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BRIEF ARTICLE

Nur-related receptor 1 gene polymorphisms and alcohol dependence in Mexican Americans

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Abstract

AIM: To investigate the association of polymorphisms of nur-related receptor 1 (*Nurr1*) and development of alcohol dependence in Mexican Americans.

METHODS: Peripheral blood samples were collected from 374 alcoholic and 346 nonalcoholic Mexican Americans; these two groups were sex- and age-matched. Sample DNA was extracted and genomic DNA was amplified by polymerase chain reaction. The *-2922(C) 2-3* polymerase chain reaction products were digested with *Sau96I*, alleles of *1345(G/C)*, and *-1198(C/G)* in the regulatory region as well as *Ex+132 (G/T/A/C)* and *Ex+715(T/-)* in exon 3 were studied by sequencing.

RESULTS: The *C2/C2, C2/C3, C3/C3* genotype distribution of *-2922(C) 2-3* was 34.4%, 38.2% and 27.5% in the nonalcoholic group compared to 23.3%, 51.2% and 25.4% in the alcoholic group (P = 0.001). The *C/C, C/G, G/G* genotype distribution of *-1198(C/G)* was 23.5%, 46.1% and 30.3% in the nonalcoholic group compared to 13.9%, 50.9% and 35.3% in the alcoholic group (P = 0.007). However, the *-1345 (G/C)*, *Ex3+132(G/T/A/C)* and *Ex3+715(T/-)* alleles were not polymorphic in Mexican Americans, and all those studied had *G/G, G/G* and *T/T* genotype for these three alleles, respectively. The *-2922(C) 2-3* did not show allele level difference between alcoholic and nonalcoholic individuals, but *-1198 (C/G)* showed a significant allele frequency difference between alcoholic (39.3%) and nonalcoholic (46.6%) populations (P = 0.005). Excluding obese individuals, significant differences were found at both genotypic and allelic levels for the *-2922(C) 2-3* polymorphism (P = 0.000 and P = 0.049) and the *-1198 (C/G)* polymorphism (P = 0.008 and P = 0.032) between nonobese alcoholics and nonobese controls. Excluding smokers, a significant difference was found only at the genotypic level for the *-2922(C) 2-3* polymorphism (P = 0.037) between nonsmoking alcoholics and nonsmoking controls, but only at the allelic level for the *-1198(C/G)* polymorphism (P = 0.034).

CONCLUSION: Polymorphisms in the regulatory region of *Nurr1* are implicated in pathogenesis of alcohol dependence and the *Nurr1*/dopamine signaling pathway might be important for this dependence development in Mexican Americans.

Key words: Nur-related receptor 1; Polymorphism; Alcohol dependence; Obesity; Smoking; Nuclear receptor

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INTRODUCTION

It is widely accepted that genetic factors play an important role in the development of alcohol dependence. Family clustering surveys have shown that alcohol dependence rates are higher among persons who are biologically related to an alcoholic[1], and that a family alcohol history is a strong predictor of alcohol dependence[2]. Twin pair studies have revealed a significantly greater concordance rate for alcohol dependence in monozygotic compared with dizygotic twins[3]. Half sibling and adoption studies have demonstrated that half brothers with different fathers and adopted sons of alcoholic men show a rate of alcohol dependence more like that of the biological father than that of the foster father[4]. On the other hand, there is no classic Mendelian pattern of inheritance for alcohol dependence. Environmental factors are also linked to alcohol dependence. Thus, alcohol dependence is a polygenic complex disorder resulting from an intricate interaction of multiple genes and various environmental factors.

Genes encoding alcohol metabolizing enzymes alcohol dehydrogenase (*ADH1B)* and aldehyde dehydrogenase (*ALDH2*) are the only genes that have been firmly linked to alcoholism[5,6]. However, data suggest that genes involved in the brain reward pathways are also strong candidates for a predisposition to alcohol dependence. These pathways, in particular, those utilizing dopamine and opioids as neurotransmitters, mediate positive reinforcement of activities, such as eating, love and reproduction. When engaging in such activities, the "natural reward" center releases dopamine from brain pathways in the nucleus accumbency and frontal cortex[7,8]. However, these same pathways can also be activated by "unnatural rewards" such as alcohol abuse. Only a minority of individuals become addicted to these various substances and certain types of behavior, therefore, it should be possible to identify factors, such as genes, that distinguish them from others not affected. Dopamine is the primary neurotransmitter in the reward pathway, genes that control dopamine signaling, including dopamine receptors and transporters, are particularly tempting candidates. Alcohol dependence is likely to be a polygenic and multifactorial disease, therefore, certain genes might have a small effect, whereas others have a greater impact in terms of increasing the risk for this disorder.

Our previous studies have shown that certain alleles of the brain dopamine receptors 2 and 4 (*DRD2* and *DRD4*) and of the serotonin transporter (*5-HTT*) as well as of opioid receptor are associated with alcohol dependence[9-13]. These findings indicate the importance of polymorphism of the gene in the reward pathway in contributing to the development of alcohol dependence in Mexican Americans.

Alcohol consumption, compulsive overeating and smoking are all associated with dysfunction of rewards pathways; there might be common risk factors in reward genes for these behaviors. *DRD2 TaqI A1* allele was identified at a higher frequency in alcoholic and nonalcoholic smokers than nonsmoking controls[14]. Smoking and obesity related to overeating might serve as confounding factors when association is analyzed between reward genes and alcohol dependence.

The Nur-related receptor 1 (*Nurr1*), *NR4A2*, is a transcription factor in the orphan nuclear receptor family[15-17], and is important for development of dopaminergic neurons. Ablation of *Nurr1* leads to agenesis of midbrain dopaminergic neurons as demonstrated by an absence of dopaminergic cell markers including tyrosine hydroxylase, as well as a loss of striatal dopamine neurotransmitter[18]. *Nurr1* knockout mice failed to develop ventral mesencephalic dopaminergic neurons and died within 12-48 h[19]. In addition, *Nurr1* increases expression of the human dopamine transporter gene in the mature brain; whereas other members of the nerve growth factor-induced clone B subfamily of nuclear receptors have lesser or even no effects[19]. Expression of *Nurr1* continues in mature dopaminergic neurons during adulthood, suggesting that *Nurr1* is also required for normal function of mature dopaminergic neurons[20]. Thus, *Nurr1* is an upstream signaling molecule for regulating the dopamine pathway and plays a broad-spectrum role in brain development.

Nurr1 involvement in the regulation of the dopaminergic system makes it a good candidate to study neuropsychiatric disorders. Several polymorphisms in the gene have been identified: *-469delG*, *M97V*, *H103R*, *DY122*, *-2922(C)2-3*, *IVS6+17* approximately *+18insG* and *EX8+657(CA)9-10*[21-24]. Among these polymorphisms, *-2922(C)2-3*, *IVS6+17* approximately *+18insG* and *EX8+657* variants are common (frequency of minor alleles > 15%) in both Caucasian and Asian populations. Two variants (*-291Tdel* and *-245T→G*) of *Nurr1* are associated with Parkinson's disease[25]. However, another study failed to identify any of the described variants in Parkinson's patients or controls[26]. In the regulatory region of the *Nurr1* gene, at least five polymorphic sites [*P1(A/T*, reference SNP number rs1462374), *P2(G/C)*, *P3(C/G)*, *P4(C/A/G)*, *P5(del C)*] have been identified, with no significant association between the genotype or allele frequency of these variants and schizophrenia or Parkinson's disease. In addition, *P2(G/C)*, *P3(C/G)*, and *P5(del C)* are in linkage disequilibrium (LD) with each other[27]. Further investigation of common *Nurr1* variants [*-2922(C)2-3*, *IVS6+17* approximately *+18insG*, *EX8+657(CA) 9-10*] has not supported their pathogenic role for schizophrenia among Japanese individuals[28]. There are two coding synonymous polymorphism sites in exon 3 (rs16840266) and exon 5 (rs61748236), and three coding nonsynonymous polymorphism sites in exon 3 (rs36083712 and rs35100271) and exon 8 (rs61729997); some other rare missense mutations have been studied in psychiatric disorders[22], but none of them has been found to be associated with alcohol addiction.

The role of *Nurr1* in alcohol dependence was studied in Japanese individuals[29], and polymorphisms including *-2922(C) 2-3* and *EX8 +657(CA)9-10* were examined. They have reported that the genotypic distribution of these two polymorphisms was in Hardy-Weinberg equilibrium in the controls and alcoholics. The allele frequency of the *(C) 2* and *(CA) 9* and allele in the alcoholics was similar to that in the controls. Significant LD between these two polymorphisms was observed in both controls ($P = 0.007$) and alcoholics ($P < 0.0001$), but the LD was much stronger in alcoholics than in controls (D' = 0.84 *vs* D' = 0.30). There was a significant difference in haplotype distributions between the alcohol dependence and control groups. The haplotypic association was not based on an increased frequency of a specific haplotype, but rather based on stronger LD in alcoholics. This finding implies that more recent ancestral chromosome sharing by the alcoholics than by the controls, and that the *Nurr1* locus is one of the genome regions that contribute to alcohol dependence.

Nurr1 is involved in the regulation of the dopaminergic system, which is the primary neurotransmitter in the reward pathway. The dopamine signaling can be activated by alcohol. Thus, we hypothesize the presence of an association between the polymorphism of the *Nurr1* gene and alcohol dependence. We selected three alleles in the regulatory region of the *Nurr1* gene that include *-2922(C)2-3*, *-1345(G/C)* and *-1198(C/G)* (promoter P2 and P3 in reference literature from Andrea Carmine) as well as coding synonymous *Ex+132(G/T/A/C*, rs16840266) and coding nonsynonymous *Ex+715(T/-*, rs35100271) in exon 3. These alleles are supposed to contribute to the function of *Nurr1* and may have an impact on alcohol dependence in Mexican Americans.

MATERIALS AND METHODS

Participants

All participants in this project were enrolled from unrelated Mexican Americans who lived in Los Angeles County, CA. The study cohorts included 374 alcoholics and 346 nonalcoholics, and the ratio of cases to controls was about 1.08. The groups were sex- and age-matched. All subjects provided informed consent for their participation in this study. This study was approved by the University of Kansas Medical Center Human Subjects Committee. All participants were investigated, diagnosed, and assigned based on the Diagnostic and Statistical Manual of Mental Disorder, Fourth Edition (DSM-IV). The alcoholic participants were interviewed by the Semi-Structured Assessment for the Genetics of Alcohol dependence II in English or Spanish[30]. The inclusion criteria for alcoholic participants included: (1) the ability to give informed consent; (2) between 21 and 76 years of age; (3) no less than three of four biological grandparents of Mexican heritage; (4) fluency in either Spanish or English; (5) no current use of other substances (except tobacco and caffeine), or history of such use within the past 6 mo; (6) no current or past diagnosis of mental illness such as schizophrenia, schizophrenia disorder, schizoaffective disorder, schizotypal disorder, major depression, bipolar disorder, or Parkinson's disease; and (7) no other clinical unacceptable disease based on physical examinations. The inclusion criteria for nonalcoholic participants included: (1) no current or history of diagnosis of DSM-IV alcohol dependence or alcohol abuse; and (2) no other clinical unacceptable disease based on physical examinations. The interview information also covered body weight, height, smoking status, tea and coffee intake, as well as marriage, and education status. Body mass index (BMI) equals body weight in kilograms divided by height in square meters (kg/m^2). Smoking status was defined as having smoked one or more cigarettes per day during the past 30 d[31].

Genotyping

Participants' peripheral blood samples were collected into tubes containing K_2-EDTA. Genomic DNA was extracted with GeneCatcher gDNA Blood kits (Invitrogen, Carlsbad, CA, United States). The primers used for PCR were: *-2922(C)2-3*, forward: 5'-AAAAGGGGATGAACCGGGTAGG-3', reverse: 5'-TTTTCCGAAAGAGGTGTGACCT-3'; *-1345(G/C)* and *-1198(C/G)*, forward: 5'-ATCCCGAATAGTTCCACGGAG-3', reverse: 5'-CACGAGTTTTAAGGGAATAAATCG-3'; *Ex3+132(G/T/A/C)* and *Ex3+715(T/-)*, forward: 5'-GCTGAGTGTGTTATCACCTGTTT-3', reverse: 5'-GCTGAGTGTGTTATCACCTGTTT-3'.

PCR amplification was carried out in a 25-μL reaction mix containing 100 ng genomic DNA, 1× Go Taq Flexi buffer, 0.2 μmol/L each primer, 0.2 mmol/L dNTP, 2.0 mmol/L $MgCl_2$, 0.2 mmol/L dGTP, and 1 U GoTaq DNA polymerase. The thermal cycling conditions were set at 95 ℃ for 5 min, then 35 cycles for 30 s at 95 ℃, 30 s at 50 ℃ (for the three alleles in the regulatory region) or 55 ℃ (for the two alleles in exon 3), and 60 s at 72 ℃, with a final extension step of 10 min at 72 ℃. PCR products were examined on a 2% agarose gel.

The *-2922(C)2-3* PCR products were digested with *Sau96I*, which generated 176- and 158-bp fragments for wild-type alleles. Positive and negative digestion controls were set in each PCR and each agarose gel to ensure correct digest results. Retest was needed when results could not be read clearly. Other genotyping was studied by sequencing. All samples were sequenced in two directions. Results where two direction sequences did not match each other were excluded. Sequencing results were read by Finch TV (1.4 Version, Geospiza, Inc.) graphical viewer.

Statistical analysis

Statistical analyses were performed with SPSS Version 15.0 software (SPSS Inc., Chicago, IL, United States). Two-sided analyses were conducted and $P < 0.05$ was used as the significance threshold. Pearson's χ^2 tests were used to compare sex, BMI, smoking, genotype, and allele distribution between alcoholics and their controls.

Table 1 Genotype and allele frequency of the *-2922(C)2-3* and *-1198(C/G)* allele in Mexican American alcoholics and nonalcoholics *n* (%)

Groups	*-2922(C)2-3* allele					*-1198(C/G)* allele				
	Genotype			Allele frequency	Total	Genotype			Allele frequency	Total
	(C)2/(C)2	*(C)2/(C)3*	*(C)3/(C)3*	*(C)3*		*C/C*	*C/G*	*G/G*	*C*	
Non-alcoholic	119 (34.4)	132 (38.2)	95 (27.5)	322 (46.5)	346	76 (23.5)	146 (46.1)	98 (30.3)	298 (46.6)	320
Alcoholic	87 (23.3)	192 (51.3)	95 (25.4)[a]	382 (51.1)	374	48 (13.9)	176 (50.9)	122 (35.3)[a]	272 (39.3)[b]	346

[a]$P = 0.007$; [b]$P = 0.005$ *vs* nonalcoholic.

RESULTS

Population characteristics

There were 374 alcoholics and 346 nonalcoholic controls. The alcoholic group had 299 (79.9%) men and 75 (20.1%) women, and the nonalcoholic control group had 260 (75.1%) men and 86 (24.9%) women. The number of young (≤ 30 years), middle-aged (30-60 years), and old (> 60 years) participants in the control group was 93 (26.9%), 245 (70.8%), and 8 (2.3%), respectively, and 99 (26.5%), 266 (71.1%), and 9 (2.4%) in the alcoholic group. The mean ± SD age of the alcoholics and controls was 38.3 ± 10.5 years (range: 21-76 years) and 37.3 ± 10.4 years (range: 19-65 years), respectively. No significant differences were found between the two cohorts regarding sex or age distribution with Pearson's χ^2 test.

BMI is the measurement of choice to determine obesity, and the clinical diagnosis of obesity is BMI ≥ 30 kg/m^2. The BMI was not significantly different between the alcoholic and nonalcoholic groups [BMI < 30 kg/m^2, 198 (57.6%) *vs* 213 (63.6%); BMI ≥ 30 kg/m^2, 146 (42.4%) *vs* 122 (36.4%), $P = 0.108$]. However, the smoker distribution was significantly different between the alcoholic and nonalcoholic groups [non-smoker, 197 (52.7%) *vs* 282 (82.7%); smoker, 177 (47.3%) *vs* 59 (17.3%), $P = 0.000$]. The alcoholic group had more smokers than the control group had.

Genotypes of -2922(C)2-3, -1345(G/C), -1198(C/G), Ex3+132(G/T/A/C) and Ex3+715(T/-) in Mexican Americans

The genotypes of *-2922(C)2-3*, *-1345(G/C)*, *-1198(C/G)*, *Ex3+132(G/T/A/C)* and *Ex3+715(T/-)* were studied in Mexican American alcoholics and controls. The *-2922(C)2-3* and *-1198(C/G)* alleles were polymorphic in Mexican Americans, but *-1345(G/C)*, *Ex3+132(G/T/A/C)* and *Ex3+715(T/-)* were shown to be present in the studied population (*G/G*, *G/G*, and *T/T*, respectively). These results are consistent with reports which come from Swedish[27], Japanese[29] and Canadian[32] populations with neurological disorders. This is believed to be the first research reporting the absence of polymorphisms in Mexican Americans at the three genomic sites mentioned above.

The genotypes of *-2922(C)2-3* and *-1198(C/G)* as well as their minor allele frequencies are shown in Table 1. The Hardy-Weinberg equilibrium *P* values of *-2922(C)2-3* and *-1198(C/G)* in alcoholics were 0.6914 and 0.2751, respectively, and the *P* values for these two alleles in nonalcoholic controls were 0.00002 and 0.1854. The genotype distribution of the *-2922(C)2-3* allele was significantly different between alcoholics and nonalcoholics ($P = 0.001$); however, there was no significant difference at the allelic level. The genotype as well as the allele frequency of the *-1198(C/G)* allele was significantly different between alcoholics and nonalcoholics ($P = 0.005$ and $P = 0.007$), suggesting the importance of sequence variations in the regulatory region of the *Nurr1* gene in differentiating these two cohorts. In addition, the *-2922(C)2-3* and *-1198(C/G)* alleles showed a strong LD (D' = 0.88) in the alcoholic population, but not in the nonalcoholic population.

Association of -2922(C)2-3 and -1198(C/G) with alcohol dependence after controlling for confounding effect of smoking and obesity

Alcoholics and nonalcoholic controls were stratified according to the smoking and obesity status. When obese individuals were excluded, a significant difference was found at both the genotypic and allelic level between non-obese alcoholics and non-obese controls, for the *-2922(C)2-3* polymorphism (Table 2, $P = 0.000$ and $P = 0.049$) and *-1198(C/G)* polymorphism (Table 3, $P = 0.008$ and $P = 0.032$). When smokers were excluded, between alcoholics and controls, a significant difference was found only at the genotypic level for the *-2922(C)2-3* polymorphism (Table 2, $P = 0.037$), but only at the allelic level for the *-1198(C/G)* polymorphism (Table 3, $P = 0.034$).

DISCUSSION

Hispanics are one of the fastest growing ethnic groups in the United States and were expected to become the largest minority group by the year 2010[33]. About two-thirds of Hispanic population is of Mexican American or Mexican origin. These population demographics have shown evidence of a serious problem of alcohol dependence with a prevalence rate of heavy drinking in Mexican American men that is three times higher than in non-Hispanic male populations[34]. Excessive alcohol drinking carries a high risk of developing various types of chronic diseases. Alcohol-related problems in this population, such as alcoholic liver disease, malignant neoplasms, psychiatric conditions, neurological impairment, and cardiovascular disease show a significantly higher

Table 2 Genotype and allele frequency of the *-2922(C)2-3* allele in Mexican American alcoholics and non-alcoholics controlling for confounding effect of smokers and obesity *n* (%)

Groups	Genotype			Allele frequency
	(C)2/(C)2	*(C)2/(C)3*	*(C)3/(C)3*	*(C)3*
Non-alcoholic				
BMI < 30 kg/m^2 (213)	73 (34.3)	78 (36.6)	62 (29.1)	202 (47.4)
BMI ≥ 30 kg/m^2 (122)	41 (33.6)	48 (39.3)	33 (27.0)	114 (46.7)
Alcoholic				
BMI < 30 kg/m^2 (198)	37 (18.7)	107 (54.0)	54 (27.3)[a]	215 (54.3)[b]
BMI ≥ 30 kg/m^2 (146)	37 (21.5)	74 (50.7)	35 (25.9)	144 (49.3)
Non-alcoholic				
Non-smokers (282)	100 (35.5)	101 (35.8)	81 (28.7)	263 (46.6)
Smokers (59)	17 (28.8)	29 (49.2)	13 (22.0)	55 (46.6)
Alcoholic				
Non-smokers (197)	49 (24.9)	88 (44.7)	60 (30.5)[c]	208 (52.8)
Smokers (177)	38 (21.5)	104 (58.8)	35 (19.8)[d]	174 (49.2)

[a]*P* = 0.000 *vs* nonalcoholic body mass index (BMI) < 30 kg/m^2; [b]*P* = 0.049 *vs* nonalcoholic BMI < 30 kg/m^2; [c]*P* = 0.037 *vs* non-alcoholic non-smokers; [d]*P* = 0.016 *vs* alcoholic non-smokers.

Table 3 Genotype and allele frequency of the *-1198(C/G)* allele in Mexican American alcoholics and non-alcoholics controlling for confounding effect of smoking and obesity *n* (%)

Groups	Genotype			Allele frequency
	C/C	*C/G*	*G/G*	*C*
Non-alcoholic				
BMI < 30 kg/m^2 (199)	54 (27.1)	81 (40.7)	64 (32.2)	189 (47.5)
BMI ≥ 30 kg/m^2 (115)	21 (18.3)	62 (53.9)	32 (27.8)	104 (45.2)
Alcoholic				
BMI < 30 kg/m^2 (181)	26 (14.4)	92 (50.8)	63 (34.8)[a]	144 (39.8)[b]
BMI ≥ 30 kg/m^2 (136)	18 (13.2)	73 (53.7)	45 (33.1)	109 (40.1)
Non-alcoholic				
Non-smokers (265)	61 (23.0)	122 (46.0)	82 (30.9)	244 (46.0)
Smokers (55)	15 (18.3)	24 (53.9)	16 (27.8)	54 (49.1)
Alcoholic				
Non-smokers (185)	27 (14.6)	90 (48.6)	68 (36.8)	144 (38.9)[c]
Smokers (161)	21 (13.0)	86 (53.4)	54 (33.5)[d]	128 (39.7)

[a]*P* = 0.008 *vs* nonalcoholic body mass index (BMI) < 30 kg/m^2; [b]*P* = 0.032 *vs* nonalcoholic BMI < 30 kg/m^2; [c]*P* = 0.034 *vs* nonalcoholic non-smokers; [d]*P* = 0.05 *vs* non-alcoholic smokers.

incidence than when compared with those from other ethnic backgrounds[35]. Apart from environmental factors, using family, twin pair, half sibling, and adoption studies, alcohol dependence has been proved to be a polygenic disorder[1-4].

Candidate gene associations approach has been widely used to explore related genetic factors. We have previously studied genes involved in both alcohol metabolism and reward pathways in Mexican American alcoholics and have established associations between certain phenotypes of alcohol dependence and the polymorphism of these genes[9-13,36,37]. We showed that most Mexican Americans carry the *ADH1B*1* (95%) and *ALDH2*1* (99.4%) genes. Thus, the *ADH1* and *ALDH2* genotypes do not distinguish those more prone to alcohol drinking from those who are not[9]. Our data also showed the importance of polymorphism of *DRD2 (-141C Del/Ins)* and *5-HTTLPR* in contribution to alcohol dependence[9-12]. Other components of the dopamine pathway have also been associated with alcohol dependence. *DRD4 VNTR* genotypes without the 7-repeat allele have been found to be risk factors for alcohol dependence in Mexican Americans[13]. In the current study, to the best of our knowledge, we reported for the first time a significant association between the polymorphisms in the promoter region of the *Nurr1* gene and alcohol dependence in Mexican Americans. Our findings indicated that polymorphisms of *-2922(C)2-3* and *-1198(C/G)* are associated with alcohol dependence in the Mexican American population, even when the confounding effects of smoking and obesity were controlled, suggesting the reliability of our findings. The frequency of the *-1198 G* allele was significantly higher in alcoholics than in controls, suggesting that the *G* allele of *-1198(C/G)* might potentially have a pathogenic effect on alcohol drinking. The functional significance of these two polymorphisms still needs to be explored. Taken together, since *Nurr1* is upstream of the dopamine pathway, our data indicate the importance of *Nurr1*/dopamine signaling in alcohol abuse in this important minority group.

Smoking and obesity might represent significant confounding factors in the relationship between risk factors and alcohol dependence. Alcohol and constituents of tobacco are potent inducers of *CYP2E1*. When smokers were excluded from both the control and alcoholic groups, the **5B* allele of the *CYP2E1* gene was significantly associated with alcohol dependence[38]. Similar findings were observed for the *DRD2 -141C Ins/Del* allele[10]. In the present studied population, 47.3% of alcoholics and 17.3% of nonalcoholics were smokers. The distribution of smokers and nonsmokers in alcoholic and nonalcoholic groups showed a significant difference. When all participants were considered, distribution of both *-2922(C)2-3* and *-1198(C/G)* polymorphisms showed significant differences between the alcoholic and nonalcoholic groups. When smokers were excluded, the association of alcohol dependence with these two polymorphisms remained. When obesity was considered, *-2922(C)2-3* and *-1198(C/G)* polymorphisms were associated with alcohol dependence in the non-obese groups. Associations of these two polymorphisms and alcohol dependence are consistently found in the nonsmoking and non-obese group, therefore, this increases the reliability of our findings.

In conclusion, the polymorphism of *-2922(C)2-3* and *-1198(C/G)* in the regulatory region of the *Nurr1* gene were shown to be associated with alcohol dependence in Mexican Americans. The functional significance of these two polymorphisms still needs to be studied. Alcohol dependence is a multifactorial disease, therefore, it is also important to study the interaction of *Nurr1* with other components of the dopamine signaling pathway in contributing to alcohol dependence, which might lead to effective treatment strategies.

COMMENTS

Background

It is widely accepted that genetic factors play an important role in the development of alcohol dependence. Family clustering surveys have shown that alcohol dependence rates are higher among persons who are biologically related to an alcoholic. Nur-related receptor 1 (*Nurr1*) is a transcription factor in the orphan nuclear receptor family that can regulate dopamine neurotransmission and influence the expression of genes important for human brain development.

Research frontiers

Nurr1 regulates the dopaminergic system. Several polymorphisms in the gene have been identified. Two variants (*-291Tdel* and *-245T→G*) of *Nurr1* are associated with Parkinson's disease. The *Nurr1* locus is located in the genome region that contributes to alcohol dependence. Thus, the authors hypothesize the presence of an association between polymorphism of the *Nurr1* gene and alcohol dependence.

Innovations and breakthroughs

Several polymorphisms of *Nurr1* have been identified: *-469delG*, *M97V*, *H103R*, *DY122*, *-2922(C)2-3*, *IVS6+17* approximately *+18insG* and *EX8+657(CA)9-10*. *-2922(C)2-3*, *IVS6+17* approximately *+18insG* and *EX8+657* variants are common in both Caucasian and Asian populations. Two variants (*-291Tdel* and *-245T→G*) of *Nurr1* are associated with Parkinson's disease. Further investigation of common *Nurr1* variants did not support their pathogenic role, and none was found to be associated with alcohol addiction. In the present study, the authors showed that genotype distribution of *-2922(C) 2-3* and *-1198(C/G)* was significantly different between nonalcoholic and alcoholic Mexican Americans. The *-1198C* frequency was found to be significantly higher in nonalcoholics than that in alcoholics. However, the *-1345(G/C)*, *Ex3+132(G/T/A/C)*, and *Ex3+715(T/-)* alleles were not polymorphic in Mexican Americans; all the studied Mexican Americans had *G/G*, *G/G and T/T* genotype for these three alleles, respectively.

Applications

This study suggested that polymorphisms in the regulatory region of the *Nurr1* gene are implicated in pathogenesis of alcohol dependence of Mexican Americans. The *Nurr1*/dopamine signaling pathway might be important for the development of alcohol dependence in Mexican Americans.

Terminology

NR4A2 is a transcription factor in the orphan nuclear receptor family, and is important for development of dopaminergic neurons. *Nurr1* is an upstream signaling molecule for regulating the dopamine pathway and plays a broad-spectrum role in brain development.

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This is a good descriptive study in which the authors analyzed the polymorphisms of the *Nurr1* gene in 374 alcoholic and 346 nonalcoholic Mexican Americans. They found that the genotype distributions of *-2922(C) 2-3* and *-1198(C/G)* were different between alcoholics and non-alcoholics. The presented data suggest that the *Nurr1*/dopamine signaling pathway might be important for the development of alcohol dependence in Mexican Americans.

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