American Journal of Medical Genetics (Neuropsychiatric Genetics) 74:439–442 (1997)

High-Activity Catechol-O-Methyltransferase Allele is More Prevalent in Polysubstance Abusers

David J. Vandenbergh,[1] Lawrence A. Rodriguez,[1] Ivan T. Miller,[2] George R. Uhl,[1,3] and Herbert M. Lachman[2]*

[1]*Molecular Neurobiology Branch, Intramural Research Program, National Institute on Drug Abuse, NIH, Baltimore, Maryland*
[2]*Departments of Medicine and Psychiatry, Albert Einstein College of Medicine, Bronx, New York*
[3]*Departments of Neurology and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland*

Allelic variants at the catechol-O-methyltransferase (COMT) locus are candidates to contribute to genetic components of interindividual differences in vulnerability to substance abuse. COMT plays a prominent role in dopaminergic circuits important for drug reward, and COMT alleles encode enzymes whose activities vary from three- to fourfold. We compared COMT allele frequencies in control research volunteers reporting insignificant lifetime use of addictive substances with those in volunteers reporting substantial polysubstance use. Homozygosity for the high-activity COMT allele was found in 18% of controls, 31% of volunteers with high lifetime substance use, and 39% meeting DSMIII-R substance abuse criteria [odds ratio (relative risks) 2.0 (control vs. use; 95% confidence interval 1.2–3.5; *P* < 0.013) and 2.8 (control vs. DSM; 1.3–6.1; *P* < 0.008)]. Individuals with the high-activity COMT variant may have greater genetic vulnerability to drug abuse. Am. J. Med. Genet. 74:439–442, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: abuse; cocaine; opiates; polymorphism; dopamine

INTRODUCTION

The use of addictive substances is likely to represent complex interactions between genetic and environmental factors [Uhl et al., 1995]. Evidence from twin studies indicates that drug abuse phenotypes, including quantity/frequency of use and features responsible for Diagnostic and Statistical Manual of Mental Disorders III-Revised (DSMIII-R) diagnoses of substance abuse/dependence, are each likely to reflect significant genetic contributions [Goldberg et al., 1993; Pickens et al., 1995; Johnson et al., 1996; Tsuang et al., 1996].

Dopaminergic brain systems play prominent roles in drug reward [Gardner, 1992], focusing attention on genes expressed in these circuits as candidates to contribute to substance abuse vulnerability. Catechol-O-methyltransferase (COMT) is expressed in dopaminergic brain regions, where its activity provides a pathway by which extraneuronally released dopamine is inactivated [Kopin, 1994]. Three- to fourfold differences in human COMT activities are attributed to codon 158 polymorphisms that encode either a valine (GTG) that produces an enzyme with higher activity or a methionine (ATG) that produces a markedly lower activity variant [Spielman and Weinshilboum, 1981; Boudikova et al., 1990; Aksoy et al., 1993; Lotta et al., 1995; Lachman et al, 1996a]. To seek influences of this COMT allelic variation on substance abuse vulnerability, we have compared COMT genotypes in a group of unrelated polysubstance abuser research volunteers, whose use was defined on the bases of 1) quantity/frequency of self-reported peak lifetime drug use and 2) DSMIII-R criteria for substance abuse/dependence, with the genotypes of control research volunteers free from significant use of addictive substances.

MATERIALS AND METHODS

Patient Ascertainment

Three hundred nine caucasian research volunteers were recruited from the National Institute on Drug Abuse Intramural Research Program (263), an adjacent hemodialysis unit (4), and a public health facility studying HIV infection in Baltimore (42). Lifetime histories of use of each class of licit and illicit substances were assessed for each research volunteer by using the Drug Use Survey (DUS), an instrument with a test-retest reliability coefficient of 0.78 and an interrater reliability coefficient of 0.94, as described previously [Smith et al., 1992]. The DUS assessed volunteers'

*Correspondence to: H.M. Lachman, Departments of Medicine and Psychiatry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail: lachman@aecom.yu.edu

Received 1 January 1997; Revised 4 April 1997

peak lifetime use of each of 15 classes of addictive substances, assigned a 0–3+ rating for each drug class and produced a composite score for total use of addictive substances [Smith et al., 1992]. Control volunteers analyzed here displayed total drug use scores of 0 or 1; no subject with a DSM diagnosis of abuse or dependence was included. A DUS score of 0 allowed minimal use of alcohol, nicotine, or marijuana and no use of other drugs, whereas a score of 1 allowed moderate use of alcohol, nicotine, or marijuana and/or minimal use of other drugs. Data from these control volunteers were contrasted with data from heavy substance abusers, who rated total DUS drug use scores of 3 based on heavy use of at least one illicit drug, often associated with heavy use of nicotine and/or alcohol. The controls were also contrasted with a subgroup of 41 of these individuals who achieved DSMIII-R drug abuse or dependence diagnoses. Concordance between the two measures of drug use was high: Ninety-two percent of the individuals in this study with DSMIII-R drug abuse or dependence diagnoses also received a DUS total drug use score of 3, and 92% of the individuals with DUS scores of 0 or 1 were free from diagnosis of drug abuse or dependence.

Mean ages were 29 ± 0.5 years for controls and 34 ± 0.4 years for drug users, statistically significantly different values that both fall after the age of incidence of most addictive substance use. Analyses using only controls 25 years of age and older removed the differences in mean ages (33 vs. 34) while retaining the significant differences in allelic frequencies (see below) between these controls and abusers characterized by quantity/frequency assessments ($X^2 = 9.71$, df = 2, $P = 0.008$).

COMT Polymorphisms

COMT genotypes were determined by restriction fragment length polymorphism (RFLP) analysis from DNA extracted from the subjects' peripheral blood by an investigator who was unaware of phenotype. The polymorphism is generated by the presence of a G or A encoding a valine or a methionine at codon 158 of the membrane-bound form, a codon equivalent to 108 of the cytoplasmic form of the enzyme [Tenhunen et al., 1994; Lotta et al., 1995; Lachman et al., 1996a]. A 210-base pair (bp) radiolabelled PCR product was generated by using the primers 5′-CTCATCACCATCGAGATCAA and 5′-GATGACCCTGGTGATAGTGG, corresponding to nucleotides 1,881–1,900 and 2,071–2,090 (GenBank accession number z2649) [Bertocci et al., 1991; Lundstrom et al., 1991; Tenhunen et al., 1994]. The PCR product (10 μl) was treated with 5 units of *Nla*III for 3 hours at 37°C, and an 85-bp fragment characteristic of the GTG (valine) codon 158 allele and a 67-bp fragment characteristic of the ATG (methionine) allele were separated by electrophoresis using 8% nondenaturing polyacrylamide gels [Lachman et al., 1996a]. Statistical analyses used SPSS (SPSS Inc., Chicago, IL) and Linkage Utility (Jurg Ott).

RESULTS

Primary analyses revealed significant differences in the distributions of both COMT genotypes and allele frequencies between controls (n = 124) and substance abusers defined by quantity/frequency of use (n = 185; genotypes: $X^2 = 6.51$, df = 2, $P = 0.038$; allele frequencies: $X^2 = 5.75$, df = 1, $P = 0.02$; this and other comparisons are evident in Table I). This difference was found in males; the small female sample (n = 59) did not allow adequate power for statistical significance (data not shown). A second analysis was calculated by using the subgroup of the drug abusing volunteers who had also been administered the Diagnostic Interview Schedule (DIS) for DSMIII-R diagnoses of substance abuse or dependence (n = 41). This analysis also revealed significant differences between COMT genotypes in abuser and control groups, accompanied by a trend in allele frequency distributions that did not reach statistical significance (genotypes: $X^2 = 7.92$, df = 2, $P = 0.02$; allele frequencies: $X^2 = 2.95$, df = 1, $P = 0.1$).

Examining genotypes that encode extreme phenotypic values may help reveal the nature of a single gene's contribution to a complex disorder. The work of Spielman and Weinshilbaum [1981] and Lachman et al. [1996a] both support a good predictive relationship between COMT genotype and COMT enzymatic activity determinations. These workers' data suggest an additive model for COMT activity; homozygote genotypes represent high and low COMT activities that can differ by three- to fourfold. However, several sorts of models could describe plausible relationships between COMT activities and substance abuse vulnerability, including threshold models. Accordingly, a third analysis examined the proportions of the highest activity homozygotes (G/G) to the lower activity genotypes (A/A and A/G) in substance abuser and control groups. G/G homozygotes are nearly twice as frequent in volunteers who report high quantity/frequency drug use than in controls free from such use ($X^2 = 6.29$, df = 1, $P = 0.012$). The sample defined on the basis of DSM diagnoses also shows a similar, statistically significant difference from control values ($X^2 = 7.16$, df = 1, $P = 0.007$). Analysis of the frequency of A/A homozygotes in

TABLE I. Catechol O-Methyltransferase Genotypes and Allele Frequencies in Drug Users and Controls

	Genotype frequencies (n)[a]			Allele frequencies (n)	
Sample	G/G%	A/G%	A/A%	G%	A%
Controls	18 (23)	51 (63)	31 (38)	44 (109)	56 (139)
Abusers (DUS quantity/ frequency)[b]	31 (58)	45 (83)	24 (44)	54 (199)	46 (171)
Diagnosis (DSM abuse/ dependence)[c]	39 (16)	32 (13)	29 (12)	55 (45)	45 (37)

[a]There was no significant deviation from the expected Hardy-Wienberg proportions for our data. DUS, Drug Use Survey; DSM, Diagnostic and Statistical Manual.

[b]Controls vs. abusers (DUS quantity/frequency measure). genotypes: $\chi^2 = 6.51$, $P = 0.038$ (df = 2); alleles: $\chi^2 = 5.75$, $P = 0.02$ (df = 1); G/G homozygote: $\chi^2 = 6.29$, $P = 0.012$ (df = 1).

[c]Controls vs. abusers (DSM diagnosis). genotypes: $\chi^2 = 7.92$, $P = 0.02$ (df = 2); alleles: $\chi^2 = 2.95$, $P = 0.11$ (df = 1); G/G homozygote: $\chi^2 = 7.16$; $P = 0.007$ (df = 1).

the samples reveals only a modest trend toward control/abuser differences, with 1.2–1.4-fold higher frequencies in the control sample ($X^2 = 1.79$, $P = 0.18$ for quantity/frequency; $X^2 = 0.03$, $P = 0.87$ for DSM diagnoses).

DISCUSSION

These results support the concept that inheritance of higher activity *COMT* alleles may increase drug abuse vulnerability. Results of association studies need to be considered in relationship to the nature of the populations investigated. In prior studies that assessed COMT genotypes or enzymatic activities in individuals, screening for use of addictive substances was not conducted. In these studies, the frequency of the high-activity allele was 0.47 in 78 British Caucasians [Daniels et al., 1996] and 0.6 in 87 Caucasian research volunteers recruited largely in New York [Lachman et al., 1996b]. Enzyme activity values in 893 caucasian volunteers collected at the Mayo Clinic found a frequency of 0.54 [Spielman and Weinshilboum, 1981]. However, this frequency is dependent on the breakpoints chosen to assign an enzyme activity level to a genotype. Our current data and the differences in *COMT* allelic frequencies among previously reported Caucasian samples may reflect sample-to-sample differences due to problems of stratification.

We have attempted to minimize the opportunity for stratification based on race or ethnicity by sampling control and polysubstance abusing research volunteers of self-reported Caucasian ethnicity from a restricted geographic area that provides widespread availability of abused substances: metropolitan Baltimore. In addition, the subjects had largely passed through the ages of risk for initiating drug use [Bennett, 1983]. Interim analysis revealed similar allele frequencies in the first 2/3 and the last 1/3 of the volunteers sampled here (data not shown).

It is important that provisional results from any association study be replicated in independent samples. A replicate analysis of drug abuse phenotypes and COMT genotypes that seeks differences in the same proportions reported here should reach significance ($\alpha < 0.05$) with a minimum $n = 171$ if the current results correctly identify the magnitude and the variability of a true association.

Recent data have documented that different features of drug abuse phenotypes, including those that contribute to DSMIII-R diagnoses and quantity-frequency assessments, may display different degrees of genetic loading [Johnson et al., 1996]. Thus, we have studied individuals assessed for two substance abuse phenotypes. Because quantity/frequency assessments provide distributed values, we contrasted individuals self-reporting high-level use of addictive substances with those reporting minimal use. It is possible for an individual to use significant quantities of illicit drugs and yet not fulfill the criteria for a diagnosis of drug abuse based on DSMIII-R. Thus, we used the same group of controls with minimal drug use for comparison with individuals diagnosed as drug abusing or drug dependent. The positive association obtained by using DSMIII-R criteria was obtained from a subset of individuals characterized by DUS and thus, is not entirely independent. However, the DSMIII-R represents an alternative method for determining phenotype widely used in clinical settings. Although further studies could analyze individuals assessed separately by using these two approaches, the initial data from the present report at least suggest that both approaches might yield positive associations.

Transgenic mice with altered expression of genes important for dopaminergic reward pathways respond to abused substances differently from wild type littermates [Miner et al., 1995]. Frequencies of markers at several other, but not all, dopaminergic gene loci can also differ between human drug abusers and controls [Smith et al., 1992; Noble et al., 1993; Persico et al., 1993; Gelernter et al., 1994; Uhl, 1994; Muramatsu and Higuchi, 1995]. The current work is part of ongoing efforts to identify genes involved in vulnerability to substance abuse that has focused on dopaminergic genes. Failure of DNA from subsets of the same individuals to display associations with markers at the DAT or VMAT loci provides some evidence for the specificity of the current results. Shortening the persistance of released dopamine in brain reward circuits through expression of a high-activity COMT variant could provide a specific and plausible mechanism for gene-neurotransmission-behavior association. It is also important to note that the current data cannot exclude a conceivable role for a functional polymorphism nearby and in linkage disequilibrium with the COMT codon 158 polymorphism used in this work.

COMT enzymatic activity in the brain can be altered by some currently available drugs. Identification of functional COMT allelic variants predisposing to substance abuse vulnerability could have significant therapeutic implications. Replication and extension of the current findings that COMT provides a strong candidate locus for substance abuse vulnerability could open the way for both improved understanding and enhanced therapeutic opportunities in addressing the difficult problems that drug abuse poses to individuals and society.

ACKNOWLEDGMENTS

This work was supported by the Intramural Research Program, NIDA, NIH, the Carmel Hill Fund, and the New York State Office of Alcohol and Substance Abuse Services (H.M.L.). We thank Elisabeth Bendahhou, Brenda Campbell, Judy Hess, and Carlo Contoreggi for their assistance.

REFERENCES

Aksoy S, Klener J, Weinshilboum RM (1993): Catechol-O-methyltransferase pharmacogenetics: Photoaffinity labelling and Western blot analysis of human liver samples. Pharmacogenetics 3:116–122.

Bennett G (1983): Substance abuse in adulthood. In Bennett G, Vourakis C, Woolf DS (eds). "Substance Abuse: Pharmacological, Developmental, and Clinical Perspectives." New York: John Wiley and Sons. pp. 175–191.

Bertocci B, Miggiano V, Da Prada M, Dembric Z, Lahm H-W, Malherbe P (1991): Human catechol-O-methyltransferase: Cloning and expression of the membrance associated form. Proc Natl Acad Sci USA 88:1416–1420.

Boudikova B, Szumlanski C, Maidak B, Weinshilboum RM (1990): Human liver catecholamine O-methyltransferase pharmacogenetics. Clin Pharmacol Ther 48:381–389.

Daniels JK, Williams NM, Williams J, et al. (1996): No evidence for allelic association between schizophrenia and a polymorphism determining high or low catechol O-methyltransferase activity. Am J Psychiatr 153: 268–270.

Gardner EL (1992): Brain reward mechanisms. In Lowinson JH, Ruiz P, Millman RB, Langrod JG (eds): "Substance Abuse: A Comprehensive Textbook, 2nd Ed." Baltimore: Williams and Wilkins, pp. 70–99.

Gelernter J, Kranzler HR, Satel SL, Rao PA (1994): Genetic association between dopamine transporter protein alleles and cocaine-induced paranoia. Neuropsychopharmacology 11:195–200.

Goldberg J, Lyons MJ, Eisen SA, True WR, Tsuang M (1993): Genetic influence on drug use: A premiminary analysis of 2674 Vietnam era veteran twins. Behav Genet 23:552.

Johnson EO, van den Bree MBM, Uhl GR, Pickens RW (1996): Indicators of genetic and environmental influences in drug abusing individuals. Drug Alcohol Depend 41:17–23.

Kopin IJ (1994): Monoamine oxidase and catecholamine metabolism. J Neural Transm 41(Suppl):57–67.

Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM (1996a): Human catechol-O-methyltransferase pharmacogenetics: Description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6:243–250.

Lachman HM, Morrow B, Shprintzen R, Veit S, Parsia S, Faedda G, Goldberg R, Kucherlapati R, Papolos DF (1996b): Association of codon 108/158 catechol-O-methyltransferase gene polymorphism with the psychiatric manifestations of velo-cardio-facial-syndrome. Am J Med Genet (Neuropsychiatr Genet) 67:468–472.

Lotta T, Vidgren J, Tilgmann C, Umanen I, Melen K, Julkunen I, Taskinen J (1995): Kinetics of human soluble and membrane bound catechol-O-methyltransferase: A revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry 34:4202–4210.

Lundstrom K, Salminen M, Jalanko A, Savolainen R, Ulmanen I (1991): Cloning and characterization of human placental catechol-O-methyltransferase cDNA. DNA Cell Biol 10:181–189.

Miner LL, Drago J, Chamberlain PM, Donovan D, Uhl GR (1995): Retained cocaine conditioned place preference in D1 receptor deficient mice. Neuroreport 6:2314–2316.

Muramatsu T, Higuchi S (1995): Dopamine transporter gene polymorphism and alcoholism. Biochem Biophys Res Comm 211:28–32.

Noble EP, Blum K, Khalsa ME, Ritchie T, Montgomery A, Wood RC, Fitch RJ, Ozkaragoz T, Sheridan PJ, Anglin MD, et al. (1993): Allelic association of the D2 dopamine receptor gene with cocaine dependence. Drug Alcohol Depend 33:271–285.

Persico AM, Vandenbergh DJ, Smith SS, Uhl GR (1993): Dopamine transporter gene polymorphisms are not associated with polysubstance abuse. Biol Psychiatr 34:265–267.

Pickens RW, Svikis DS, McGue M, LaBuda M (1995): Common genetic mechanisms in alcohol, drug and mental disorder comorbidity. Drug Alcohol Depend 39:129–138.

Smith SS, O'Hara BF, Persico AM, Gorelick DA, Newlin DB, Vlahov D, Solomon L, Pickens R, Uhl GR (1992):Genetic vulnerability to drug abuse. The D2 dopamine receptor TaqI B1 restriction fragment length polymorphism appears more frequently in polysubstance abuse. Arch Gen Psychiatr 49:723–727.

Spielman RS, Weinshilboum RM (1981): Genetics of red cell COMT activity: Analysis of thermal stability and family data. Am J Med Genet 10:279–290.

Tenhunen J, Salminen M, Lundstrom K, Kiviluoto T, Savolainen R, Ulmanen I (1994): Genomic organization of the human catechol-O-methyltransferase gene and its expression from two distinct promoters. Eur J Biochem 223:1049–1054.

Tsuang MT, Lyons MJ, Eisen SA, Goldberg J, True W, Lin N, Meyer JM, Toomey R, Faraone SV, Eaves L (1996): Genetic influences on DSM-III-R drug abuse ad dependence: A study of 3,372 twin pairs. Am J Med Genet 67:473–477.

Uhl GR (1994): Association strategies in substance abuse. In Jansson B, Jornvall H, Rydberg U, Terenius L, Vallee BL (eds): "Toward a Molecular Basis of Alcohol Use and Abuse." Basel: Berkhauser Verlag. 71:133–141.

Uhl GR, Elmer GI, Labuda MC, Pickens RW (1995): Genetic influences in drug abuse. In Bloom FE, Kupfer DJ (eds): "Psychopharmacology: The Fourth Generation of Progress." New York: Raven Press. pp. 1793–1806.