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Brief report

The association of exon 3 VNTR polymorphism of the dopamine receptor D4 (DRD4) gene with alcoholism in Mexican Americans

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ABSTRACT

In this study, the variable number tandem repeats (VNTR) polymorphism of a 48-bp sequence located in exon 3 of the dopamine receptor D4 (DRD4) gene was genotyped in 365 alcoholic and 337 non-alcoholic Mexican Americans. Logistic regression showed that genotypes without the 7-repeat allele were risk factors for alcoholism. However, linear regression did not find an association between DRD4 VNTR and MAXDRINKS, which was defined as the maximum number of drinks consumed within 24 h. Our results indicate the presence of an association between DRD4 VNTR and alcoholism in Mexican Americans.

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1. Introduction

Dopamine receptor D4 (DRD4) is regarded as one of the most important candidate genes for alcoholism, in which a variable number tandem repeats (VNTR) polymorphism of a 48-bp sequence located in exon 3 has been extensively studied. Both alleles with 2–6 repeats and with 7 repeats were shown to be the risk factors of alcoholism in non-Hispanic populations (George et al., 1993; Muramatsu et al., 1996; Skowronek et al., 2006; Laucht et al., 2007). The association of DRD4 VNTR with alcoholism has not been studied in Mexican Americans who represent a fast growing ethnic group in the United States and who seem to be at a high risk for alcohol problems. MAXDRINKS, which is defined as the largest number of drinks a person has ever consumed within 24 h, is closedly related to alcoholism diagnosis. The relationship between the heritable phenotype of MAXDRINKS and DRD4 has never been explored, either. The current study addresses these two issues.

2. Materials and methods

2.1. Participants

Unrelated Mexican Americans including 365 DSM-IV alcoholics and 337 controls who were gender- and age-matched were enrolled in the Los Angeles County. All participants gave informed consent and the protocol was approved by Harbor-UCLA Research and Education Institute and University of Kansas Medical Center Human Subject Committees.

2.2. Genotyping

DRD4 VNTR was genotyped by PCR amplification (Mitsuyasu et al., 2001).

2.3. Statistical analysis

Binary or multinomial logistic regression and linear regression was performed to explore the association of DRD4 VNTR with alcoholism and with MAXDRINKS, respectively. A Log (10) transform of the MAXDRINKS raw data was used to correct distribution skewness.

3. Results

3.1. Characteristics of participants

MAXDRINKS of alcoholics ranged between 4 and 100 with the kurtosis and skewness being 1.544 and 1.203, respectively. In 363 alcoholics, 113 participants (31.0%) had severe withdrawal symptoms that are alcoholic withdrawal seizure (AWS) and/or delirium tremens (DT).

3.2. Overall genotype and allele distribution

Eight different DRD4 VNTR alleles with 2 to 8 and 10 tandem repeats as well as twenty-four genotypes were present in our study population. The 4- and 7-repeat allele was the first and second most

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Table 1
The association of DRD4 VNTR with alcoholism by logistic regression controlling for the effect of gender, age, and smoking status.

		Overall				Alcoholism without severe withdrawal symptoms				Alcoholism with severe withdrawal symptoms			
		Controls/ alcoholics	B	*P*	OR (95% CI)	Controls/ alcoholics	B	*P*	OR (95% CI)	Controls/ alcoholics	B	*P*	OR (95% CI)
DRD4 VNTR	X/X 7/X&7/7	274/324 63/41	0.601	*0.009*	1.825 (1.165–2.859)	274/223 63/29	0.546	*0.030*	1.726 (1.055–2.825)	274/101 63/12	0.736	*0.036*	2.088 (1.050–4.152)
Gender	Male Female	257/296 80/69	0.205	0.302	1.228 (0.831–1.814)	257/196 80/56	0.010	0.962	1.010 (0.668–1.528)	257/100 80/13	0.836	*0.013*	2.309 (1.196–4.444)
Age		37.34 ± 10.42/ 38.31 ± 10.57	0.011	0.171	1.011 (0.995–1.026)	37.34 ± 10.42/ 37.29 ± 10.36	0.001	0.936	1.001 (0.984–1.018)	37.34 ± 10.42/ 40.60 ± 10.73	0.033	*0.002*	1.034 (1.012–1.056)
Smoking status	Smoking Non-smoking	57/166 274/199	1.351	*<0.001*	3.862 (2.707–5.512)	57/106 274/146	1.238	*<0.001*	3.448 (2.353–5.050)	57/60 274/53	1.601	*<0.001*	4.950 (3.086–8.000)

X: alleles other than the 7-repeat. Italicized data mean $P<0.05$.

common allele in both controls and alcoholics (69.1% *vs.* 72.1% for 4-repeat, and 12.3% *vs.* 9.3% for 7-repeat). The 4/4 genotype was the most common genotype in both controls and alcoholics with a frequency of 58.5% and 65.5%, respectively.

3.3. Association of DRD4 VNTR with alcoholism by logistic regression controlling for effect of confounders

After DRD4 VNTR genotypes were grouped into two categories: with and without 7-repeat allele, logistic regression controlling for the effect of gender, age, and smoking status was performed. Genotypes without the 7-repeat allele were associated with a higher risk (OR>1) for alcoholism as well as for different alcoholic subgroups (with or without severe withdrawal symptoms, Table 1).

3.4. Association of DRD4 VNTR with MAXDRINKS

With log (10) transform and linear regression controlling for effect of gender, age, and smoking status, no significant association was detected between DRD4 VNTR and MAXDRINKS ($P=0.750$).

4. Discussion

For the first time, association of DRD4 exon 3 VNTR with alcoholism and MAXDRINKS was studied in Mexican Americans, and genotypes without the 7-repeat allele were shown to be risk factors for alcoholism in Mexican Americans.

In our studied Mexican American population, the 4- and 7-repeat were found to be the most common alleles, and the 4/4 was the most frequent genotype. Similar allele and genotype distributions have been reported in other ethnic populations (Chang et al., 1996), suggesting little inter-ethnic differences.

A pathogenic effect of genotypes without the 7-repeat allele was observed when all the alcoholics were considered. Such association also exists in both alcoholic subgroups with and without severe withdrawal symptoms. Thus, this polymorphism may have a crucial role in alcoholism because the association was found even in alcoholics who did not have severe withdrawal symptoms.

Our findings are consistent with the results of the studies that were conducted in Canadians (George et al., 1993) and Japanese (Muramatsu et al., 1996), but in conflict with other association studies (Skowronek et al., 2006; Laucht et al., 2007). The controversy might be due to different ways to group the DRD4 VNTR alleles (McGeary, 2009). Some studies defined alleles as “long” or “short” with a specific cut off point, while other studies grouped the genotypes according to the presence or absence of a particular allele, e.g. the 7-repeat allele (Skowronek et al., 2006; Laucht et al., 2007). It seems that the 7-repeat allele harbors special functional significance for DRD4. It has been shown that the 7-repeat allele is related to decreased expression and reduced sensitivity to endogenous dopamine in comparison with the 2- and 4-repeat alleles (Asghari et al., 1995; Schoots and Van Tol, 2003). Based on these findings, in the current study, we grouped genotypes according to the presence or absence of the 7-repeat allele. The sample size, ethnicity, gene–gene interactions, etc. may also explain the conflicting findings.

According to previous functional studies mentioned above (Asghari et al., 1995; Schoots and Van Tol, 2003), the 7-repeat allele may lead to functional deficiency of dopamine neurotransmission and consequently the risk for alcoholism. These functional assumptions are in conflict with our findings. Thus, the functional significance of DRD4 VNTR should be re-examined and the presence of linkage disequilibrium (LD) of DRD4 VNTR with another genuine disease locus should be considered and studied.

Twin studies have shown that MAXDRINKS has a heritability of 50% (Slutske et al., 1999), and a MAXDRINKS locus has been identified on chromosome 4 in the vicinity of the alcohol dehydrogenase (ADH) gene cluster (Saccone et al., 2000). However, no association of DRD4 with MAXDRINKS was found in the current study, which suggests that alcohol tolerance is probably mainly related to the alcohol metabolizing genes rather than the neurotransmission genes.

In conclusion, our results demonstrate an association between DRD4 VNTR genotypes without the 7-repeat allele and alcoholism in Mexican Americans. Additional functional study is warranted to confirm the finding.

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