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Brief Research Communication

Lack of Association Between *Taq*I A1 Allele of Dopamine D2 Receptor Gene and Alcohol-Use Disorders in Atayal Natives of Taiwan

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Association studies between the A1 allele of the dopamine D2 receptor (DRD2) gene *Taq*I A polymorphism and alcoholism remain controversial. A recent study from Japan demonstrated that the A1 allele is associated with severe alcoholism in the Japanese population. We were interested in knowing if this association also exists in the Atayals of Taiwan, who were found to have a higher prevalence of alcohol-use disorders than the Han Chinese in Taiwan. Genotype and allele frequencies were determined in alcohol-abusing, alcohol-dependent, and nonalcoholic control Atayal natives in Taiwan. A1 allele frequencies in alcohol-dependent, alcohol-abusing, and normal control Atayals were 0.39, 0.42, and 0.39, respectively. No difference in A1 allele frequency was found among these three groups. Our data do not support the hypothesis that the A1 allele of the *Taq*I A polymorphism of the DRD2 gene increases susceptibility to alcohol-use disorders in the Atayals of Taiwan.

KEY WORDS: restriction fragment length polymorphism, susceptible gene, association

INTRODUCTION

Alcoholism is a complex, multifactorial disease, with both environmental and biological origins. Family, twin, and adoption studies have elucidated the importance of the genetic component of alcoholism [Merikangas, 1990; Pickens et al., 1991; Kendler et al., 1992]. However, no gene has yet been identified which increases susceptibility to alcoholism. Blum et al. [1990, 1991] first reported a higher frequency of the A1 allele of *Taq*I A polymorphism of the DRD2 gene in alcoholics compared to nonalcoholic controls. This finding implied that a molecular variant of the DRD2 gene may increase susceptibility to alcoholism. Since neurobiological studies have provided evidence that alcohol works on the central dopaminergic system, which is involved in drug-mediated reinforcement behavior, and which may be related to the pathogenesis of alcoholism, many researchers have tried to replicate the initial observations of Blum et al. [1990]. Some groups were able to find the association [Comings et al., 1991; Parsian et al., 1991; Amadeo et al., 1993; Neiswanger et al., 1995a]. However, many groups were unable to obtain the same result [Gelernter et al., 1993; Noble, 1993]. Therefore, the finding of allelic association between DRD2 *Taq*I A and alcoholism remains controversial.

Atayal natives of Taiwan are aboriginal people of Malayo-Polynesian heritage, who colonized Taiwan several centuries before the arrival of Han Chinese immigrants from mainland China. Previous epidemiological studies have revealed a higher prevalence of alcohol abuse (11.6%) and alcohol dependence (11.4%) among Atayals than Han Chinese in Taipei (3.4% for alcohol abuse, and 1.5% for alcohol dependence) [Hwu et al., 1990], which is a serious social and family issue among the Atayals of Taiwan. Recently, an association study between the A1 allele of DRD2 *Taq*I A polymorphism and alcoholism carried out in the Japanese population demonstrated the existence of an association between the A1 allele and severe alcoholism [Arinami et al., 1993]. The severity of alcoholism increased in the order of the genotypes A2/A2, A1/A2, and A1/A1 in Japanese alcoholics. We were interested in understanding if the association and correlation between the A1 allele and alcoholism also exists in Atayal natives of

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Taiwan. To address this issue, genotype and allele frequencies of the *Taq*I A polymorphism of Atayals with alcohol-use disorders and of nonalcoholic Atayal controls were determined in this study.

MATERIALS AND METHODS

Subjects

Subjects were Atayal people who participated in our serial Taiwan Aborigine Alcoholism Studies. These individuals were enrolled with informed consent, and were interviewed by well-trained interviewers using the section on alcoholism of the modified Chinese Diagnostic Interview Schedule [Hwu et al., 1990; Lin et al., 1995], which was shown to have satisfactory interrater reliability. The diagnosis of alcohol-use disorders, i.e., alcohol dependence and alcohol abuse, was based on clinical assessment according to the criteria of DSM-III-R criteria. Nonalcoholic controls were also Atayals living in the same community, and they were evaluated by the interviewers to exclude the diagnosis of alcohol-use disorder, but not other neuropsychiatric disorders. One hundred and ninety-nine unrelated Atayals were recruited in this study, including 85 diagnosed as alcohol-abusing, 73 as alcohol-dependent, and 41 as nonalcoholic controls. The alcohol-abusing group consisted of 44 males and 41 females with a mean age of 40 years. The alcohol-dependent group consisted of 51 males and 22 females, with a mean age of 40.5 years. The non-alcoholic control group consisted of 15 males and 26 females, with an average age of 41 years.

Genotype Determination

Blood samples were collected with EDTA anticoagulant, and genomic DNA was prepared using the standard method. Restriction fragment length polymorphism (RFLP) analysis of *Taq*I A polymorphism of the DRD2 gene was carried out by using a PCR-based restriction analysis according to the method described by Grandy et al. [1993], with modifications. In brief, amplification reactions were carried out in a volume of 50 μl, containing 100 ng genomic DNA as template, 200 μM dNTP, 1 μM of each sense and antisense primer, 1 × DynaZyme II reaction buffer (Finnzymes, Oy, Finland), 1.5 mM $MgCl_2$, and 1 unit of DynaZyme II Taq polymerase (Finnzymes, Oy, Finland). The sequence of sense and antisense primers were 5′-CCGTCGACGGCTGGCCAAGTTGTCTA-3′, and 5′-CCGTCGACCCTTCCTGAGTGTCATCA-3′, respectively. After initial denaturation at 94°C for 5 min, 30 cycles of PCR reaction were performed under conditions of denaturation at 94°C, 1 min, annealing at 56°C, 1 min, and extension at 72°C, 1 min. Ten μl of PCR products were digested with 5 units of *Taq*I restriction enzyme (Boehringer Mannheim, Germany) in buffer B in a total volume of 20 μl at 65°C for at least 6 hr. Digested PCR products were separated by electrophoresis in a 2% MetaPhor (FMC BioProducts, Rockland) agarose gel, and visualized with ethidium bromide staining under ultraviolet light. The A1 allele shows a band of 310 bp, whereas the A2 allele shows bands of 180 bp and 130 bp.

Statistical analysis of the fitness of the Hardy-Weinberg equilibrium, and the allelic association of *Taq*I A polymorphism and alcohol-use disorders, were evaluated by chi-square test.

RESULTS

The genotype and allele frequencies of the *Taq*I A polymorphism of the DRD2 gene of the three groups are listed in Table I. The distribution of DRD2 genotypes of the three groups, namely alcohol-dependent ($\chi^2 = 2.36$, df = 1, $P = 0.12$), alcohol-abusing ($\chi^2 = 0.31$, df = 1, $P = 0.58$), and nonalcoholic control ($\chi^2 = 0.67$, df = 1, $P = 0.41$), did not depart from the Hardy-Weinberg equilibrium. There was no difference in genotype ($\chi^2 = 0.08$, df = 2, $P = 0.96$) and allele frequencies ($\chi^2 = 0.00$, df = 1, P = 1.00) between the alcohol-dependent group and normal controls. Also, no difference in genotype ($\chi^2 = 0.40$, df = 2, $P = 0.82$) and allele frequencies ($\chi^2 = 0.13$, df = 1, $P = 0.71$) was detected between the alcohol-abusing group and normal controls.

DISCUSSION

In this study, we found no evidence to support an association between the A1 allele of *Taq*I A polymorphism of the DRD2 gene and alcoholism in a genetically isolated aboriginal population, the Atayals, of Taiwan. Our results are in line with several other research groups, who also did not find an association between the A1 allele and alcoholism [reviewed in Gelernter et al., 1993]. As discussed by many researchers, association studies are liable to errors of sampling bias and different allele distributions in different ethnic groups. In the present study, our subjects were homogeneous in ethnic background, and lived in an isolated geographic area, avoiding possible population stratification. Moreover, our sample size was large enough to provide enough power to detect an association, if present.

The A1 allele frequency of normal Atayals of Taiwan is about 0.39, which is comparable to Han Chinese normal controls (0.37) (our unpublished data) and Japanese normal controls (0.42) [Arinami et al., 1993]. Orientals seem to have a significantly higher A1 allele frequency than Caucasians. However, unlike the study which reported an association between the A1 allele of the DRD2 gene and severe alcoholism in the Japanese population [Arinami et al., 1993], no association was found in our study. The reason for the discrepancy may

TABLE I. Genotype and Allele Frequencies of *Taq*I A Polymorphism of the DRD2 Gene Among Atayal Natives of Taiwan*

	Genotypes				Allele frequency	
	A1/A1	A1/A2	A2/A2	N	A1	A2
AD	8	41	24	73	57	87
	11%	56%	33%		39%	61%
AA	14	44	27	85	72	98
	16%	52%	32%		42%	58%
C	5	22	14	41	32	50
	12%	54%	34%		39%	61%

* AD, alcohol dependence; AA, alcohol abuse; C, nonalcoholic control; N, number of individuals.

lie in the differing severities of alcohol dependence of in our study and in the Japanese study of Arinami et al. [1993]. As Noble et al. [1994] reported that severity of alcohol dependence in alcoholics and of substance-abuse behaviors in controls are important variables in DRD2 allelic associations, our alcoholic subjects were recruited from the community, instead of from hospital, and were not evaluated for severity of alcohol dependence. Therefore, our alcoholic subjects may comprise *patients with differing* severities of alcohol dependence, who would be different from patients with severe medical complications recruited from hospitals. In addition, as appropriate controls are important in population-based association studies [Neiswanger et al., 1995b], nonalcoholic controls with other neuropsychiatric disorders were not excluded from our control group in the present study. Thus, the real association may be obscured. Even so, our results are consistent with the finding that no association exists between the A1 allele and alcoholism in Han Chinese alcoholics in Taiwan [Lu et al., 1993].

In summary, we did not find an association between the A1 allele of *Taq*I A polymorphism of the DRD2 gene and alcohol-use disorders in Atayal natives of Taiwan. However, the association with severe alcohol dependence cannot be ruled out in the present study.

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