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The Interaction of Reward Genes With Environmental Factors in Contribution to Alcoholism in Mexican Americans

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Abstract

Background—Alcoholism is a polygenic disorder resulting from reward deficiency; polymorphisms in reward genes including serotonin transporter (5-HTT)-linked polymorphic region (5-HTTLPR), A118G in opioid receptor mu1 (OPRM1), and –141C Insertion/Deletion (Ins/Del) in dopamine receptor D2 (DRD2) as well as environmental factors (education and marital status) might affect the risk of alcoholism. Objective of the current study was to examine the main and interacting effect of these 3 polymorphisms and 2 environmental factors in contribution to alcoholism in Mexican Americans.

Methods—Genotyping of 5-HTTLPR, OPRM1 A118G, and DRD2–141C Ins/Del was performed in 365 alcoholics and 338 nonalcoholic controls of Mexican Americans who were gender- and age-matched. Alcoholics were stratified according to tertiles of MAXDRINKS, which denotes the largest number of drinks consumed in one 24-hour period. Data analysis was done in the entire data set and in each alcoholic stratum. Multinomial logistic regression was conducted to explore the main effect of 3 polymorphisms and 2 environmental factors (education and marital status); classification tree, generalized multifactor dimensionality reduction (GMDR) analysis, and polymorphism interaction analysis version 2.0 (PIA 2) program were used to study factor interaction.

Results—Main effect of education, OPRM1, and DRD2 was detected in alcoholic stratum of moderate and/or largest MAXDRINKS with education 12 years, OPRM1 118 A/A, and DRD2 –141C Ins/Ins being risk factors. Classification tree analysis, GMDR analysis, and PIA 2 program all supported education*OPRM1 interaction in alcoholics of largest MAXDRINKS with education 12 years coupled with OPRM1 A/A being a high risk factor; dendrogram showed synergistic interaction between these 2 factors; dosage-effect response was also observed for

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Results of HWE Test

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education*OPRM1 interaction. No definite effect of marital status and 5-HTTLPR in pathogenesis of alcoholism was observed.

Conclusions—Our results suggest main effect of education background, OPRM1 A118G, and DRD2 –141C Ins/Del as well as education*OPRM1 interaction in contribution to moderate and/or severe alcoholism in Mexican Americans. Functional relevance of these findings still needs to be explored.

Keywords

Alcoholism; Serotonin Transporter; Opioid Receptor mu-1; Dopamine Receptor D2; Education; Marriage; Interaction; Mexican American

Alcoholism has a significant impact on the nation's health and welfare especially in Mexican Americans. Mexican Americans represent a prominent segment of the American mosaic and appear to be at a high risk for alcohol problems. The prevalence rate of past heavy drinking among male Mexican Americans is 3 times higher than that reported for non-Hispanic male populations (Lee et al., 1997). Furthermore, Hispanics with alcohol-induced problems, such as alcoholic liver disease (ALD), appear to fare significantly less well than those with other ethnic backgrounds. For example, the survival rate of Hispanic ALD patients after 4.5 years of follow-up is only 28%, in contrast to 66% for African Americans and 40% for Caucasians (Mendenhall et al., 1989). Studying the risk factors of alcoholism in Mexican Americans will lead to the development of diagnostic, therapeutic, and preventive strategies.

Based on family, twin pair, half sibling, and adoption studies, it has been proved that alcoholism is a polygenic complex disorder with heritability ranging between 49% and 64% (Cotton, 1979; Goodwin, 1978; Heath et al., 1997; McGue, 1999). Complex interaction of multiple genetic and environmental factors plays an important role in the development of alcoholism. Candidate gene association approach has been widely used to study the genetic risk factors for polygenic complex disorders including alcoholism. Although the pathogenic mechanism of alcoholism is not clearly understood, genes in the reward system are important and are frequently considered as candidates in association studies.

The reward system, which is a collection of brain neuro-transmission pathways, reinforces behavior by inducing pleasurable effects after stimulation of "rewards." Primary natural rewards include those that are necessary for the survival of human beings such as food and sex. Drugs, alcohol, risky sports, etc. can also stimulate the reward system, and are referred to as "unnatural rewards" (Comings and Blum, 2000). In 1996, the reward deficiency hypothesis was proposed (Blum et al., 1996a,b) which postulates that individuals who have dysfunctions of 1 or more components of the reward system tend to be less satisfied with natural rewards and tend to abuse unnatural rewards including alcohol as a way to seek enhanced stimulation of the reward pathways. The final result of such a process is "reward deficiency syndrome" which may be comprised of negative behaviors such as alcoholism, substance abuse, and smoking.

The reward system is mainly composed of 4 neurotransmission pathways: serotonin (5-HT), opioid, dopamine, and gamma-aminobutyric acid (GABA) pathways, and these 4 pathways form a “reward cascade” (Blum et al., 1996a,b). Serotonergic neurons release 5-HT, then 5-HT activates the opioidergic neurons and endogenous opioid is released which will inhibit the release of GABA. The primary role of GABA is to inhibit activation of dopamine neurons, so the result of inhibiting GABA activity is an increase of dopamine release. Finally, dopamine, a “pleasure molecule” (Blum et al., 2000), will produce a feeling of elation.

The common mechanism of reward deficiency is inhibition of dopaminergic neurotransmission, and utilizing natural dopaminergic repletion therapy to promote long-term dopaminergic activation will ultimately lead to a common, safe and effective modality to treat reward deficiency syndrome behaviors (Blum et al., 2008). At the same time, the final outcome of the reward cascade is decided by the function of all the 4 neurotransmission pathways. Theoretically, genetic variations leading to dysfunction of any component in these 4 pathways will likely result in the reward deficiency syndrome. Most of the reward genes are highly polymorphic, and a number of genetic polymorphisms in the functional regions of these genes have been implicated as being responsible for altering the gene functions and have been associated with alcoholism, among which are 5-HT transporter (5-HTT) linked polymorphic region (5-HTTLPR) in 5-HTT, A118G polymorphism (rs1799971) in opioid receptor mu1 (OPRM1), and –141C Insertion/Deletion (Ins/Del) polymorphism (rs1799732) in dopamine receptor D2 (DRD2).

5-HTT plays an important role in the serotonergic transmission. It is responsible for reuptake of 5-HT in the synaptic cleft back into the presynaptic neuron, which thus terminates the synaptic actions of serotonin and recycles it into the neurotransmitter pool (Ramamoorthy et al., 1993). 5-HTTLPR is a functional polymorphism involving the insertion or deletion of a 44-bp motif in the promoter region, resulting in a long (L) allele and a short (S) allele, respectively. The short allele has a markedly reduced transcriptional efficiency than its longer counterpart (Bradley et al., 2005; Smith et al., 2004). Regarding the association between 5-HTTLPR and alcoholism, many studies in non-Mexican American populations have yielded conflicting results, while a meta-analysis based on 17 published studies (including 3,489 alcoholics and 2,325 controls mainly of European ancestry) concluded that the short allele was significantly associated with increased risk to alcoholism with an odds ratio (OR) of 1.18 (Feinn et al., 2005). For Mexican Americans, our previous study, which has a smaller sample size compared with the current study, has consistent finding with the meta-analysis (Konishi et al., 2004b).

OPRM1 is the primary site of action for opiates. The most frequently studied single nucleotide polymorphism (SNP) in this gene is A118G. OPRM1 A118G results in an amino acid change from asparagine (Asn) to aspartic acid (Asp) at position 40 of the receptor protein which is a putative *N*-glycosylation site. The Asp-containing receptor has 3 times higher affinity to the β -endorphin than the Asn form (Bond et al., 1998). In addition, OPRM1 118A mRNA is 1.5- to 2.5-fold more abundant than the 118G mRNA in heterozygous brain autopsy tissues, and 118G causes a 10-fold reduction in OPRM1 protein level (Zhang et al., 2005). Previous association studies regarding relationship between

A118G in OPRM1 and alcoholism have produced conflicting results. In some studies, no association was found (Bergen et al., 1997; Franke et al., 2001; Zhang et al., 2006), while in some other studies 118G or 118A was found to increase alcoholism risk (Bart et al., 2005; Schinka et al., 2002). An association study of OPRM1 A118G and alcoholism in Mexican Americans has not been conducted.

Although other neurotransmitters may be important in determining the rewarding and stimulating effects of ethanol, dopamine may be critical for initiating substance use. A deficiency or absence of DRD2 receptors then predisposes individuals to a high risk for multiple addictive, impulsive, and compulsive behaviors (Bowirrat and Oscar-Berman, 2005). DRD2 is a G protein-coupled receptor located on postsynaptic dopaminergic neurons that are centrally involved in reward signal transduction (Neville et al., 2004). The DRD2 -141C Ins/Del polymorphism is located in the 5' promoter region 141 base pairs upstream from exon1 and involves insertion or deletion of a cytosine. Compared with the insertion allele (Ins), the deletion allele (Del) is related to lower transcription activity (Arinami et al., 1997), but higher striatal DRD2 density (Jonsson et al., 1999). Ishiguro and colleagues (1998) found that the Ins allele increased the risk for alcoholism, while many other studies failed to replicate such an association (Chen et al., 2001; Parsian et al., 2000; Sander et al., 1999). In Mexican American populations, the Ins allele was found to be a risk factor for alcoholism by our previous study, in which 200 alcoholics were studied (Konishi et al., 2004b).

In the GABA pathway, GABA receptor (GABR) genes have been considered as candidates for alcoholism. The GABA receptor $\beta 3$ gene (GABR $\beta 3$) encodes a major subunit of the GABA_A receptor. There is a CA repeat polymorphism in GABR $\beta 3$. Noble and colleagues (1998) reported that 1-repeat allele (G1) contributed to the risk of alcoholism, but our previous study, which included 200 Mexican American alcoholics and 251 non-alcoholics, did not find an association between the G1 variant and alcoholism (Konishi et al., 2004b). Thus, GABR $\beta 3$ is not included in the current study. Besides, lack of functional information for this polymorphism, as well as sample size limitation for a multiallelic polymorphism, is the reason why GABR $\beta 3$ is excluded from further study.

Environmental factors, such as education background and marital status, also play an important role in pathogenesis of reward deficiency syndrome including alcoholism and drug abuse. In the National Longitudinal Alcohol Epidemiology Study, the risk of alcoholism among participants with <12 years of education is significantly higher (odds ratio = 1.24) than among participants with 16 years or more (Grant, 1997). With the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC, 2001–2002) participants, individuals without a college degree have higher risk of alcoholism than individuals with a college degree or more; the magnitude of risk, however, varies significantly by race-ethnicity, and there is no association between education and alcoholism among Hispanics (Gilman et al., 2008). As to marital status, never having been married was strongly associated with alcohol dependence and abuse in Swedish women (Thundal and Allebeck, 1998), and women who married or remarried decreased drinking, whereas those who became separated or divorced increased drinking (Hanna et al., 1993). For males,

transition into marriage is significantly associated with greater decreases in drug use during the mid to late 20s (Flora and Chassin, 2005).

The findings regarding association of an individual polymorphism in reward genes or a single environmental factor with alcoholism have been very inconsistent partly due to the low-penetrance and minor-effect nature of every individual factor coupled with a failure to consider the complexity of gene-gene or gene-environment interactions (Goode et al., 2002; Wu et al., 2007). As what has been described above, the function of different neurotransmission pathways in reward system is closely linked with each other, so it is highly possible that the effect of a polymorphism in 1 pathway can be modified by polymorphisms in other pathways. It has been shown that after stratification of DRD2 TaqIA genotypes, a significant difference for 5-HTTLPR polymorphisms between antisocial alcoholics and antisocial non-alcoholics was found, indicating an interaction between DRD2 TaqI A and 5-HTTLPR (Wu et al., 2008). Interaction between reward genes and environmental factors has also been reported. The risk of alcoholism associated with the GABRA2 genotype varies as a function of marital status. Among individuals not currently married, the influence of GABRA2 on alcohol dependence is not detectable. However, among individuals who report a stable marriage, the influence of GABRA2 is significant (Dick et al., 2006). The interactions among 5-HTTLPR, OPRM1 A118G, DRD2 –141C Ins/Del, education background, and marital status in the development of reward deficiency syndrome have not been reported in any populations.

In the current study, for the first time, we examine the interactions among 5-HTTLPR, OPRM1 A118G, DRD2 –141C Ins/Del, education background, and marital status in contribution to alcoholism in Mexican Americans.

MATERIALS AND METHODS

Study Population

Unrelated Mexican Americans living in the Los Angeles County including 338 controls and 365 alcoholics were recruited (109 controls and 200 alcoholics were from our previous studies) (Konishi et al., 2004b; Luo et al., 2005). Controls and alcoholics were gender-and age-matched. Participants were recruited from a variety of sources including: (1) Human services, substances dependence, and mental health programs; (2) driving schools and the Alcohol Anonymous Groups organized and/or associated with these schools; (3) bars and liquor stores; (4) Hispanic churches with counseling services programs; (5) day labor centers; and (6) 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: (1) no current or past diagnosis of DSM-IV alcohol dependence (303.90) or alcohol abuse (305.00); and (2) no clinically unacceptable findings from physical examinations and vital signs. The inclusion criteria for both controls and alcoholics were as follows: (1) ability to give informed consent; (2) between 21 and 79 years; (3) 3 of 4 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 months;

and (6) no current or past diagnosis of mental illnesses such as schizophrenia, schizophreniform disorder, schizoaffective disorder, schizotypal disorder, major depression, 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 information including gender, age, marital status, duration of education, medical history, history of smoking and alcohol consumption. Marital status was categorized into 3 types: being married, being single or living with a partner. Having been divorced, separated, and widowed was regarded as being single. Current smoking was defined as having smoked 1 or more cigarettes on 1 or more days during the past 30 days (Christophi et al., 2008). Each alcoholic was interviewed with The Semi-Structured Assessment for the Genetics of Alcoholism II (SSAGA-II) (Bucholz et al., 1994). Information of MAXDRINKS was collected according to participants' response to the question in SSAGA "What's the largest number of drinks you have had in a 24-hour period?"

Genotyping

Peripheral venous blood samples were collected for all participants and kept at -70°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).

OPRM1 A118G and -141C Ins /Del genotypes were determined by PCR followed by restriction fragment length polymorphism analysis according to Gelernter and colleagues (1999) and Arinami and colleagues (1997), respectively. The 5-HTTLPR genotype was determined by PCR amplification according to Collier and colleagues (1996). 5-HTTLPR and DRD2 -141C Ins /Del genotypes of 109 controls and 200 alcoholics had been determined in our previous studies (Konishi et al., 2004b).

Statistical Analysis

Hardy-Weinberg equilibrium (HWE) in controls as well as in alcoholics was tested for all the 3 polymorphisms with the HWSIM program. Pearson's chi-square test was conducted to compare the gender, smoking status, and marital status distribution between controls and alcoholics. Age and education background difference was evaluated with both Pearson's chi-square test and Wilcoxon rank sum test. Main effect of each factor or factor combination was explored with binary or multinomial logistic regression. Interaction among different factors was studied with classification tree analysis, generalized multifactor dimensionality reduction (GMDR) program, and polymorphism interaction analysis program version 2.0 (PIA2). The grayscale interaction dendrogram was adapted from the colorful dendrogram made by multifactor dimensionality reduction (MDR) program version 2.0-alpha (MDR 2.0-

alpha). Pearson's chi-square test, Wilcoxon rank sum test, and logistic regression were performed with SPSS 15.0 software package (SPSS Inc., Chicago, IL). $p < 0.05$ was taken as significance level for all statistical analyses. Bonferroni correction was applied for multiple comparisons.

RESULTS

Demographics of Participants

There were 80 (23.7%) and 69 (18.9%) females in 338 non-alcoholic controls and 365 alcoholics, respectively. There was no significant difference in gender distribution between these 2 cohorts ($\chi^2 = 2.385$, $p = 0.122$).

The number of young (< 30 years), middle-aged (30 to 60 years), and old (>60 years) participants in 338 controls was 93 (27.5%), 237 (70.1%), and 8 (2.4%), respectively. In the alcoholic group (365), there were 99 (27.1%) young, 257 (70.4%) middle-aged, and 9 (2.5%) old participants. The median age for controls was the same as that of alcoholics (37 years). No significant difference was found regarding age distribution with Pearson's chi-square test ($\chi^2 = 0.019$, $p = 0.991$) and Wilcoxon rank sum test ($Z = -1.032$, $p = 0.301$).

Six control participants did not provide the information of smoking history. There were significantly more current smokers in 365 alcoholics than in 332 controls (45.5% vs. 17.2%, $\chi^2 = 64.045$, $p < 0.001$).

Seventeen controls and 8 alcoholics did not provide the information of marital status. The number of participants who were married, single and living with a partner was 120 (37.4%), 185 (57.6%), and 16 (5.0%) in 321 controls, while the number was 113 (31.7%), 215 (60.2%), and 29 (8.1%) in 357 alcoholics. No significant difference was found between these 2 cohorts regarding distribution of marital status ($\chi^2 = 4.317$, $p = 0.116$).

As to education background, fifteen controls and 4 alcoholics did not provide the information. The median duration of education for 323 controls and 361 alcoholics was 12 and 11 years, respectively. There were 111 (34.4%) controls and 75 (20.8%) alcoholics who had received education of more than 12 years. Significant difference was found between these 2 cohorts with Wilcoxon rank sum test ($Z = -2.663$, $p = 0.008$) and chi-square test ($\chi^2 = 15.902$, $p < 0.001$).

There were 2 alcoholics who did not provide the information of MAXDRINKS. For the rest 363 alcoholics, the minimum, maximum, and median MAXDRINKS was 4, 100 and 25, respectively. The skewness value was 1.203 with a standard error of 0.128, and the kurtosis value was 1.544 with a standard error of 0.255. The first and second tertile of MAXDRINKS was 22 and 35, respectively. There were 124 (34.2%) alcoholics with MAXDRINKS < 22, 121 (33.3%) with MAXDRINKS 23 to 35, and 118 (32.5%) with MAXDRINKS > 36.

Main Effect of Each Individual Factor on Alcoholism

Genotype distribution in controls as well as in alcoholics for each of the 3 studied polymorphisms was in HWE ($p > 0.05$, Table 1 and Table S1).

Controlling for the confounding effect of gender, age, and smoking status, the binary logistic regression model was constructed to explore the main effect of marriage status, education background, 5-HTTLPR, OPRM1 A118G and DRD2 –141C Ins/Del on alcoholism. With all alcoholics included, the model showed that only duration of education was associated with alcoholism ($p = 0.002$, Table 2). Compared with education of more than 12 years, education of less than or equal to 12 years was a risk factor for alcoholism with an odds ratio (OR) of 1.792 (95%CI 1.236 to 2.604).

When alcoholics were divided into 3 subgroups according to tertiles of MAXDRINKS, the multinomial logistic regression analysis controlling for the effect of confounding factors indicated that alcoholics with different MAXDRINKS were related to different risk factors (Table 2). For alcoholics with the largest MAXDRINKS (≥ 36), education of less than or equal to 12 years, OPRM1 118 A/A, and DRD2 –141C Ins/Ins were all risk factors with the ORs being 1.966, 2.162, and 1.847, respectively. For alcoholics with medium MAXDRINKS (23 to 35), only education of less than or equal to 12 years was found to be a risk factor with an OR of 2.032. For alcoholics with the smallest MAXDRINKS (≤ 22), neither the environmental factors (marital status and education background), nor the genetic factors (5-HTTLPR, OPRM1 A118G and DRD2 –141C Ins/Del) were associated with alcoholism.

Interaction Among Environmental and Genetic Factors

Classification Tree Analysis—Classification tree analysis including the 2 environmental (marital status and education background) and 3 genetic factors (OPRM1, HTTLPR, and DRD2) was performed through consecutive data splitting. No factor interaction was detected when all alcoholics, alcoholic subgroup with MAXDRINKS ≥ 22 or with MAXDRINKS 23 to 35 were considered. For overall alcoholics and alcoholic subgroup with MAXDRINKS 23 to 35, only a splitting by education background can be made ($p < 0.001$ and $p = 0.001$, respectively). For alcoholic subgroup with MAXDRINKS ≥ 22 , no splittings can be made. For alcoholic subgroup of MAXDRINKS ≥ 36 , 3 consecutive splittings by education background, OPRM1 and HTTLPR were made, and a Bonferroni corrected p -value which was less than 0.05 was observed for each splitting (Fig. 1). Binary logistic regression controlling for effect of gender, age, smoking and DRD2 was adopted to compare the effect of each factor combination (Fig. 1). Education ≤ 12 years had to be combined with at least 1 genetic risk factor (OPRM1 A/A and/or HTTLPR S/S) to become a risk factor, although it was found to have main effect on pathogenesis of alcoholism, indicating interactions among education background, OPRM1 and HTTLPR.

GMDR Analysis—With the 2 environmental (marital status and education background) and 3 genetic factors (OPRM1, HTTLPR, and DRD2), GMDR analysis controlling for effect of gender, age, and smoking constructed different risk prediction models for alcoholic subgroups of different MAXDRINKS (Table 3). For overall alcoholics and alcoholics of

MAXDRINKS 23 to 35, the best prediction model contained only 1 factor, the education background, whose testing balanced accuracy was 56.25 and 57.04%, respectively. For alcoholics of MAXDRINKS 22, no environmental or genetic factors were suitable for risk prediction. For alcoholics of MAXDRINKS 36, the 2-factor model consisting of education background and OPRM1 showed the best prediction efficacy with sign test p -value less than 0.05, highest cross-validation consistency (10/10) and highest testing balanced accuracy (63.16%), suggesting interaction between education background and OPRM1. Combination of education 12 years and OPRM1 A/A served as a high risk factor for alcoholism of MAXDRINKS 36, while the other combinations served as low risk factors (Fig. 2).

Analysis With PIA2 Program—With 7 different scoring functions including % correct, sensitivity + specificity, positive predictive value (PPV) + negative predictive value (NPV), risk ratio, odds ratio, and Gini Index, PIA2 software was used to further evaluate the efficacy of risk prediction models containing different number of factors (Table 4). For alcoholics of MAXDRINKS 36, education*OPRM1 and education*OPRM1*HTTLPR were shown to be the best 2-factor and 3-factor model with highest overall score, respectively, supporting the findings regarding factor interaction revealed by classification tree and GMDR analysis.

Dendrogram Regarding Education*OPRM1*HTTLPR Interaction—A dendrogram regarding the relationship among education background, OPRM1, and HTTLPR for alcoholics of MAXDRINKS 36 was made by MDR 2.0-alpha software (Fig. 3). Education background and HTTLPR were shown to be redundant with each other, while OPRM1 was synergistic with both education background and HTTLPR.

Dose-Effect Response of Factor Interaction—For education*OPRM1 interaction detected with alcoholics of MAXDRINKS 36, dosage-effect response was observed by binary logistic regression controlling for effect of gender, age, smoking, and DRD2 (Table 5). Between individuals containing no and 1 risk factor, the risk for severe alcoholism with MAXDRINKS 36 was not significantly different ($p = 0.694$). Individuals containing both 2 risk factors, however, were more susceptible to severe alcoholism ($p = 0.003$, OR = 3.258).

DISCUSSION

In this study, we found the main effect of education background, OPRM1 A118G, and DRD2 -141C Ins/Del as well as the interaction between education background and OPRM1 A118G in modulating risk for alcoholism in Mexican Americans. Education 12 years, OPRM1 A/A, and DRD2 Ins/Ins were shown to be risk factors. No definite effect of marital status and 5-HTTLPR in pathogenesis of alcoholism was observed.

Individuals of lower socioeconomic status are at greater risk for alcoholism. Duration of education reflects the individual's socioeconomic background, insofar as opportunities for schooling are influenced by parental resources (Gilman et al., 2008), so it's not surprising to find education 12 years was a risk factor for alcoholism in Mexican Americans. Improving education level might serve as a preventive strategy for alcoholism in this minority group.

With respect to OPRM1 A118G and DRD2 –141C Ins /Del, functional significance is not well established (Arinami et al., 1997; Bond et al., 1998; Jonsson et al., 1999; Zhang et al., 2005); the mechanism accounting for the causative effect of OPRM1 A/A and DRD2 –141C Ins/Ins still remains to be elucidated.

Regarding the main effect of DRD2 –141C Ins/Del on alcoholism in Mexican Americans, findings of our current study are similar to those revealed by our previous study with a smaller sample size (251 controls and 200 alcoholics) and chi-square test. The current results about main effect of 5-HTTLPR are conflicting with our previous ones which indicated S/S to be a risk factor, probably due to different sample sizes and statistical methods. For the main effect of the 2 environmental factors and 3 genetic factors on alcoholism considered in this study, a number of studies conducted in other ethnic groups have yielded conflicting results. This controversy might be caused by: (1) different sample size, ethnicity, and linkage disequilibrium (LD) patterns; (2) different status of population stratification and sample heterogeneity; and (3) neglect of gene-gene interactions. In our current study participants with 3 of 4 biological grandparents of Mexican heritage were recruited to ensure ethnic homogeneity and avoid population stratification. In addition, we have performed genotyping for many other polymorphisms in this population (Konishi et al., 2003, 2004a,b; Luo et al., 2005; Mendoza et al., 2001; Yang et al., 2007) finding that the allele frequencies were consistent with those reported by other studies of Mexican Americans. In addition, effect of confounding factors was controlled and factor interactions were considered, which also enhance the reliability of our findings.

Three strategies with different advantages were used simultaneously in this study to identify interactions among the 2 environmental (education background and marital status) and 3 genetic factors (OPRM1, HTTLPR, and DRD2). The main advantage of classification tree analysis is that multiple comparisons can be corrected in a stringent way; GMDR analysis can not only control for the effect of confounders, but also verify the interactions by dividing the data into training and testing set; PIA2 program includes 7 different scoring functions for evaluation of prediction models. Combination of these 3 strategies will lead to a more reliable result. Classification tree analysis indicated education*OPRM1*HTTLPR interaction, while GMDR analysis suggested education *OPRM1 interaction. For PIA2, both the 2-factor and 3-factor interactions mentioned above were supported with both education*OPRM1 and education*OPRM1*HTTLPR being the top prediction models. In summary, all the 3 strategies converged on the education*OPRM1 interaction. Combination of education 12 years and OPRM1 A/A was shown to be a high risk factor for alcoholism by GMDR, which is consistent with the dendrogram illustrating synergy between education background and OPRM1. The synergistic interaction was further confirmed by dosage-effect response analysis of education*OPRM1 with logistic regression, which showed individuals with both 2 risk factors had higher risk for alcoholism than those with no or 1 risk factor. The education*OPRM1 interaction further underscores the importance of improving education level for alcoholism prevention in Mexican Americans. Polygenic disorders, such as alcoholism, are a result of the complex interplay of genetic and environmental factors. Education*OPRM1 interaction found in this current study actually represents such an environment*gene interplay. The mechanism accounting for such an interaction may be the incomplete penetrance of genotypes. OPRM1 A/A has main effect on pathogenesis of

alcoholism, but the expression of genetic effect depends on environment. Only when individuals have low education level, the pathogenic genetic effect can be expressed resulting in the occurrence of alcoholism. Another possibility is that high level education can improve the brain function, including function of reward system, so the pathogenic effect of reward gene variants may be masked. These hypotheses still need to be validated with functional studies. At the same time, the reason why only OPRM1 in the reward pathway interacts with education background still remains to be elucidated.

According to previous studies (Bradley et al., 2005; Smith et al., 2004), 5-HTTLPR S allele results in decreased expression of 5-HTT and less reuptake of 5-HT, so more 5-HT will be degraded in the synaptic cleft. As a result, 5-HT in the neurotransmitter pool may not remain abundant and stable serotonergic neurotransmission may not be maintained, breaking normal function of the reward system and increasing the risk for alcoholism. In our current study, HTTLPR was shown to be involved in the factor interaction by classification tree analysis, and the dendrogram indicated synergy between HTTLPR and OPRM1. Although the involvement of HTTLPR in the interaction was not supported by GMDR analysis, possible effect of HTTLPR on alcoholism still deserves further investigation with a larger sample size.

PIA2 program is designed to be highly sensitive at modest interactions. Even in absence of interacting factors, PIA2 will identify top scoring models, so observed interactions with PIA2 must be interpreted with caution and confirmed with alternative methods (Mechanic et al., 2008). In our current study, PIA2 indicated a top 4-factor model, which was not supported by classification tree and GMDR analysis, so the 4-factor interaction is considered as a false-positive result.

Main effects of and interactions among risk factors were detected with alcoholics of moderate or/and large MAXDRINKS. MAXDRINKS, which denotes the maximum number of drinks a person consumes in a 24-hour period, is an important phenotype used in the Collaborative Study on the Genetics of Alcoholism (COGA) study. Larger MAXDRINKS may reflect an increased tolerance for high levels of alcohol (Edenberg, 2002), so it is reasonable to take MAXDRINKS as an indicator of severity of alcoholism. For a polygenic disorder such as alcoholism, patients with severe forms of the disease always harbor more genetic and environmental risk factors, therefore, it is not surprising to detect main effect of and interaction among reward gene polymorphisms and environmental factors only in alcoholics who have the large MAXDRINKS.

The main effect and interaction of genetic factors identified in the current study might be race-specific and only exist in Mexican Americans. For other ethnic populations with high morbidity of alcoholism, the allele frequency of OPRM1 118G is very low in Africans (0.8 to 2.2%) (dbSNP), and the allele frequency of DRD2 -141C Del is as low as 2.1% in Europeans [Allele Frequency Database (ALFRED), 1999]. Low allele frequency suggests a minor effect of these polymorphisms in pathogenesis of alcoholism in Africans and Europeans.

Although the current study might have the largest collection of Mexican Americans in the literature, given the nature of alcoholism as a polygenic disorder, the limited number of studied polymorphisms and participants might still be the limitation of the current study. Further validations from independent populations are necessary to confirm our findings. More genes in the reward pathways such as serotonin receptors, opioid receptor kappa 1, GABA receptor β 1, dopamine transporter, dopamine receptor D4, etc. can be included in the interaction analysis to produce a more robust result. Alcoholics were stratified arbitrarily according to tertiles of MAXDRINKS in the current study, and the results will be more accurate if we define the severity of alcoholics with Severity of Alcohol Dependence Questionnaire (SADQ) score. None of the strategies used for interaction analysis are perfect, and development of more powerful statistical tools will be critical for future studies. More importantly, findings of current study need to be verified with functional research.

In conclusion, our results suggest a main effect of education background, OPRM1 A118G, and DRD2 –141C Ins/Del as well as education*OPRM1 interaction in contribution to moderate and/or severe alcoholism in Mexican Americans. Functional relevance of these findings still needs to be explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Interaction among genetic and environmental factors with alcoholics of MAXDRINKS 36 by classification tree analysis. p -Value and OR of each factor combination were decided by binary logistic regression controlling for effect of gender, age, smoking, and DRD2.

Fig. 2.

Interaction between OPRM1 and education by GMDR analysis. Dark gray and light gray boxes correspond to the high- and low-risk factor combinations, respectively. The left and right bars within each box correspond to cases and controls, respectively. The top number is the sum of scores for the corresponding group of individuals (cases or controls with particular factor combination). The heights of the bars are proportional to the sum of scores in each group. For education, 0 means ≤ 12 years, and 1 means >12 years. For OPRM1, 0 means A/A, and 1 means A/G&G/G.

Fig. 3.

Dendrogram of interaction among education, OPRM1, and HTTLPR. The gray scale represents a continuum from Synergy to Redundancy. It ranges from black representing a high degree of synergy (positive information gain) to white representing the highest degree of redundancy (negative information gain).

Table 1

Genotype Distribution of 5-HTTLPR, OPRM1 A118G, and DRD2 –141C Ins/Del in Controls and Alcoholics

5-HTTLPR	<i>n</i>	S/S (%)	S/L (%)	L/L (%)
Controls	338	108 (32.0)	173 (51.2)	57 (16.9)
Alcoholics	365	136 (37.3)	163 (44.6)	66 (18.1)
MAXDRINKS 22	124	38 (30.6)	61 (49.2)	25 (20.2)
MAXDRINKS 23–35	121	46 (38.0)	56 (46.3)	19 (15.7)
MAXDRINKS 36	118	51 (43.2)	45 (38.1)	22 (18.6)
OPRM1 A118G	<i>n</i>	A/A (%)	A/G (%)	G/G (%)
Controls	338	198 (58.6)	120 (35.5)	20 (5.9)
Alcoholics	365	230 (63.0)	123 (33.7)	12 (3.3)
MAXDRINKS 22	124	70 (56.5)	48 (38.7)	6 (4.8)
MAXDRINKS 23–35	121	71 (58.7)	47 (38.8)	3 (2.5)
MAXDRINKS 36	118	88 (74.6)	27 (22.9)	3 (2.5)
DRD2 –141C Ins/Del	<i>n</i>	Ins/Ins (%)	Ins/Del (%)	Del/Del (%)
Controls	338	235 (69.5)	97 (28.7)	6 (1.8)
Alcoholics	365	270 (74.0)	84 (23.0)	11 (3.0)
MAXDRINKS 22	124	93 (75.0)	29 (23.4)	6 (1.8)
MAXDRINKS 23–35	121	82 (67.8)	36 (29.8)	2 (1.6)
MAXDRINKS 36	118	94 (79.7)	18 (15.3)	3 (2.5)

Table 2
Main Effects of Environmental and Genetic Factors on Alcoholism With Multinomial Logistic Regression Controlling for the Effect of Gender, Age, and Smoking Status

Factors	Overall			MAXDRINKS 36		
	Controls/ alcoholics	p-Value	OR (95% CI)	Control/ alcoholics	p-Value	OR (95% CI)
Marital status						
Single	185/215	0.254	NA	185/68	0.446	NA
Married	120/113			120/36	Reference	
Living with a partner	16/29			16/10	0.183	
Duration of education						
12 years	212/286	0.002	1.792 (1.236–2.604)	212/96	0.019	1.966 (1.117–3.461)
>12 years	111/75		Reference	111/21		Reference
5-HTTLPR						
S/S	108/136	0.373	NA	108/51	0.082	NA
S/L & L/L	230/229			230/67		
OPRM1						
A/A	198/230	0.292	NA	198/88	0.003	2.162 (1.300–3.597)
A118G A/G & G/G	140/135		140/30		Reference	
DRD2–141C						
Ins/Ins	235/270	0.111	NA	235/94	0.030	1.847 (1.062–3.211)
Ins/Del	103/95			103/24		Reference
Factors	MAXDRINKS			23–35 MAXDRINKS 22		
	Controls/ alcoholics	p-Value	OR (95% CI)	Control/ alcoholics	p-Value	OR (95% CI)
Marital status						
Single	185/75	0.180	NA	185/70	0.971	NA
Married	120/34	Reference		120/43	Reference	
Living with a partner	16/11	0.051		16/8	0.677	
Duration of education						
12 years	212/98	0.01	2.032 (1.186–3.481)	212/90	0.155	NA
>12 years	111/22		Reference	111/32		
5-HTTLPR						
S/S	108/46	0.396	NA	108/38	0.552	NA
S/L & L/L	230/75			230/86		
OPRM1						
A/A	198/71	0.952	NA	198/70	0.605	NA
A118G						
A/G & G/G	140/50			140/54		
DRD2–141C						
Ins/Ins	235/82	0.926	NA	235/93	0.160	NA
Ins/Del	103/39			103/31		

Table 3
Risk Prediction of Alcoholism by GMDR Analysis With Different Alcoholic Subgroups

MAXDRINKS	Number of factors in the model	Best model	Testing balanced accuracy (%)	Cross-validation consistency	Sign test <i>p</i> -value
Overall	1	<i>Education</i>	56.25	10/10	0.0010
	2	Education*Marriage	51.95	4/10	0.1719
	3	Marriage*Education*OPRM1	52.25	6/10	0.1719
	4	Marriage*Education*OPRM1*HTTLPR	51.74	7/10	0.6230
	5	Marriage*Education*OPRM1*HTTLPR*DRD2	50.54	10/10	0.6230
36	1	OPRM1	56.54	9/10	0.0107
	2	<i>Education*OPRM1</i>	63.16	10/10	0.0107
	3	Marriage*Education*OPRM1	59.88	6/10	0.0547
	4	Education*OPRM1*HTTLPR*DRD2	59.79	5/10	0.0547
	5	Marriage*Education*OPRM1*HTTLPR*DRD2	62.05	10/10	0.0547
23–35	1	<i>Education</i>	57.04	10/10	0.0107
	2	Education*Marriage	50.84	5/10	0.1719
	3	Marriage*Education*HTTLPR	52.82	5/10	0.0547
	4	Marriage*Education*OPRM1*HTTLPR	48.22	5/10	0.6230
	5	Marriage*Education*OPRM1*HTTLPR*DRD2	49.91	10/10	0.3770
22	1	Education	51.51	8/10	0.3770
	2	HTTLPR*OPRM1	48.35	7/10	0.6230
	3	Marriage*HTTLPR*DRD2	49.54	7/10	0.6230
	4	Marriage*OPRM1*HTTLPR*DRD2	44.25	5/10	0.9893
	5	Marriage*Education*OPRM1*HTTLPR*DRD2	43.58	10/10	0.9990

Italicized lines mean the best prediction models for the corresponding alcoholic subgroup.

Table 4

Overall Score of Factor Combinations by PIA2 Program With Alcoholics of MAXDRINKS 36

	Single-factor model (overall score)	Two-factor model (overall score)	Three-factor model (overall score)	Four-factor model (overall score)
1st	Education (296.82)	Education*OPRM1 (292.26)	Education*OPRM1*HTTLPR (297.76)	Marriage*Education*OPRM1*HTTLPR (298.73)
2nd	OPRM1 (277.05)	OPRM1*HTTLPR (259.91)	Marriage*Education*OPRM1 (287.06)	Marriage*OPRM1*HTTLPR*DRD2 (286.29)
3rd	HTTLPR (235.82)	Education*DRD2 (246.23)	OPRM1*HTTLPR*DRD2 (266.74)	Education*OPRM1*HTTLPR*DRD2 (277.91)
4th	DRD2 (224.38)	Marriage*Education (245.81)	Education*OPRM1*DRD2 (266.11)	Marriage*Education*HTTLPR*DRD2 (250.94)
5th	Marriage (186.71)	Education*HTTLPR (242.27)	Marriage*OPRM1*HTTLPR (264.18)	Marriage*Education*OPRM1*DRD2 (249.32)

Table 5

Dosage–Effect Response of Education*OPRM1 Interaction With Alcoholics of MAXDRINKS 36

	Factor combination	<i>p</i>-Value	OR (95% CI)
No risk factors *	Both education>12 years and OPRM1 A/G & G/G	Reference	NA
One risk factor *	Either education 12 years or OPRM1 A/A	0.694	NA
Two risk factors *	Both education 12 years and OPRM1 A/A	0.003	3.258 (1.509–7.035)

* Risk factors include education 12 years and OPRM1 A/A.