Lack of Association of Alcohol Dependence and Habitual Smoking With Catechol-O-methyltransferase

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Objective: To test whether variation in the gene encoding the enzyme catechol-O-methyltransferase (COMT), which catalyzes the breakdown of dopamine and other catecholamine neurotransmitters, is associated with the risk for alcohol dependence and habitual smoking.

Methods: Single nucleotide polymophisms (SNPs) were genotyped in a sample of 219 multiplex alcohol-dependent families of European American descent from the Collaborative Study on the Genetics of Alcoholism (COGA). Family-based tests of association were performed to evaluate the evidence of association between the 18 SNPs distributed throughout *COMT,* including the functional Val158Met polymorphism, and the phenotypes of alcohol dependence, early onset alcohol dependence, habitual smoking, and comorbid alcohol dependence and habitual smoking.

Results: No significant, consistent evidence of association was found with alcohol dependence, early onset alcohol dependence, habitual smoking or the comorbid phenotype. There was no evidence that the functional Val158Met polymorphism, previously reported to be associated with these phenotypes, was associated with any of them.

Conclusion: Despite the substantial size of this study, we did not find evidence to support an association between alcohol dependence or habitual smoking and variation in *COMT*.

Key Words: Alcoholism, COMT, Genetic Association, Family Study, Smoking, SNP.

ALCOHOLISM IS A complex disorder with a significant genetic contribution to risk of this disease (Heath et al., 1997; Kendler et al., 1994; Pickens et al., 1991; Pollock et al., 1987). Recently, several genes have been associated with the risk for alcoholism, including *GABRA2* (Covault et al., 2004; Edenberg et al., 2004; Fehr et al., 2006; Lappalainen et al., 2005), *ADH4* (Edenberg et al., 2006; Guindalini et al., 2005; Luo et al., 2005b), and *CHRM2* (Luo et al., 2005a; Wang et al., 2004), and their role in alcoholism susceptibility has been confirmed in multiple studies. Additional genes have also been implicated in disease risk, including *GABRG3* (Dick et al., 2004) and a bitter taste receptor, *TAS2R16* (Hinrichs et al., 2006). Despite these successes in dissecting the genetic susceptibility to alcoholism, it is clear that additional genes must also contribute because of the relatively small influence of the individual genes identified thus far.

There is also substantial evidence for a genetic contribution to nicotine dependence (Carmelli et al., 1992; Heath et al., 1995; Kendler et al., 1999; Lessov et al., 2004; Li et al., 2003; Maes et al., 2004; Sullivan and Kendler, 1999; Swan et al., 1997; True et al., 1999; Tyndale, 2003) with 50% of the liability estimated to be due to genetic factors (Sullivan and Kendler, 1999; True et al., 1999). However, it appears that different sets of overlapping genes contribute to variation in smoking initiation and persistence of smoking (Heath et al., 2002; Kendler et al., 1999; Madden et al., 1999; Tyndale, 2003). Linkage analyses have been performed using several different tobacco-related phenotypes, including habitual smoking (Bierut et al., 2004), number of cigarettes smoked per day (smoking quantity), heaviness of smoking index on a 7-point scale (Li et al., 2006), maximum number of cigarettes smoked in a 24-hour period (Saccone et al., 2007) and the quantitative scale from the Fagerstrom Tolerance Questionnaire (Swan et al., 2006). Most of these studies identified linkage to unique chromosomal regions. Two studies (Bierut et al., 2004; Swan et al., 2006) have reported linkage to the same regions on chromosomes 6, 7, and 8 using the phenotypes of habitual smoking and the Fagerstrom Tolerance Questionnaire. One study found significant evidence of linkage to chromosome 10 when analyzing the heaviness of smoking index (Li et al., 2006). Employing the maximum number of cigarettes smoked in a 24-hour period yielded significant evidence of linkage to chromosome 22 (22 to 27 cM),

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although not in the *COMT* region. The dopa decarboxylase (DDC) gene has been associated with smoking phenotypes in several studies (Ma et al., 2005; Yu et al., 2006).

Studies have shown substantial comorbidity of tobacco and alcohol dependence. This observation led to further analyses which suggest that there are also likely to be some common genetic risk factors that contribute to both addictions (Hopfer et al., 2001; Swan et al., 1996; True et al., 1999). The identification of genes with pleiotropic effects on both tobacco and alcohol could provide important insight regarding the mechanisms of addiction.

Abnormal dopaminergic neurotransmission has been hypothesized to play just such a role in the risk for addictions (Volkow et al., 1996). The enzyme catechol-O-methyltransferase (COMT) plays an important role in the metabolism of central nervous system catecholamines, including dopamine and norepinephrine. A common G to A polymorphism (rs4680) in the *COMT* gene at codon 158 results in the replacement of the amino acid methionine (Met) for valine (Val) in exon 4. This amino acid substitution results in 3- to 4-fold variation in enzyme activity (Chen et al., 2004; Weinshilboum et al., 1999). The Val158 allele is commonly referred to as the high (H) activity allele, while the Met158 allele is termed the low (L) activity allele. The COMT activity distribution is trimodal, corresponding to the 3 *COMT* genotypes at this single nucleotide polymorphism (SNP) (Lachman et al., 1996), indicating additive effects on activity.

Numerous studies have explored the potential linkage and/or association of *COMT* with psychiatric disorders, including bipolar affective disorder, schizophrenia, panic disorder, obsessive compulsive disorder, drug dependence, and alcohol dependence (Craddock et al., 2006); however, results have been inconsistent. Most association studies have focused on the Val158Met polymorphism that affects enzyme activity. The Met158 allele has been associated with late onset alcoholism in men (Hallikainen et al., 2000; Tiihonen et al., 1999). This same allele has been associated with early onset alcoholism in one study (Wang et al., 2001) but not in others (Hallikainen et al., 2000; Ishiguro et al., 1999). The Met158 allele has also been associated with elevated weekly alcohol consumption in male social drinkers (Kauhanen et al., 2000). In a Plains Indian sample, the Val158 allele, in contrast, was associated with the risk of alcoholism (Enoch et al., 2006).

Some studies have found an association of the Val158Met polymorphism with a subject's Fagerstrom score (Beuten et al., 2006). Further analyses indicated that the haplotypes with the Val158 (i.e., G allele) were protective while those with the Met158 (i.e., A allele) were at higher risk for nicotine dependence. Analyses in the Plains Indian samples (Enoch et al., 2006) indicated that the Met158 allele had its highest frequency in the female subset of the sample that was not only alcohol dependent but also smoked heavily. Genotyping of additional SNPs in *COMT* in the Plains Indian samples suggested that the *COMT* haplotypes associated with alcohol dependence and smoking were not solely defined by the presence of the valine polymorphism, but that additional sequence variation in *COMT* may contribute to the risk for alcohol dependence and smoking (Enoch et al., 2006).

Because the evidence regarding the association of COMT and various psychiatric disorders is inconsistent, we have rigorously evaluated the role of many variations in COMT as a risk factor for alcohol dependence and habitual smoking in a large sample of multiplex alcohol-dependent families.

MATERIALS AND METHODS

Sample

The Collaborative Study on the Genetics of Alcoholism (COGA) is an ongoing multi-site study that has recruited families at 6 centers across the United States: Indiana University, State University of New York Downstate Medical Center, University of Connecticut, University of Iowa, University of California/San Diego, and Washington University, St Louis. This study was approved by the institutional review boards of all participating institutions.

Probands were identified through alcohol treatment programs. A poly-diagnostic interview instrument, the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994; Hesselbrock et al., 1999), was administered to the proband and their family members. Details of the ascertainment and assessment of the sample have been described elsewhere (Foroud et al., 2000; Reich et al., 1998). A subset of families that included at least 3 first degree relatives who met both lifetime DSM-IIIR criteria for alcohol dependence (American Psychiatric Association, American Psychiatric Association and Work Group to Revise DSM-III, 1987) and lifetime Feighner (Feighner et al., 1972) criteria for definite alcoholism, participated in the genetic phase of this study. Due to racial differences in SNP allele frequency and patterns of linkage disequilibrium (LD), we restricted all analyses to a sample of 1,923 European American subjects from 219 families, which was used in this analysis.

Phenotypes

Two phenotypes were employed in the family-based genetic analyses: alcohol dependence and habitual smoking (Table 1). From the SSAGA data, subjects were classified as alcohol dependent according to the DSM-IV criteria (American Psychiatric Association, American Psychiatric Association and Task Force on DSM-IV, 2000) and included 753 affected individuals. Individuals without a completed SSAGA ($n = 123$) were classified as unknown. In a previous study, an association was found with early onset alcoholism, defined as

Table 1. Phenotypic Characteristics of the 1,923 Genotyped Individuals in 219 Families[a]

	Male	Female	Total
Alcohol dependent[b]	499	254	753
Habitual smoker[c]	481	348	829
Not habitual smoker[d]	139	260	399
Alcohol dependent and habitual smoker	327	127	454
Nonalcohol dependent and nonhabitual smoker	60	182	242

[a]There are 123 individuals, 64 males and 59 females, who have genotypic information but did not complete a SSAGA and therefore are classified as unknown for both the phenotype of alcohol dependence and habitual smoking.

[b]Alcohol dependence defined by DSM-IV criteria.

[c]Habitual smokers defined as ever smoking at least 1 pack (20 cigarettes) daily for 6 months or more.

[d]Nonhabitual smokers defined as smoked fewer than 1 pack (20 cigarettes) or smoked for <6 months.

onset of DSM-IV alcohol dependence prior to 25 years of age (Wang et al., 2001). To test the potential association of COMT with early onset alcohol dependence, we identified a large subset of our sample (n = 503; 67% of the DSM-IV alcohol-dependent sample) with early onset alcohol dependence (by the same criteria); for these specific analyses using the restricted definition, individuals with onset of DSM-IV alcohol dependence at age 25 years or greater were coded as unknown. Although some studies (Hallikainen et al., 2000; Tiihonen et al., 1999) found evidence of association of the Met158 allele with late onset alcoholism in men, we did not have the power to test for that specific association because our sample was predominantly of early onset (average age of onset in genotyped sample = 23.5 years, median age = 21 years).

Corroborative analyses were performed using 2 additional alcohol dependence diagnoses: a narrower alcohol dependence definition using the criteria of the International Classification of Disease [ICD-10; (World Health Organization 1993)] that included 565 affected individuals and a less restrictive definition using criteria for both DSM-III-R alcohol dependence (American Psychiatric Association, American Psychiatric Association and Work Group to Revise DSM-III, 1987) and Feighner definite alcoholism (Feighner et al., 1972) that included 884 affected individuals.

Nicotine dependence per se was not initially assessed in the COGA study sample. Therefore, habitual smoking was employed as a related phenotype (Bierut et al., 2004), defined as ever smoking at least one pack (20 cigarettes) daily for 6 months or more (n = 829). Individuals who smoked fewer than 1 pack or smoked for $<$6 months were classified as nonhabitual smokers (n = 399). All others were coded as unknown for the analysis (n = 695). To test the possibility of association with the comorbid phenotype of alcohol dependence and habitual smoking, additional analyses were performed wherein individuals who met criteria for both DSM-IV alcohol dependence and habitual smoking (n = 454) were classified as affected. To draw a strong contrast with the affected group, only subjects meeting criteria for neither phenotype (n = 242) were classified as unaffected in those analyses. All others (n = 1127) were classified as unknown.

SNP Selection and Genotyping

COMT contains 6 exons and spans 27 kb on chromosome 22q (Fig. 1*A*). SNPs distributed throughout the gene were selected from public databases, primarily dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP). At the time when some SNPs were selected, allele frequencies were not available. To determine allele frequencies and to test the assays, SNPs were genotyped in 2 sets of samples, each consisting of 40 unrelated individuals from the Coriell European- and African-American diversity samples. All genotyped SNPs were in Hardy-Weinberg equilibrium in both test populations. Most SNPs were located in noncoding regions of the genes; known-coding SNPs were also genotyped, in particular rs4680, which is the well-studied Val158Met polymorphism (Table 2). The location of each SNP was determined from the annotations in the NCBI human genome assembly Build 35.1.

Genotyping was done using a modified single nucleotide extension reaction with allele detection by mass spectrometry (Sequenom MassArray system; Sequenom, San Diego, CA). The assays were designed and run with either of 2 formats, hME™ or iPLEX™. With the exception of rs9332381 (success rate 87%), all other SNPs had a genotypic success rate of 93.4% or greater. All SNP genotypes were checked for Mendelian inheritance using the program PEDCHECK (O'Connell and Weeks, 1998). Marker allele frequencies and heterozygosities were computed in the COGA sample using the program USERM13, part of the MENDEL linkage computer programs (Boehnke, 1991). All markers were assessed for significant (p < 0.001) deviations from Hardy Weinberg equilibrium.

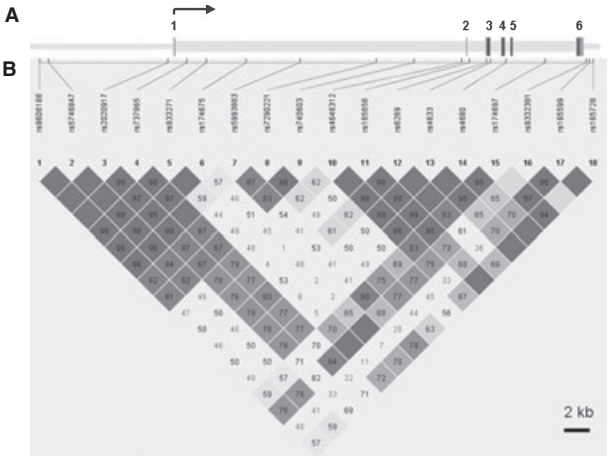

Fig. 1. (**A**) Genomic structures of catechol-O-methyltransferase (*COMT*). The gene structure of *COMT* is based on transcript NM_000754.The direction of transcription and the exons are indicated in arrow and numerical number, respectively. The size of the gene is indicated in scale at the lower right. (**B**) Pairwise linkage disequilibrium (LD). Pairwise LD (D′) estimates among the 18 genotyped SNPs. Darkly shaded boxes have strong evidence of LD, defined as a pair of SNPs with the 1-sided upper 95% confidence bound on D′ of 0.98 and the lower bound above 0.7. Lightly shaded boxes have lower LD.

Statistical Analyses

SNP coverage across *COMT* was evaluated using the program HAPLOVIEW (Barrett et al., 2005) which examined the extent of LD between pairs of SNPs. The program Tagger (de Bakker et al., 2005) (http://www.broad.mit.edu/mpg/tagger/) was used to estimate how well the SNPs genotyped in this study represented the genetic information contained in nongenotyped SNPs. Because some of the SNPs genotyped in this study were not in the HapMap database and therefore could not be evaluated by Tagger, this method underestimates the extent to which the genotyped SNPs in this study also carry information on the nongenotyped variation in the gene.

The Pedigree Disequilibrium Test (PDT) (Martin et al., 2000), as implemented in the program UNPHASED (Dudbridge, 2003), was used to test for association with alcohol dependence and habitual smoking in the extended, multiplex COGA pedigrees. The PDT utilizes data from all available trios in a family, as well as from discordant sibships. Evidence for association is assessed based on (1) the overtransmission of a particular allele to affected individuals and (2) the greater frequency of the allele in affected individuals as compared with their unaffected siblings. Results from the "avg-PDT" statistic, which weighs each family equally in computing the overall test statistic (Martin et al., 2001), are reported.

RESULTS

Eighteen SNPs covering the entire COMT gene and spanning 10 kb in the promoter and upstream flanking region (Fig. 1*A*) were genotyped. The LD between adjacent genotyped SNPs was high (D′ ≥ 0.57) (Fig. 1*B*). Twelve of the 18 genotyped SNPs were in the HapMap database, and using the HapMap data we were able to estimate that these SNPs captured the information from 25 of the 40 SNPs (63%) in the region (minor allele frequency [MAF] ≥ 0.05) at $r^2 \geq 0.8$, with a mean r^2 of 0.77 (and from 30 of the 40 SNPs (75%) in

Table 2. Association of Single Nucleotide Polymophisms (SNPs) With Alcoholism and Smoking

SNP ID	Chromosome position[a]	SNP location[b]	MAF[c]	Alcohol dependence[d]			
				DSM-IV	Early onset DSM-IV	DSM-IV + habitual smoking	Habitual smoking[d,e]
rs9606186	18294913	Upstream	0.43	0.37	0.86	0.78	0.53
rs5746847	18295557	Upstream	0.43	0.30	0.78	0.74	0.39
rs2020917	18303438	Upstream	0.27	0.11	0.18	0.97	0.71
rs737865	18304675	Intron 1	0.27	0.06	0.08	0.75	0.35
rs933271	18305961	Intron 1	0.30	0.82	0.44	0.71	0.75
rs174675	18308605	Intron 1	0.30	0.52	0.25	0.73	0.92
rs5993883	18312192	Intron 1	0.48	0.36	0.93	0.42	0.42
rs7290221	18317234	Intron 1	0.49	0.10	0.45	0.86	0.69
rs740603	18319731	Intron 1	0.47	0.52	0.93	0.83	0.74
rs4646312	18322891	Intron 1	0.39	0.43	0.26	0.78	0.94
rs165656	18323417	Intron 2	0.47	0.48	0.19	0.93	0.48
rs6269	18324506	Intron 2	0.39	0.22	0.16	0.97	0.74
rs4633	18324789	Exon 3, His62	0.47	0.24	0.12	0.93	0.94
rs4680[f]	18325825	Exon 4, Val158Met	0.47	0.15	0.08	0.88	0.88
rs174697	18328386	Intron 5	0.05	0.54	0.62	0.09	0.34
rs9332381	18331107	Downstream	0.04	0.31	0.04	0.96	0.59
rs165599	18331335	Downstream	0.30	0.05	0.07	0.13	0.60
rs165728	18331577	Downstream	0.05	0.20	0.64	0.10	0.08

[a]Chromosome positions are based on NCBI Human Genome Assembly v. 35.1.
[b]SNP location relative to the exons of *COMT*, based on transcript NM_000754.
[c]Minor allele frequency in European Americans.
[d]*p*-Value of avg-PDT statistic for associations between the SNPs and phenotypes shown.
[e]Habitual smoking defined as ever smoking at least 1 pack (20 cigarettes) daily for 6 months or more.
[f]Val158Met polymorphism.

the region at $r^2 \geq 0.5$). Because 6 of the 18 genotyped SNPs were not considered in the HapMap analysis, these estimates are a conservative estimate of the SNP gene coverage. There was no significant deviation from Hardy Weinberg equilibrium for any of the SNPs.

The primary analysis examined the evidence for an association between the SNPs in *COMT* and the phenotype of alcohol dependence, as defined by DSM-IV. As shown in Table 1, despite the large number of SNPs genotyped throughout the gene and the range of SNP informativeness as measured by minor allele frequency, no SNPs provided evidence of association with alcohol dependence (all $p \geq 0.05$). Narrowing the sample of affected individuals to only those with early onset alcohol dependence, defined as onset before 25 years of age, did not substantially alter the results. A single SNP, rs9332381, resulted in a nominally significant association ($p = 0.04$). This SNP had low informativeness (MAF = 0.04), and other SNPs in LD with this SNP did not provide significant association with the early onset alcohol dependence phenotype. Taken together, these data would suggest that the nominally significant association with rs9332381 is likely to be a false-positive result. No other SNP, including rs4680, was significant (all $p \geq 0.05$). Corroborative analyses employing a narrower (ICD-10) and broader (DSM-IIIR + Feighner definite) definition of alcohol dependence yielded only a single SNP with evidence of a significant association. This SNP, rs9332381, was significant when employing the broadest definition of alcohol dependence and likely represented a false-positive finding. All other tests of association were not significant (all $p \geq 0.05$).

Secondary analyses focused on the evaluation of association with habitual smoking. There was no evidence of association with any of the SNPs genotyped in *COMT* (all $p > 0.08$). Further analyses considering as affected only those individuals with both DSM-IV alcohol dependence and habitual smoking were also negative (all $p \geq 0.10$).

DISCUSSION

The enzyme COMT is a good candidate gene for influencing both alcohol dependence and smoking, because it plays an important role in the metabolism of central nervous system catecholamines, including dopamine and norepinephrine. Because the results from previous studies have been inconsistent, we evaluated the possible association of variations across the COMT gene with alcohol dependence and habitual smoking in a large sample of multiplex alcohol-dependent families Importantly, we did not limit our analysis of *COMT* to only the Val158Met polymorphism. While this SNP was included in the genotyped SNPs, 17 additional SNPs were also genotyped throughout *COMT*, allowing comprehensive evaluation of association. We did not find consistent, significant evidence of association between multiple SNPs in *COMT* and alcohol dependence, habitual smoking or the comorbid phenotype of alcohol dependence, and habitual smoking in our large sample of European Caucasian multiplex alcohol-dependent families. Restricting the analysis to early onset alcohol dependence did not provide consistent evidence of association, nor did analyses using narrower (ICD-10) or broader (DSM-IIIR + Feighner definite) criteria for alcohol depen-

dence. Despite reports from previous studies, we also did not find evidence of association ($p = 0.15$) with the Val158Met polymorphism located in exon 4, which has substantial effect on enzyme activity (Lachman et al., 1996).

In reviewing the association results from this study, we have considered not only the strength of association based on the *p*-value, but also the patterns of LD in *COMT*. One SNP, rs9332381, yielded nominally significant evidence ($p < 0.05$) for an association with early onset alcohol dependence, and one ($p = 0.05$) with broadly defined alcohol dependence. There are several additional SNPs which yielded association results that are not statistically significant but might be considered to be a trend ($0.05 \leq p \leq 0.10$). These trend level results were scattered throughout COMT and were observed with different phenotypes. Our interpretation of these results is that they do not appear to be consistent with patterns of LD and seem most likely to be due to false-positive association results.

In several previous studies, association analyses focused on only men (Hallikainen et al., 2000; Tiihonen et al., 2000) or women (Enoch et al., 2006), or stratified the sample based on age of onset (Hallikainen et al., 2000; Tiihonen et al., 2000; Wang et al., 2001) or other comorbid phenotypes such as cigarette smoking (Enoch et al., 2006). To reduce the potential increase in false-positive results due to population stratification, our study was designed to employ family-based tests of association. Due to the ascertainment and recruitment strategy, it was possible to perform analyses considering only those with early onset alcohol dependence. Previous analyses (Wang et al., 2001) reported that although there was no overall association between the Val158Met polymorphism and alcoholism in a study of 70 trios, nor in other subsets of the data based on gender or age, the Met158 allele was preferentially associated with early onset alcoholism in a subset of 28 trios having a male proband. In the present, larger family study, we found no evidence of association of the Val158Met polymorphism with either alcoholism or early onset alcoholism (Table 1), although the latter discrepancy may be due to our inclusion of females. We also analyzed a common alcohol-related comorbid phenotype, habitual smoking, and did not detect evidence of association with this phenotype either.

Enoch et al. (2006) genotyped several SNPs within *COMT* and found the greatest evidence of association with the Val158 allele in women who were both alcohol dependent and smokers. Further examination of *COMT* haplotypes indicated that the association was not entirely due to the Val158Met polymorphism, as some haplotypes carrying the Val158 allele were not significantly associated (Enoch et al., 2006). The present study genotyped more SNPs than the number reported by Enoch et al. (2001). However, even with a dense set of SNPs, we were not able to detect significant, consistent evidence of association in any portion of the *COMT* gene with the phenotypes of alcohol dependence, habitual smoking or habitual smokers who are also alcohol dependent.

This study has several advantages over previous studies evaluating the role of *COMT* in alcohol dependence and smoking. First, we employed a large sample that included 1,923 individuals from 219 families in which family-based tests of association were to be performed. Second, we analyzed only European American families to reduce the potential effects of racial differences in SNP allele frequencies and in the patterns of LD in the *COMT* gene. Third, in addition to evaluating one primary alcohol dependence phenotype, we also performed confirmatory analyses in a subset of early onset subjects as well as 2 definitions of alcohol dependence. Fourth, we performed extensive genotyping throughout *COMT* to ensure maximal power to detect association.

The data from the large number of multiplex alcohol-dependent families do not support an association between alcohol dependence or habitual smoking and variation in *COMT*. Despite the substantial size of this sample, we cannot eliminate the possibility that variation in *COMT* might have a very small effect on the risk for alcohol dependence or habitual smoking, although this effect would be unlikely to be clinically relevant. There is one alternative explanation for the variable pattern of association of COMT with different alcohol-related phenotypes. It is possible that *COMT* may act on an intermediate endophenotype related to alcohol dependence, such as cognitive ability or personality (Barnett et al., 2007; Enoch et al., 2003; Stein et al., 2005).

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