechol-*O*-methyltransferase gene on chromosome 22. *Biol Psychiatry* 2002;51:591–601. [PubMed: 11950461]
- Horowitz R, Kotler M, Shufman E, Aharoni S, Kremer I, Cohen H, Ebstein RP. Confirmation of an excess of the high enzyme activity COMT Val allele in heroin addicts in a family-based haplotype relative risk study. *Am J Med Genet* 2000;96:599–603. [PubMed: 11054766]

- Jiang H, Xie T, Ramsden DB, Ho SL. Human catechol-*O*-methyltransferase down-regulation by estradiol. *Neuropharmacology* 2003;45:1011–1018. [PubMed: 14573393]
- Karayorgou M, Altemus M, Galke BL, Goldman D, Murphy DL, Ott J, Gogos JA. Genotype determining low catechol-*O*-methyltransferase activity as a risk factor for obsessive-compulsive disorder. *Proc Natl Acad Sci USA* 1997;94:4572–4575. [PubMed: 9114031]
- Karayorgou M, Sobin C, Blundell ML, Galke BL, Malinova L, Goldberg P, Ott J, Gogos JA. Family-based association studies support a sexually dimorphic effect of COMT and MAOA on genetic susceptibility to obsessive-compulsive disorder. *Biol Psychiatry* 1999;45:1178–1189. [PubMed: 10331110]
- Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-*O*-methyltransferase pharmacogenetics: Description of functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 1996;6:243–250. [PubMed: 8807664]
- Li T, Chen CK, Hu X, Ball D, Lin SK, Chen W, Sham PC, Loh el-W, Murray RM, Collier DA. Association analysis of the DRD4 and COMT genes in methamphetamine abuse. *Am J Med Genet Part B* 2004;129B:120–124. [PubMed: 15274053]
- Lipsky RH, Sparling MB, Ryan LM, Xu K, Salazar AM, Goldman D, Warden DL. Association of COMT Val158Met genotype with executive functioning following traumatic brain injury. *J Neuropsychiatry Clin Neurosci* 2005;17:465–471. [PubMed: 16387984]
- Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen K, Julkunen I, Taskinen J. Kinetics of human soluble and membrane-bound catechol *O*-methyltransferase: A revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* 1995;34:4202–4210. [PubMed: 7703232]
- Maggos CE, Tsukada H, Kakiuchi T, Nishiyama S, Myers JE, Kreuter J, Schlussman SD, Unterwald EM, Ho A, Kreek MJ. Sustained withdrawal allows normalization of in vivo [11C]N-methylspiperone dopamine D2 receptor binding after chronic binge cocaine: A positron emission tomography study in rats. *Neuropsychopharmacology* 1998;19:146–153. [PubMed: 9629568]
- Maisonneuve IM, Ho A, Kreek MJ. Chronic administration of a cocaine “binge” alters basal extracellular levels in male rats: An in vivo microdialysis study. *J Pharmacol Exp Ther* 1995;272:652–657. [PubMed: 7853178]
- Männistö PT, Kaakkola S. Catechol-*O*-methyltransferase (COMT): Biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol Rev* 1999;51:593–628. [PubMed: 10581325]
- Massat I, Souery D, Del-Favero J, Nothen M, Blackwood D, Muir W, Kaneva R, Serretti A, Lorenzi C, Rietschel M, Milanova V, Papadimitriou GN, Dikeos D, Van Broekhoven C, Mendlewicz J. Association between COMT (Val158Met) functional polymorphism and early onset in patients with major depressive disorder in a European multicenter genetic association study. *Mol Psychiatry* 2005;10:598–605. [PubMed: 15583702]
- Mattay VS, Goldberg TE, Fera F, Hariri AR, Tessitore A, Egan MF, Kolachana B, Callicott JH, Weinberger DR. Catechol *O*-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc Natl Acad Sci USA* 2003;100:6186–6191. [PubMed: 12716966]
- Matthysse S, Baldessarini RJ. S-adenosylmethionine and catechol-*O*-methyltransferase in schizophrenia. *Am J Psychiatry* 1972;128:1310–1312. [PubMed: 5013751]
- McLellan AT, Kushner H, Metzger D, Peters R, Smith I, Grissom G, Pettinati H, Argeriou M. The fifth edition of the addiction severity index. *J Subst Abuse Treat* 1992;9:199–213. [PubMed: 1334156]
- Niehaus DJ, Kinnear CJ, Corfield VA, du Toit PL, van Kradenburg J, Moolman-Smook JC, Weyers JB, Potgieter A, Seedat S, Emsley RA, Knowles JA, Brink PA, Stein DJ. Association between a catechol-*O*-methyltransferase polymorphism and obsessive-compulsive disorder in the Afrikaner population. *J Affect Disord* 2001;65:61–65. [PubMed: 11426511]
- Reilly DK, Rivera-Calimlim L, Van Dyke D. Catechol-*O*-methyltransferase activity: A determinant of levodopa response. *Clin Pharmacol Ther* 1980;28:278–286. [PubMed: 7398195]
- Reuter M, Peters K, Schroeter K, Koebke W, Lenardon D, Bloch B, Hennig J. The influence of the dopaminergic system on cognitive functioning: A molecular genetic approach. *Behav Brain Res* 2005;164:93–99. [PubMed: 16026865]

- Rivera-Calimlim L, Reilly DK. Difference in erythrocyte catechol-*O*-methyltransferase activity between Orientals and Caucasians: Difference in levodopa tolerance. *Clin Pharmacol Ther* 1984;35:804–809. [PubMed: 6734032]
- Rivett AJ, Roth JA. Kinetic studies on the *O*-methylation of dopamine by human brain membrane-bound catechol *O*-methyltransferase. *Biochemistry* 1982;21:1740–1742. [PubMed: 7082642]
- Rivett AJ, Francis A, Roth JA. Localization of membrane-bound catechol-*O*-methyltransferase. *J Neurochem* 1983;40:1494–1496. [PubMed: 6834074]
- Roth JA. Membrane-bound catechol-*O*-methyltransferase: A reevaluation of its role in the *O*-methylation of the catecholamine neurotransmitters. *Rev Physiol Biochem Pharmacol* 1992;120:1–29. [PubMed: 1519017]
- Stephens M, Donnelly PA. Comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003;73:1162–1169. [PubMed: 14574645]
- Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001;68:978–989. [PubMed: 11254454]
- Tenhunen J, Salminen M, Lundström K, Kiviluoto T, Savolainen R, Ulmanen I. Genomic organization of the human catechol *O*-methyltransferase gene and its expression from two distinct promoters. *Eur J Biochem* 1994;223:1049–1059. [PubMed: 8055944]
- Tiihonen J, Hallikainen T, Lachman H, Saito T, Volavka J, Kauhanen J, Salonen JT, Ryyänänen OP, Koulou M, Karvonen MK, Pohjalainen T, Syvälahti E, Hietala J. Association between the functional variant of the catechol-*O*-methyltransferase (COMT) gene and type 1 alcoholism. *Mol Psychiatry* 1999;4:286–289. [PubMed: 10395222]
- Tsukada H, Kreuter J, Maggos CE, Unterwald EM, Kakiuchi T, Nishiyama S, Futatsubashi M, Kreek MJ. Effects of binge pattern cocaine administration on dopamine D1 and D2 receptors in the rat brain: An in vivo study using positron emission tomography. *J Neurosci* 1996;16:7670–7677. [PubMed: 8922423]
- Vandenbergh DJ, Rodriguez LA, Miller IT, Uhl GR, Lachman HM. High-activity catechol-*O*-methyltransferase allele is more prevalent in polysubstance abusers. *Am J Med Genet* 1997;74:439–442. [PubMed: 9259381]
- Volkow ND, Fowler JS, Wang GJ, Swanson JM. Dopamine in drug abuse and addiction: Results from imaging studies and treatment implications. *Mol Psychiatry* 2004;9:557–569. [PubMed: 15098002]
- Volkow ND, Wang GJ, Ma Y, Fowler JS, Wong C, Ding YS, Hitzemann R, Swanson JM, Kalivas P. Activation of orbital and medial prefrontal cortex by methylphenidate in cocaine-addicted subjects but not in controls: Relevance to addiction. *J Neurosci* 2005;25:3932–3939. [PubMed: 15829645]
- Weickert TW, Goldberg TE, Mishara A, Apud JA, Kolachana BS, Egan MF, Weinberger DR. Catechol-*O*-methyltransferase val108/158met genotype predicts working memory response to antipsychotic medications. *Biol Psychiatry* 2004;56:677–682. [PubMed: 1552252]
- Weinshilboum R, Dunnette J. Thermal stability and the biochemical genetics of erythrocyte catechol-*O*-methyltransferase and plasma dopamine-beta-hydroxylase. *Clin Genet* 1981;19:426–437. [PubMed: 7296933]
- Weir, BS. Genetic data analysis: Methods for discrete population genetic data. Sunderland: Sinauer Associates, Inc; 1990.
- Westfall, PH.; Young, SS. Examples and methods for P-value adjustment. New York: J Wiley and Sons; 1993. Resampling-based multiple testing.
- Woo JM, Yoon KS, Choi YH, Oh KS, Lee YS, Yu BH. The association between panic disorder and the L/L genotype of catechol-*O*-methyltransferase. *J Psychiatr Res* 2004;38:365–370. [PubMed: 15203287]
- Xie T, Ho S-L, Ramsden D. Characterization and implications of estrogenic down-regulation of human catechol-*O*-methyltransferase gene transcription. *Mol Pharmacol* 1999;56:31–38. [PubMed: 10385681]
- Yuferov V, Fassel D, LaForge KS, Nielsen DA, Gordon D, Ho A, Leal SM, Ott J, Kreek MJ. Redefinition of the human kappa opioid receptor gene (*OPRK1*) structure and association of haplotypes with opiate addiction. *Pharmacogenetics* 2004;14:793–804. [PubMed: 15608558]

Zhang Y, Schlussman SD, Ho A, Kreek MJ. Effect of chronic “binge cocaine” on basal levels and cocaine-induced increases of dopamine in the caudate putamen and nucleus accumbens of C57BL/6J and 129/J mice. *Synapse* 2003;50:191–199. [PubMed: 14515336]

TABLE I

Demography of Study Subjects

Category	Opioid-dependent	Control	Total
Ethnic/cultural demographics			
Caucasian	98	75	173
Hispanic	93	29	122
African American	58	42	100
Asian	1	20	21
Mixed ethnicity	14	7	21
Other	2	0	2
Total	266	173	439
Gender			
Male	149	93	242
Female	117	80	197
Total	266	173	439

TABLE II
Association of the Genotypes of Polymorphisms of the *COMT* Gene With Heroin Addiction

Ethnic/cultural group	Group of subjects	Genotype				Allele				
		GG (freq.)	GA (freq.)	AA (freq.)	P	G	A	95% CI	OR	P
Polymorphism G304A (rs5031015), Ala102Thr substitution										
Caucasians	Control.	75 (1.00)	0	0	NA	150 (1.00)	0	NA	NA	NA
	Op-dep.	97 (0.99)	1 (0.01)	0		195 (0.99)	1 (0.01)			
Hispanics	Control	29 (1.00)	0	0	NA	58 (1.00)	0	NA	NA	NA
	Op-dep.	92 (0.99)	1 (0.01)	0		185 (0.99)	1 (0.01)			
African Americans	Control	41 (0.98)	1 (0.02)	0	NA	83 (0.99)	1 (0.01)	NA	NA	NA
	Op-dep.	56 (0.97)	2 (0.03)	0		114 (0.98)	2 (0.02)			
Allele										
Ethnic/cultural group		Genotype				Allele				
Group of subjects		CC (freq.)	CG (freq.)	GG (freq.)	P	C	G	95% CI	OR	P
Polymorphism C408G (rs4818), Leu136Leu synonymous										
Caucasians	Control	24 (0.32)	36 (0.48)	15 (0.20)	0.492	84 (0.56)	66 (0.44)	0.823, 1.953	1.268	0.282
	Op-dep.	40 (0.94)	41 (0.42)	17 (0.17)		121 (0.62)	75 (0.38)			
Hispanics	Control	13 (0.45)	11 (0.38)	5 (0.17)	0.420	37 (0.64)	21 (0.36)	0.804, 2.807	1.502	0.200
	Op-dep.	51 (0.55)	33 (0.35)	9 (0.10)		135 (0.73)	51 (0.27)			
African Americans	Control	25 (0.60)	13 (0.31)	4 (0.10)	1.000	63 (0.75)	21 (0.25)	0.523, 1.913	1.000	1.000
	Op-dep.	34 (0.59)	19 (0.33)	5 (0.09)		87 (0.75)	29 (0.25)			
Allele										
Ethnic/cultural group		Genotype				Allele				
Group of subjects		AA (freq.)	AC (freq.)	CC (freq.)	P	A	C	95% CI	OR	P
Polymorphism C438T (rs8192488), Ala146Val substitution										
Caucasians	Control	75 (1.00)	0	0	NA	150 (1.00)	0	NA	NA	NA
	Op-dep.	98 (1.00)	0	0		196 (1.00)	0			
Hispanics	Control	28 (0.97)	1 (0.03)	0	0.420	57 (0.98)	1 (0.02)	0.224, 16.114	1.900	1.000
	Op-dep.	87 (0.84)	6 (0.06)	0		180 (0.97)	6 (0.03)			
African Americans	Control	35 (0.83)	7 (0.17)	0	0.872	77 (0.92)	7 (0.08)	0.378, 2.848	1.038	0.943
	Op-dep.	49 (0.84)	8 (0.14)	1 (0.02)		106 (0.91)	10 (0.09)			
Allele										
Ethnic/cultural group		Genotype				Allele				
Group of subjects		CC (freq.)	CG (freq.)	GG (freq.)	P	C	G	95% CI	OR	P

Ethnic/cultural group	Group of subjects	Genotype				Allele				
		GG (freq.)	GA (freq.)	AA (freq.)	P	G	A	95% CI	OR	P
		GG (freq.)	GA (freq.)	AA(freq.)	P	G	A	95% CI	OR	P
Polymorphism G472A (rs4680), Val158Met substitution										
Caucasians	Control	21 (0.28)	40 (0.53)	14 (0.19)	0.543	82 (0.55)	68 (0.45)	0.664, 1.560	1.018	0.936
	Op-dep.	32 (33)	44 (0.45)	22 (0.22)		108 (0.55)	88 (0.45)			
Hispanics	Control	14 (0.48)	9 (0.31)	6 (0.21)	0.053	37 (0.64)	21 (0.36)	0.466, 1.582	0.859	0.626
	Op-dep.	30 (0.32)	52 (0.56)	11 (12)		112 (0.60)	74 (0.40)			
African Americans	Control	20 (0.48)	19 (0.45)	3 (0.07)	0.568	59 (0.70)	25 (0.25)	0.598, 2.068	1.112	0.737
	Op-dep.	32 (0.55)	20 (0.35)	6 (0.10)		84 (0.72)	32 (0.28)			

NA, data is not available; computations were not performed since the sample size was not sufficient to estimate odd ratios and confidence intervals.

TABLE III

Genotypes and Alleles of the G472A SNP Stratified by Ethnicity and Gender

Ethnic/cultural group	Gender	Genotype*				Allele frequency			
		GG	GA	AA	P	G	A	95% CI	OR
Caucasian, n = 173	Male control	13	19	8	0.969	45	35	0.569, 1.756	1.000
	Male opioid	20	32	12		72	56		
	Female control	8	21	6	0.120	37	33	0.514, 1.958	1.003
	Female opioid	12	12	10		36	32		0.992
Hispanic, n = 122	Male control	3	5	3	0.433	11	11	0.394, 3.789	1.500
	Male opioid	16	28	6		60	40		0.389
	Female control	11	4	3	0.053	26	10	0.252, 1.373	0.252
	Female opioid	14	24	5		52	34		0.217
African American, n = 100	Male control	13	10	2	0.965	36	14	0.396, 2.077	0.907
	Males opioid	15	12	3		42	18		0.818
	Female control	7	9	1	0.260	23	11	0.561, 3.670	1.435
	Female opioid	17	8	3		42	14		0.450

* Number of subjects are shown; a nominal point-wise significant ($P = 0.049$) association of opioid dependence was found for genotypes GA and AA (versus GG) in Hispanic women, but not men; odds ratios and confidence intervals computed using the method implemented in the GENESTAT webtool (URL: <http://fhg.gsf.de/cgi-bin/hw/hwa1.pl>).

TABLE IV
Case/Control Haplotype-Based Association Analysis (Polymorphisms C408G, C438T, G472A)

Method	Ethnic/cultural group	Group of subjects	Haplotypes				LRT	Df	P
			ATG	ATA	GTG	ACG			
SNPHAP	Caucasian	Opioid-dependent	0.168	0.449	0.383	0.000	2.872	3	0.412
		Control	0.108	0.453	0.439	0.000			
	Hispanic	Opioid-dependent	0.296	0.398	0.274	0.032	1.854	4	0.763
		Control	0.362	0.362	0.259	0.017			
PHASE	African American	Opioid-dependent	0.388	0.276	0.250	0.086	0.130	4	0.998
		Control	0.369	0.298	0.250	0.083			
	Caucasian	Opioid-dependent	0.168	0.449	0.383	0.000	NA	NA	0.257
		Control	0.107	0.453	0.440	0.000			
	Hispanic	Opioid-dependent	0.296	0.397	0.274	0.032	NA	NA	0.543
		Control	0.259	0.362	0.362	0.017			
African American	Opioid-dependent	0.381	0.284	0.249	0.085	NA	NA	0.994	
	Control	0.370	0.297	0.250	0.083				

Three-locus haplotype frequency estimates are provided for control and case groups in three ethnic/cultural groups using the methods implemented in SNPHAP and PHASE software. Likelihood Ratio Test (LRT) results for SNPHAP are shown, with *P* values determined assuming asymptotic distribution theory. For the PHASE method, *P* values are determined using 1,000 permutations of case/control status. NA, not applicable.