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The Association Between the *SLC6A3 VNTR 9*-Repeat Allele and Alcoholism—A Meta-Analysis

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Abstract

Background—Dopamine transporter gene (*SLC6A3*) represents a promising candidate involved in the development of alcoholism. This study aimed to explore the association between the 9-repeat allele (*A9*) of a 40-bp variable number tandem repeat (*VNTR*) polymorphism in the 3′ untranslated region (3′ UTR) of the *SLC6A3* gene and alcoholism.

Methods—The *SLC6A3 VNTR* was genotyped by PCR in unrelated Mexican Americans including 337 controls and 365 alcoholics. Pearson's chi-square test or Fisher's exact test was used to compare the genotype and allele distribution. Meta-analyses were performed for population-based case–control association studies of the *SLC6A3 VNTR* polymorphism with alcoholism. Data were analyzed under random effect models with the Comprehensive Meta-analysis (v.2) statistical software package.

Results—In Mexican Americans, no significant difference was found in allele and genotype distribution between controls and alcoholics or between controls and alcoholics with alcohol withdrawal seizure (AWS) or delirium tremens (DT) (unadjusted $p > 0.05$). A total of 13 research articles were included in the meta-analyses. No significant difference of the *SLC6A3 VNTR A9* was noted between controls and alcoholics at the genotypic and allelic level when all ethnic populations, only Caucasian populations, or only Asian populations were considered ($p > 0.05$). Significant associations were observed between *SLC6A3 VNTR A9* and alcoholics with AWS or DT at the genotypic as well as allelic level when all ethnic populations or only Caucasian populations were considered ($p < 0.05$, OR 1.5–2.1).

Conclusions—Meta-analyses suggest a possible association between the *SLC6A3 VNTR A9* and alcoholic subgroup with AWS or DT.

Keywords

Alcoholism; Dopamine Transporter; Variable Number Tandem Repeat; Meta-Analysis; Mexican American

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Alcoholism is a widespread psychiatric disorder, affecting 5.4% of the general population (Kessler et al., 2005). Family and adoption studies support the role of a genetic component in alcoholism. Furthermore, twin studies estimate that the heritability ranges between 50 and 60% (Hiroi and Agatsuma, 2005). The candidate gene approach has been widely used to study the genetic risk factors for alcoholism. Alcoholism is regarded as a "reward deficiency syndrome" (Blum et al., 1996, 2000, 2008; Bowirrat and Oscar-Berman, 2005; Comings and Blum, 2000). Thus, genes in the reward system are frequently considered as candidates in association studies, among which is the dopamine transporter (*DAT1 or DAT*), namely solute carrier family 6 member 3 (*SLC6A3*).

The dopaminergic neurotransmission pathway is a pivotal part of the reward system, and some components including dopamine receptor D2 (*DRD2*) (Blum et al., 1990; Du and Wan, 2009; Noble, 2003; Wang et al., 2007) have been associated with alcoholism. SLC6A3 protein is located at the pre-synaptic membrane of the dopaminergic synapse. Reuptake of dopamine into presynaptic neurons by means of SLC6A3 is the primary mechanism for termination of dopaminergic neurotransmission, and some studies have suggested a relationship between SLC6A3 and alcoholism. It was found that nucleus accumbens and/or striatal SLC6A3 density was significantly reduced in type-1 nonviolent late-onset alcoholics or alcoholics on admission for detoxification compared with controls (Laine et al., 1999; Repo et al., 1999; Tiihonen et al., 1995; Tupala et al., 2000, 2001). Indeed *SLC6A3* represents a promising candidate gene in the dopaminergic pathway that could be involved in the development of alcoholism.

Most association studies focused on a 40-bp variable number tandem repeat (*VNTR*) polymorphism in the 3′un-translated region (3′ UTR) of *SLC6A3*. The copy number of the *VNTR* ranges from 3 to 16 (Vandenbergh et al., 1992). In different ethnic populations, the 10-repeat allele (*A10*) is the most frequent allele followed by the 9-repeat allele (*A9*) (Bannon et al., 2001; Doucette-Stamm et al., 1995; Vandenbergh et al., 1992). The frequencies of other alleles are very low.

Both positive and negative results have been observed for the association between the *SLC6A3 VNTR* and alcoholism (Bau et al., 2001; Chen et al., 2001; Dobashi et al., 1997; Foley et al., 2004; Gorwood et al., 2003; Heinz et al., 2000; Kohnke et al., 2005; Le Strat et al., 2008; Muramatsu and Higuchi, 1995; Parsian and Zhang, 1997; Samochowiec et al., 2008; Sander et al., 1997; Ueno et al., 1999). Under the additive model, no significant association is found in Asians (Chen et al., 2001; Dobashi et al., 1997) or Caucasians (Foley et al., 2004; Heinz et al., 2000; Le Strat et al., 2008; Parsian and Zhang, 1997; Samochowiec et al., 2008) at the genotypic or allelic level. Under the dominant model, more *A9* carriers are found in Caucasian alcoholics than in controls (Kohnke et al., 2005), while another study does not find such an association (Sander et al., 1997).

The search for specific genes conferring susceptibility to alcoholism is complicated by the heterogeneity of the disease. Focusing on homogeneous alcoholic subpopulations may help elucidate the complex pathogenic mechanism. It is likely that some alcoholic subtypes are more homogeneous or genetically determined, such as severe forms of alcoholism (Walters, 2002). The physiological component of alcohol dependence, defined by tolerance or

withdrawal, has been associated with a greater severity of dependence (Schuckit et al., 2003). Severe alcohol withdrawal complications, namely alcohol withdrawal seizure (AWS) and delirium tre-mens (DT), occur in <13% of alcohol-dependent patients (Schuckit et al., 1995), who form the relatively homogeneous alcoholic subgroup. According to previous studies, the geno-types containing the *A9* allele are significantly more prevalent in alcoholics with AWS or DT than in controls (Sander et al., 1997).

Environmental factors, such as education background and marital status, also play an important role in pathogenesis of polygenic disorders including alcoholism. Marriage is significantly associated with decreases in drug and alcohol use in both men and women (Flora and Chassin, 2005; Hanna et al., 1993; Thundal and Allebeck, 1998). Our previous study suggests the main effect of education background as well as the education**OPRM1 A118G* interaction in contribution to moderate and/or severe alcoholism in Mexican Americans (Du and Wan, 2009). The *SLC6A3**environmental factors interaction has not been analyzed.

The *A9* allele seems to be associated with alcoholism, but inconsistent findings have been reported by different genetic association studies. No such association studies have been conducted in Mexican Americans who represent a prominent segment of the American mosaic and appear to be at a high risk for alcohol problems. In the current study, association of *SLC6A3 VNTR A9* with alcoholism was explored in Mexican Americans. In addition, meta-analyses were performed to further establish the relationship of *SLC6A3 VNTR A9* with alcoholism.

METHODS AND MATERIALS

Study Population

Unrelated Mexican Americans living in the Los Angeles County including 337 controls and 365 alcoholics were recruited. Controls and alcoholics were gender- and age-matched. Participants were recruited from a variety of sources including: (i) Human services, substance dependence, and mental health programs; (ii) driving schools and the Alcohol Anonymous Groups organized and/ or associated with these schools; (iii) bars and liquor stores; (iv) Hispanic churches with counseling services programs; (v) day labor centers; and (vi) Hispanic newspapers, radio stations, and television stations. The alcoholic participants fulfilled the Diagnostic and Statistical Manual of Mental Disorder, Fourth Edition (DSM-IV) criteria for a current diagnosis of either alcohol dependence (303.90) or alcohol abuse (305.00). Control participants fulfilled the following criteria: (i) no current or past diagnosis of DSM-IV alcohol dependence (303.90) or alcohol abuse (305.00); and (ii) no clinically unacceptable findings from physical examinations and vital signs. The inclusion criteria for both controls and alcoholics were as follows: (i) ability to give informed consent; (ii) between 21 and 79 years; (iii) 3 of 4 biological grandparents of Mexican heritage; (iv) fluency in either Spanish or English; (v) no current use of other substances (except tobacco and caffeine), or history of such use within the past 6 months; and (vi) no current or past diagnosis of mental illnesses such as schizophrenia, schizophreniform disorder, schizoaffective disorder, schizotypal disorder, major depression, antisocial personality disorder, anxiety disorder, or bipolar disorder. Written informed consent was obtained from

each participant. The use of participants' DNA samples was approved by the Human Subjects Committees at the University of Kansas Medical Center and Los Angeles Biomedical Research Institute at Harbor-University of California, Los Angeles Medical Center. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

Interview Instrument

Every participant was interviewed with a standard questionnaire to collect the basic information including gender, age, educational background, marital status, and alcohol consumption. All alcoholic participants were interviewed by the Semi-Structured Assessment for the Genetics of Alcoholism II (SSAGA II) (Bucholz et al., 1994). The SSAGA was translated into Spanish and then validated. The results were reviewed by a psychiatrist. Information of severe withdrawal symptoms including DT and AWS was obtained through the SSAGA interview.

Genotyping

Peripheral venous blood samples were collected for all participants and kept at −80°C until DNA extraction. The frozen blood was thawed and leukocyte DNA was isolated by a rapid nonenzymatic method (Lahiri and Nurnberger, 1991) or by GeneCatcher gDNA blood kits (Invitrogen, Carlsbad, CA). With primers 5′-TGTGGTGTAGGGAACGGCCTGAG-3′ and 5′-CTTCCTGGAGGTCACGGCTCAAGG-3′ (Vandenbergh et al., 1992), PCR amplification was carried out in 25 μl reaction mix containing 100 ng genomic DNA, 1 × Green Go Taq Flexi buffer, 0.32 μM each primer, 0.24 mM dNTP, 1.5 mM $MgCl_2$, and 2 U Go Taq DNA polymerase. The thermal cycling conditions included 95°C for 5 minutes, then 40 cycles for 30 seconds at 95°C, 30 seconds at 68°C, and 90 seconds at 72°C, with a final extension step of 7 minutes at 72°C. PCR products were examined on a 3% agarose gel. Seven alleles were distinguished according to the length of PCR product: 3 repeats—200 bp, 6 repeats—320 bp, 8 repeats—400 bp, 9 repeats—440 bp, 10 repeats—480 bp, 11 repeats—520 bp, and 12 repeats—560 bp. Approximately 15% of the samples (55 and 50 individuals in alcoholics and controls, respectively) were randomly selected for blind re-genotyping.

Selection of Studies for Meta-Analyses

Population-based case–control association studies of the *SLC6A3 VNTR* polymorphism between healthy controls and alcoholics or between healthy controls and alcoholic subgroup with severe withdrawal symptoms (AWS or DT) were included in meta-analyses. Studies reporting data on either single-sex or both male and female participants of any ethnic origin were included. Only a few family-based association studies were reported in the literature, and those studies were excluded from the current meta-analyses.

Literature Search Strategy

The literature search was performed in PubMed, BIOSIS Previews, SCI, and PsycINFO databases. Key words including all the possible combinations of "alcoholism," "alcohol dependence," "dopamine transporter," "DAT," "DAT1," and "SLC6A3" were searched in

title or abstract from the database up to July 30, 2009. Once articles were obtained, bibliographies listed in the articles were then hand-searched for additional references. The abstracts of studies identified by these search strategies were then examined with reference to the above inclusion and exclusion criteria. Duplications were deleted and the full text of each reference was then checked to further establish whether the study met the inclusion criteria. The articles we retrieved as well as the results of current study in Mexican Americans were included in the meta-analyses.

Statistical Analysis

For analysis in our Mexican American samples, Hardy-Weinberg equilibrium (HWE) of *SLC6A3 VNTR* in controls and alcoholics was tested with the HWSIM program. Pearson's chi-square test or Fisher's exact test was used to compare the gender, genotype, and allele distribution. Age difference was evaluated with Pearson's chi-square test and Wilcoxon rank sum test. The gene–environment interaction was analyzed with classification tree through consecutive data splitting. All these analyses were 2-sided and were performed with SPSS 15.0 software package (SPSS Inc., Chicago, IL).

For meta-analyses, data were analyzed with the Comprehensive Meta-analysis (v.2) statistical software package (Biostat Inc., Englewood, NJ). The principal outcome measure was the genotypic or allelic odds ratio (OR) of *SLC6A3 VNTR* for alcoholism. The significance of the pooled OR was determined using a *Z*-test. Stratified analyses by sample ancestry were conducted. Because of heterogeneity among the samples included in the meta-analysis, which was caused by different ethnicity, gender ratio, and diagnostic criteria, random effect models instead of fixed effect models were adopted for the meta-analyses.

In current study, *SLC6A3 VNTR* alleles were divided into 2 categories: *A9* and other alleles. Genotypes were mainly analyzed under a dominant rather than a recessive model, because it seems that *SLC6A3 VNTR A9* expresses its effect in a hereditary mode of dominance. For example, individuals with the *A10/ A9* genotype were once found to have a mean 22% reduction of SLC6A3 protein availability in putamen compared with *A10* homozygous individuals (Heinz et al., 2000). $p < 0.05$ was taken as significance level for all the analyses. Bonferroni correction was adopted for multiple comparisons.

RESULTS

Characteristics of Participants

There was no significant difference in gender distribution between the control and alcoholic cohorts ($p = 0.118$); the percentage of females in controls and alcoholics was 23.7 and 18.9, respectively.

The number of young (≤30 year), middle aged (30 to 60 year), and old (>60 year) participants of the 337 controls was 93 (27.6%), 236 (70.0%), and 8 (2.4%), respectively. There were 99 (27.1%) young, 257 (70.4%) middle aged, and 9 (2.5%) old participants in 365 alcoholics. The median age for the control cohort was the same as that of the alcoholic cohort (37 years). No significant difference was found in age distribution between these 2

cohorts by Pearson's chi-square test and Wilcoxon rank sum test (p = 0.988 and 0.305, respectively).

In 365 alcoholics, 37 (10.1%) participants had AWS, 95 (26.0%) had DT, and 113 (31.0%) had AWS or DT. The remaining 252 (69.0%) participants had neither AWS nor DT history.

Genotype and Allele Distribution of SLC6A3 VNTR in Alcoholics and Controls of Mexican Americans

The overall genotyping error rate was 0.95% (1/105). Seven different *SLC6A3 VNTR* alleles were identified in our Mexican American samples including *A10, A9, A3, A6, A8, A11*, and *A12*. The first and second most frequent alleles were *A10* and *A9*, respectively, in both alcoholics and controls. The frequency of *A10* and *A9* in controls was 82.2% (554/674) and 15.9% (107/674), respectively, and the frequency in alcoholics was 80.5% (588/730) and 18.2% (133/730), respectively. In each cohort *A10* and *A9* accounted for more than 98% of all the alleles, while the other 5 alleles only accounted for <2%.

Ten different genotypes of *SLC6A3 VNTR* were identified in Mexican Americans, including A10/A10, A10/A9, A9/A9, A10/A11, A10/A12, A10/A8, A11/A9, A3/A3, A9/A6, and A9/A8. The first, second, and third most frequent genotypes were *A10/A10, A10/A9*, and *A9/A9*, respectively, in both alcoholics and controls. The frequency of *A10/A10, A10/A9*, and *A9/A9* in controls was 68.2% (230/337), 25.8% (87/337), and 2.4% (8/337), respectively, and the frequency in alcoholics was 65.2% (238/365), 29.3% (107/365), and 3.6% (13/365), respectively. In each cohort, *A10/A10, A10/A9*, and *A9/A9* accounted for more than 96% of all the genotypes, while the other 7 genotypes only accounted for <4%.

Due to their very low frequency, alleles other than *A10* and *A9* as well as genotypes containing alleles other than *A10* and *A9* were excluded from further analysis, just as previous studies had done (Chen et al., 2001). After excluding those participants who had rare alleles or genotypes, there were 325 controls and 358 alcoholics left, and the genotype distribution of *SLC6A3 VNTR* in these 2 cohorts (Table 1) was in HWE (χ^2 = 0.0045, p = 0.95 in controls; χ^2 = 0.0512, p = 0.82 in alcoholics). No significant difference was found between controls and alcoholics regarding allele or genotype distribution under both additive and dominant models (unadjusted $p > 0.05$, Table S1). Gender specific genotypic and allelic analysis did not show significant association between *SLC6A3 VNTR* and alcoholism in either gender (unadjusted $p > 0.05$, Table S2). Classification tree analysis of the *SLC6A3 VNTR*–education-marital status interaction suggested marginally significant interaction between educational background and *SLC6A3 VNTR* (Fig. S1, unadjusted p = 0.058, Bonferroni corrected p = 0.116).

Genotype and Allele Distribution of SLC6A3 VNTR in Controls and Alcoholic Subgroup with Severe Withdrawal Symptoms of Mexican Americans

Controls were compared with the homogeneous alcoholic subgroup (alcoholics with AWS or DT, Table 1). No signifi-cant difference was found in allele or genotype distribution under both additive and dominant models (unadjusted $p > 0.05$, Table S1).

Characteristics of Research Articles Included in the Meta-Analyses

Thirteen research articles were included in the meta-analyses, and their characteristics are listed in Table 2. The studied populations were Caucasians, Brazilians, or Asians, and the diagnostic criteria for alcoholism were DSM-III-R, DSM-IV, ICD-10, or Feighner criteria. Different studies have different sample size and gender ratio. *A10* and *A9* were found to be the most frequent alleles in all the studies, and other rare alleles were excluded in certain studies. HWE of the genotype distribution was only reported in some studies.

Meta-Analyses of the Association between the SLC6A3 VNTR A9 and Overall Alcoholics

To determine the association between the *SLC6A3 VNTR A9* and alcoholism with all the alcoholics, data from 13 research articles plus the current study were included in the meta-analyses (Table 3). When all ethnic populations, only Caucasian populations, or only Asian populations were considered, no significant association was found at the genotypic (under the dominant model) or allelic level ($p > 0.05$, Table S3). The forest plot of meta-analysis in all ethnic populations regarding association of *SLC6A3 VNTR A9* with alcoholism at genotypic level under dominant model is shown in Fig. 1.

Meta-Analyses of the Association Between the SLC6A3 VNTR A9 and Alcoholism Within the Alcoholic Subgroup

To determine the association between the *SLC6A3 VNTR A9* and alcoholism within the alcoholic subgroup (alcoholics with AWS or DT), data from 3 research papers plus the current study were included in the meta-analyses (Table 4). When all ethnic groups were considered together or when only Caucasians were considered, significant pathogenic effects of the *A9* for alcoholism was observed at the genotypic (dominant model) and the allelic level ($p < 0.05$, OR 1.5–2.1, Table S3). The forest plot of meta-analysis with all ethnic groups at genotypic level under the dominant model is illustrated in Fig. 2.

DISCUSSION

The current study represents the first report of *SLC6A3* and alcoholism in Mexican Americans as well as the first comprehensive meta-analysis of *SLC6A3* and alcoholism. In this study, we report the association between *SLC6A3 VNTR A9* and alcoholism with severe withdrawal symptoms through meta-analyses although no association was found in our studied Mexican American population.

In Mexican Americans, *A10* and *A9* were found to be the first and second most frequent *SLC6A3 VNTR* alleles, which was similar to the allele distribution in other ethnic populations (Bannon et al., 2001; Doucette-Stamm et al., 1995; Vandenbergh et al., 1992). No significant association between *SLC6A3 VNTR* and alcoholism was found in Mexican Americans, while association of *A9* with alcoholism was reported in Caucasians (Kohnke et al., 2005; Sander et al., 1997). Differences in ethnicity and diagnostic criteria of alcoholism might account for the inconsistent finding. Furthermore, the statistical power of the current study was found to be relatively low by GPower 3.1 software (Table S1), which might be caused by the minor effect of *A9* on polygenic disorders and small sample size. When gene–environment (marital status and educational background) interaction is considered (Fig. S1),

marginal significance (unadjusted $p = 0.058$, Bonferroni corrected $p = 0.116$) is found for *SLC6A3 VNTR**educational background interaction. Replication studies with a larger sample size and a meta-analysis will help to further elucidate the relationship between *SLC6A3 VNTR* and alcoholism.

In meta-analysis, the association between *SLC6A3 A9* and alcoholism was found only in the alcoholic subgroup with severe withdrawal symptoms, but not in all the alcoholics. This finding underscores the importance of using homogeneous subgroupings in genetic association studies. Actually, the homogeneity of alcoholism is also affected by many other factors such as family history, antisocial personality, type of alcoholism, gender, onset age, etc. For example, more *SLC6A3 A9* alleles are present in alcoholics with antisocial personality than in those without, and there are significant differences in genotype frequencies of *ADH2 C992G* and *A13543G* single nucleotide polymorphisms between familial and nonfamilial alcoholics (Choi et al., 2006). Additionally, striatal SLC6A3 density was lower in nonviolent type 1 alcoholics than in controls, but the difference was not significant for violent type 2 alcoholics (Repo et al., 1999; Tiihonen et al., 1995; Tupala et al., 2000, 2001). The more factors we use to subtype alcoholics, the more homogeneous the alcoholics will be, and the more likely a genuine relationship between genetic factors and alcoholism will be identified.

Some studies have shown that the presence of the *SLC6A3 A9* allele was correlated with decreased levels of *SLC6A3* transcript (Brookes et al., 2007; Fuke et al., 2001; Heinz et al., 2000). Moreover, the *SLC6A3 VNTR* allele is associated with function of central nervous system especially that of the reward system. Activation in the striatum was greater in carriers of *A9* than in individuals homozygous for the *A10* (Durston et al., 2008). Additionally, the *A9* carriers showed the highest response activity of brain regions involved in anticipation and reception of rewards during reward anticipation and reward delivery. These findings presumably reflect functional changes consequent to higher synaptic dopamine availability, and these responses may contribute to individual differences in reward-seeking behavior and in predisposition to neuropsychiatric disorders including alcoholism (Dreher et al., 2009).

Moreover, the dopamine transporter may be an important mediator to counteract the risk of AWS, because SLC6A3 mRNA levels in brain dopaminergic neurons are significantly lower in the genetically epilepsy-prone rats than in other strains of healthy rats. Additionally, the induction of seizures by the chemoconvulsant pentylenetrazol is associated with a reduced expression of SLC6A3 mRNA (Szot et al., 1996). More specifically, in humans an association was detected between the *SLC6A3 VNTR* and seizure, as an excess of *A9* was found in patients with idiopathic generalized epilepsy compared with control subjects (Sander et al., 2000).

SLC6A3 VNTR A9 may also be involved in the risk of DT, because hallucination and delusion processes are classically related to hyperdopaminergic states (Shaner, 1999), with dopamine receptor agonists triggering hallucinations (Moser et al., 1996; Srisurapanont et al., 2001) and, in contrast, dopa-mine receptor antagonists (such as neuroleptics) being used for counteracting sound DT (Mayo-Smith, 1997). The above findings help to further explain

why the association between *SLC6A3 VNTR A9* and alcoholism was only detected in alcoholics with AWS or DT.

Findings of the current meta-analysis help to explain the function of SLC6A3 and pathogenesis of alcoholism, but still should be interpreted with caution because of the limitations of this study. First, due to the discrepancies between included studies in ethnicity, diagnostic criteria, and gender ratio, random effect models were adopted for all the meta-analyses. Under the random effect model, effect size of the studied factor (*SLC6A3 VNTR*) could vary substantially from study to study, and the final combined effect does not represent the "common" effect, but only estimates the mean effect of a series of different effects. Consequently, the association between *A9* and alcoholism with severe withdrawal symptoms might only exist in some specific samples, but not in others.

Second, in the meta-analysis under a random effect model, each study is used to estimate the effect in a specific population and all of the effects are used to estimate the combined effect. Therefore, our ability to estimate the combined effect precisely will depend on both the number of subjects within each study and also the total number of studies. In the current study, the sample size and number of studies included in the meta-analyses were both relatively small, so the result may not be robust enough.

Third, gene–gene, polymorphism–polymorphism, and gene–environment interactions, which contribute to pathogenesis of polygenic disorders including alcoholism, were not considered in the meta-analysis because few related studies addressed these issues. Muramatsu and Higuchi (1995) showed in a Japanese sample that the frequency of the *SLC6A3 VNTR* 7-repeat allele was significantly higher in alcoholics with *ALDH2*2* than in control subjects. At the same time, among individuals with *SLC6A3 VNTR* homozygosity for the *A10*, a higher prevalence of SLC6A3 3′-UTR G2319A *A/A* homozygosity in alcoholics with a history of AWS or DT was found compared with homozygote *A10/A10* normal controls (Wernicke et al., 2002). These are good examples of gene–gene and polymorphism–polymorphism interactions. Moreover, significant association of dopamine receptor D2 (*DRD2*) *TaqI A1* allele with alcoholism was found, but only in patients with high stress levels, suggesting gene–environment interaction. When interactions were not considered in the above studies, no significant association between genetic factors and alcoholism was observed.

Finally, coexistence of addictive and psychiatric disorders in controls or alcoholics, which share some common genetic risk factors in reward system with alcoholism, might confound the association of *SLC6A3 VNTR* with alcoholism.

Most studies included in the meta-analyses failed to report the exclusion of some important comorbid psychiatric disorders including antisocial personality disorder and anxiety disorder from cases and/or controls (Table 2), which reduces the reliability of our findings.

In summary, no association between *SLC6A3 VNTR* and alcoholism was found in our Mexican American samples, while the meta-analysis suggests a possible association between *SLC6A3 VNTR A9* and alcoholics with severe withdrawal symptoms. Because of limitations of the current study, replication studies on homogenous subgroups with larger

sample size as well as consideration of gene–gene and gene– environmental interactions are warranted before reaching definite conclusions.

SUPPORTING INFORMATION

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Supplementary Material

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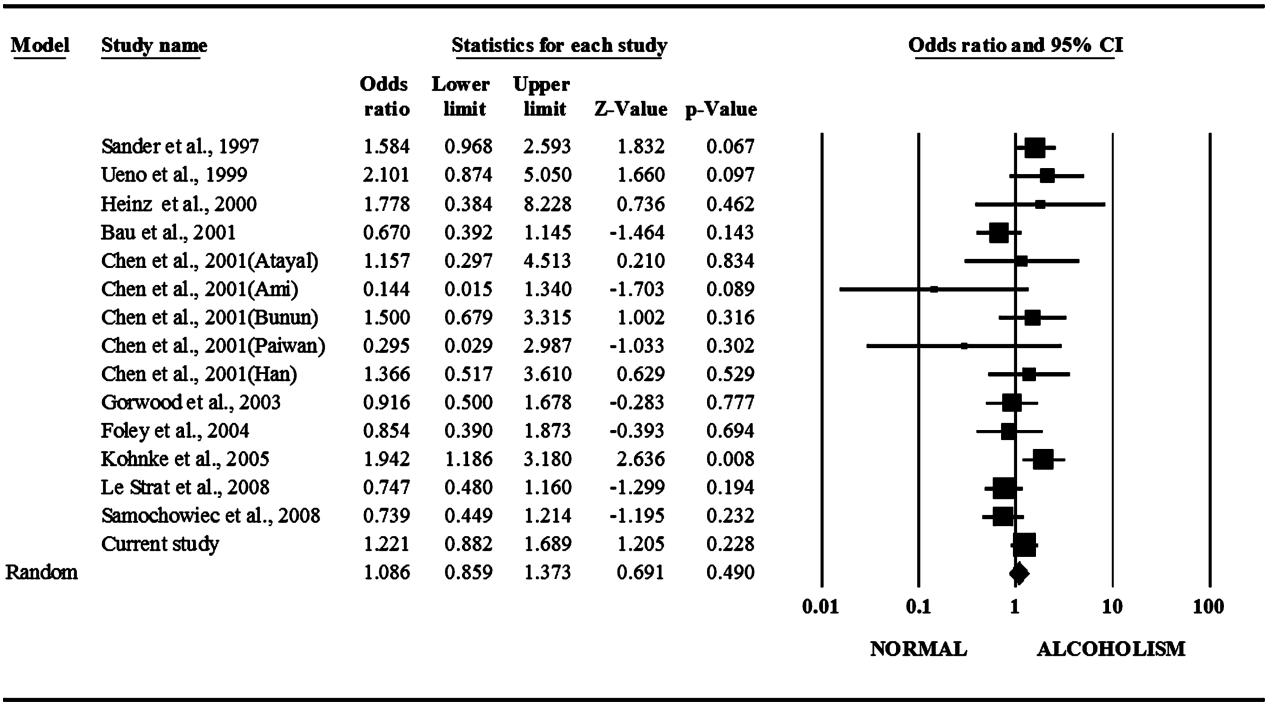

Fig. 1.

Forest plot of meta-analysis (controls vs. alcoholics, all ethnic populations, genotypic level under dominant model).

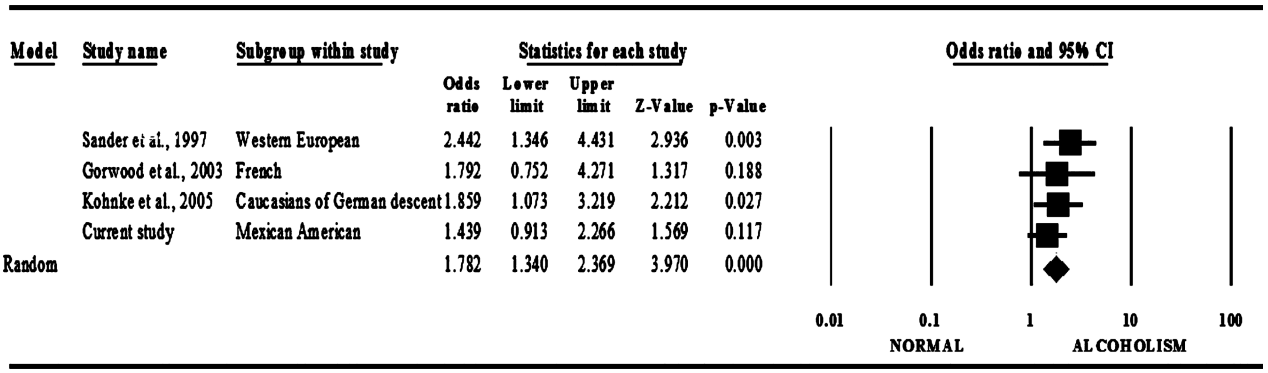

Fig. 2.

Forest plot of meta-analysis (controls vs. alcoholics with withdrawal seizure or delirium tremens, all ethnic populations, genotypic level under dominant model).

Table 1

Genotype and Allele Distribution of *SLC6A3* Variable Number Tandem Repeat in Controls and Alcoholics of Mexican Americans

		Genotype (%)					
		Additive model			Dominant model		Allele (%)
	n	*A10/A10*	*A10/A9*	*A9/A9*	*A10/A10*	*A10/A9+A9/A9*	*A9*
Controls	325	230 (70.8)	87 (26.8)	8 (2.5)	230 (70.8)	95 (29.2)	103 (15.8)
Alcoholics	358	238 (66.5)	107 (29.9)	13 (3.6)	238 (66.5)	120 (33.5)	133 (18.6)
Without AWS and DT	248	169 (68.1)	69 (27.8)	10 (4.0)	169 (68.1)	79 (31.9)	89 (17.9)
With AWS or DT	110	69 (62.7)	38 (34.5)	3 (2.7)	69 (62.7)	41 (37.3)	44 (20.0)

AWS, alcohol withdrawal seizure; DT, delirium tremens.

Table 2

Characteristics of the Studies Included in the Meta-Analyses

Literatures	Ethnicity	Diagnosis of alcoholism	Exclusion criteria	Number of cases/controls	Males: females in cases/ controls	Alleles found	HWE in cases/controls
Muramatsu and Higuchi (1995)	Japanese	DSM-III-R	NR	212/235	NR/111:124	*A6*, *A7*, *A8*, *A9*, *A10*, and *A11*	NR/NR
Sander et al. (1997)	Western European	ICD-10	Alcoholics: primary major psychiatric disorder or other substance dependence Controls: addictive disorder or previous psychiatric treatment, or an age under 26	293/93	254:39/46:47	*A9*, *A10*, and *A11*	NR/NR
Dobashi et al. (1997)	Japanese	DSM-III-R	NR	80/120	79:1/60:60	*A7*, *A9*, *A10*, and *A11*	Yes/Yes
Parsian and Zhang (1997)	Caucasians of Western European	Modified Feighner criteria	Alcoholics: NR Controls: affective disorders, schizophrenia, or other psychotic or drug use disorders	162/89	117:45/46:43	*A9*, *A10*, and *A11*	NR/NR
Ueno et al. (1999)	Japanese	DSM-III-R	Alcoholics: psychoses other than those associated with alcohol use Controls: schizophrenia	124/107	118:6/55:52	*A6*, *A7*, *A9*, *A10*, *A11*, and *A14*	NR/NR
Heinz et al. (2000)	NR	DSM-IV	Alcoholics: current drug abuse or a past history of drug dependence other than alcoholism, serious head trauma. Korsakoff's syndrome, or the presence of psychiatric (DSM-IV axis I Diagnoses) and neurological diseases unrelated to alcoholism Controls: DSM-IV axis I and II diagnoses, alcohol-dependent first-degree relatives, history of drug abuse	14/11	11:3/7:4	*A9*, and *A10*	Yes/Yes
Bau et al. (2001)	Brazilian	DSM-III-R	NR	114/112	114:0/NR	*A7*, *A9*, *A10*, and *A11*	NR/NR
Chen et al. (2001)	Taiwan Atayal	DSM-III-R	Alcoholics and controls: born from an interethnic marriage	41/31	NR/NR	*A9*, and *A10*	Yes/Yes
	Taiwan Ami			26/23	NR/NR	*A9*, and *A10*	Yes/Yes
	Taiwan Bunun			56/56	NR/NR	*A9*, and *A10*	Yes/Yes
	Taiwan Paiwan			36/34	NR/NR	*A9*, *A10*, and *A11*	Yes/Yes

Literatures	Ethnicity	Diagnosis of alcoholism	Exclusion criteria	Number of cases/controls	Males: females in cases/ controls	Alleles found	HWE in cases/controls
	Taiwan Han			68/59	NR/NR	*A9*, *A10*, and *A11*	Yes/Yes
Gorwood et al. (2003)	French	DSM-III-R	Alcoholics: comorbid schizophrenia or dementia Controls: psychiatric or addictive morbidity	120/65	120:0/NR	*A9*, *A10*	Yes/Yes
Foley et al. (2004)	Caucasian	NR	Alcoholics: NR Controls: liver disease	61/43	NR	*A9*, and *A10*	NR
Kohnke et al. (2005)	Caucasians of German descent	DSM-IV	Alcoholics and controls: primary major psychiatric disorders, severe somatic problems, substance dependence other than alcohol or nicotine, or individuals receiving psychotropic drugs Controls: a positive family history for addiction (other than nicotine) and other psychiatric disorders or an age younger than 24 years	216/102	176:40/66:36	*A9*, *A10*, and others (not specified)	Yes/Yes
Samochowiec et al. (2008)	Caucasians of Polish descent	ICD-10	Alcoholics: history of psychiatric disorders of axis I of ICD-10 other than alcohol or tobacco dependence Controls: psychiatric disorders with Prime MD questionnaire	122/150	99:23/120:30	*A9*, and *A10*	Yes/Yes
Le Strat et al. (2008)	Alcoholics: French (mainly Caucasians) Controls: Caucasian	DSM-IV	Alcoholics: schizophrenia and dementia Controls: psychiatric and addictive disorders	250/121	175:75/NR	*A9*, and *A10*	Yes/NR

NR, not reported.

Table 3

Studies Having *SLC6A3* Variable Number Tandem Repeat Data in Alcoholics and Controls

Literatures	Genotypes		Alleles
	Additive model *Ax/Ax*: *Ax/A9*:*A9/A9* in cases/controls	**Dominant model *A9–* : *A9+* in cases/controls**	***Ax*:*A9* in cases/controls**
Muramatsu and Higuchi (1995)	NR	NR	400:24/441:29 Difference: NR
Sander et al. (1997)	167:107:19/63.25.5 Difference. NR	167:126/63:30 Difference: NS	441:145/151:35 Difference: NR
Dobashi et al. (1997)	NR	NR	151:5/216:18 Difference: NS
Parsian and Zhang (1997)	NR	NR	234:84/128:46 Difference: NS
Ueno et al. (1999)	106:18:0/99:8:0 Difference. NR	106:18/99:8 Difference: NR	230:18/206:8 Difference: NR
Heinz et al. (2000)	9:8:0/8:4:0 Difference: NR	9:8/8:4 Difference: NR	26:8/20:4 Difference: NS
Bau et al. (2001)	74:31:9/62:45:5 Difference: NR	74:40/62:50 Difference: NR	179:49/169:55 Difference: NR
Chen et al. (2001) (Atayal)	35:6:0/27:3:1 Difference: NS	35:6/27:4 Difference: NR	76:6/57:5 Difference: NS
Chen et al. (2001) (Ami)	25:1:0/18:5:0 Difference: NS	25:1/18:5 Difference: NR	51:1/41:5 Difference: NS
Chen et al. (2001) (Bunun)	35:21:0/40:15:1 Difference: NS	35:21/40:16 Difference: NR	91:21/95:17 Difference: NS
Chen et al. (2001) (Paiwan)	35:1:0/31:3:0 Difference: NS	35:1/31:3 Difference: NR	71:1/65:3 Difference: NS
Chen et al. (2001) (Han)	56:11:1/51:7:1 Difference: NS	56:12/51:8 Difference: NR	123:13/109:9 Difference: NS
Gorwood et al. (2003)	58:52:10/30:26:9 Difference: NR	58:62/30:35 Difference: NR	168:72/86:44 Difference: NR
Foley et al. (2004)	35:22:4/23:14:6 Difference: NS	35:26/23:20 Difference: NR	92:30/60:26 Difference: NR
Kohnke et al. (2005)	NR	112:104/69:33 *Difference: significant*	NR
Le Strat et al. (2008)	130:86:16/59:47:15 Difference: NS	130:102/59:62 Difference: NR	346:118/165:77 Difference: NR
Samochowiec et al. (2008)	81:35:6/89:51:10 Difference: NS	81:41/89:61 Difference: NR	197:47/229:71 Difference: NS
Current study	238:107:13/230:87:8 Difference: NS	238:120/230:95 Difference: NS	583:133/547:103 Difference: NS

NS, not significant; NR, not reported; *Ax*, alleles except 9 repeat; *A9–*, genotypes without *A9* allele; *A9+*, genotypes with *A9* allele.

Table 4

Studies Having *SLC6A3* Variable Number Tandem Repeat Data in Controls and Alcoholics with Withdrawal Seizure or Delirium Tremens

	Genotypes		Alleles
Literatures	**Additive model *Ax/Ax:Ax/A9:A9/A9* in cases/controls**	**Dominant model *A9–*: *A9+* in cases/controls**	***Ax:A9* in cases/controls**
Sander et al. (1997)	43:40:10/63:25:5 Difference: NR	43:50/63:30 *Difference: significant*	126:60/151:35 Difference: NR
Gorwood et al. (2003)	11:19:4/30:26:9 Difference: NR	11:23/30:35 Difference: NR	41:27/86:44 Difference: NR
Kohnke et al. (2005)	NR	63:56/69:33 NR	NR
Current study	69:38:3/230:87:8 Difference: NS	69:41/230:95 Difference: NS	176:44/547:103 Difference: NS

NS, not significant; NR, not reported; *Ax*, alleles except 9 repeat; *A9–*, genotypes without *A9* allele; *A9+*, genotypes with *A9* allele.