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

Bronson E. Oosterhuis[1], **K. Steven LaForge**[1], **Dmitri Proudnikov**[1], **Ann Ho**[1], **David A. Nielsen**[1], **Robert Gianotti**[1], **Sandra Barral**[2], **Derek Gordon**[3], **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 [Vandenbergh 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.

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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

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TABLE II

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

		Genotype				Allele				
Ethnic/cultural group	**Group of subjects**	**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)			
		Genotype				Allele				
Ethnic/cultural group	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)			
		Genotype				Allele				
Ethnic/cultural group	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)			
Ethnic/cultural group	Group of subjects	Genotype				Allele				

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

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

*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://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

TABLE IV

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

			Haplotypes						
Method	**Ethnic/cultural group**	**Group of subjects**	**ATG**	**ATA**	**GTG**	**ACG**	**LRT**	**Df**	***P***
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			
	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			
PHASE	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.

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