Int J Clin Exp Med 2016;9(2):4527-4532
www.ijcem.com /ISSN:1940-5901/IJCEM0015894

Original Article

Dimorphisms of NPY2R gene unassociated with alcohol dependence in Chinese Han population

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Received September 3, 2015; Accepted January 12, 2016; Epub February 15, 2016; Published February 29, 2016

Abstract: Objective: Excessive and chronic alcohol use has been associated with damage to major organs and is a leading cause of death in industrialized countries. Research has indicated that alcohol dependence (AD) is related to both heritable and environmental factors. Previous studies have suggested that neuropeptide Y receptors (NPYR) are associated with AD, however, few studies have investigated the associations between NPY2R gene variants and AD in detail. The present study evaluated the association between NPY2R gene variations and AD in a genetically homogeneous Chinese Han population. Methods: All of AD patients were diagnosed using Diagnostic and Statistical Manual of Mental Disorders, 4th Edition criteria, and the two single nucleotide polymorphisms (SNPs) of NPY2R were genotyped in healthy controls (n = 633) and AD patients (n = 547) using the TaqManTM probe. The two SNPs selected, rs6857715 and rs4425326, were located on the 5'-flanking region of NPY2R. Results: The allele and genotype frequencies of all loci did not significantly differ between controls and AD patients (P > 0.05). Interestingly, linkage disequilibrium analysis revealed that one possible haplotype is present between in rs6857715 and rs4425326 on NPY2R gene (D' = 0.974, r2 = 0.488). Conclusion: These results suggest that the two SNPs of the NPY2R gene do not play a role in the northern Chinese Han population with AD.

Keyword: NPY, alcohol dependence, polymorphism

Introduction

Alcohol dependence (AD) is a common disease affecting 4-5% of the population in the United States [1], with a lifetime prevalence of 12.5% [2]. Neuropeptide Y (NPY) is a highly-conserved 36 amino-acid peptide [3] expressed abundantly within the entire mammalian nervous system [4] which plays a key role in control of alcohol intake, withdrawal, and dependence [5]. The effects of NPY are mediated by four membrane-bound, G-protein-coupled receptors: Y1R, Y2R, Y4R, and Y5R [6]. The Y1R and Y2R subtypes, specifically, are highly expressed in the central nervous system (CNS) [7].

The Y1R subtype is the first NPY binding receptor [8]. Animal studies have suggested that NPY plays a part in alcohol preference and consummatory behavior [9-11], and that Y1R and Y2R are associated with alcohol preference in mammals [12, 13]. Injecting NPY reduces ethanol intake in rats [14], and NPY-deficient mice consume more alcohol than wild-type mice [15]. Pharmacological data also show that Y2R is involved in alcohol self-administration, and that mice deficient in Y2R in their genetic backgrounds consume significantly more water than normal mice [16]. NPY and its receptors have also been reported to play a key part in nicotine [17, 18] and alcohol [5, 19, 20] addiction in animal and human studies. In addition, the NPY/R system has been associated with dependency on other drugs such as methamphetamine [21], cocaine, and amphetamine [22]. Further, accumulating evidence has clearly demonstrated a dose-response relationship between alcohol and nicotine use [23-25].

Table 1. Hardy-Weinberg equilibrium test in the two groups

	P value	
Group	rs6857715	rs4425326
AD	0.357730	0.326297
HC	0.912759	0.322713

AD, Alcohol dependence; HC, healthy control.

Table 2. Missing genotype in two loci in the two groups

Group	rs4425326	rs6857715
AD	17	2
HC	18	14

AD, Alcohol dependence; HC, healthy control.

Rs6857715 (T/C) and rs4425326 (T/C) are both located on the 5'-flanking region of NPY-2R, implying possible influence on NPY2R gene transcription regulation and representing good candidates for association study; indeed, previous research has posited that both are associated with alcohol and cocaine dependence [19]. The significance of rs4425326, which is 0.2 Mb upstream from the exon 1 of NPY2R, is unknown though the C-allele carriers have been associated with nicotine dependence in elderly Japanese individuals [17].

The primary aim of this study was to investigate the role of polymorphisms in NPY2R genes in the etiology of alcohol-dependent Chinese Han individuals. Genotyping was performed to elucidate the two SNPs, rs6857715 and rs4425326, in 547 AD patients and 633 healthy controls (HC).

Materials and methods

Subjects

A total of 547 AD inpatients were recruited from psychiatric hospitals in Northern China. All patients met AD criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV). Participants without any history of other drug abuse or dependence, with the exception of nicotine, were included in the sample. Their ages ranged from 23 to 73 years (45.61 ± 9.21).

A total of 633 unrelated HCs 19 to 63 years in age (37.84 ± 9.04) were also recruited. The significant difference was noted in age between the patient group and normal control group ($P < 0.001$). None of the HCs had any history of other drug abuse or dependence, likewise with the exception of nicotine. All of subjects were male, Chinese Hans.

Diagnoses were made by two skilled psychiatrists, and all staff was trained properly before participating in this study. Informed written consent was obtained from all subjects before participation. The entirety of the genetic study was approved by the Institutional Review Board of Inner Mongolia Medical University.

DNA extraction and genetic analysis

A salting-out method was utilized to extract genomic DNA with 5 ml peripheral blood [26]. The two SNPs in the NPY2R genes were genotyped using the 5'nuclease fluorescent TaqManTM primer (Applied Biosystems, Foster City, CA). Post-PCR fluorescence plate reads were conducted with an ABI PRISM 7900HT Sequence Detection System. The TaqMan® primers and probes were purchased from Applied Biosystems. All laboratory procedures were performed in accordance with the manufacturer's instructions, and blind to case control status. The conditions for TaqMan reaction were as follows, with two variations: 50°C for 2 min, 95°C for 10 min, 95°C for 15 s, and 60°C for 1 min, for 50 cycles. Ten percent of the samples were duplicate genotyped randomly, and no genotyping error was identified. Any missing genotypic data was automatically ignored during statistical analysis.

Statistics analysis

Student's t-tests were used to assess differences between continuous variables in the two groups. The genotype and allele frequencies, Hardy-Weinberg Equilibrium, and differences among allele and genotype frequencies between AD and control groups were all analyzed via chi-square method with the SHEsis platform [27]; SHEsis was also utilized to perform linkage disequilibrium analysis. Generalized multifactor dimensionality reduction (GMDR), a nonparametric and genetic model-free alternative to linear or logistic regression for detecting and characterizing nonlinear interactions among discrete genetics, was used to calculate the polymorphism interactions [28]. The *P*-values

Table 3. Allelic frequencies of the two SNPs in the two groups

Group		AD	HC	*P*
Locus	Allele	n (%)	n (%)	
rs6857715	C	489 (0.449)	601 (0.551)	0.239
	T	807 (0.471)	905 (0.529)	
rs4425326	C	323 (0.305)	737 (0.695)	0.667
	T	530 (0.312)	1166 (0.688)	

AD, Alcohol dependence; HC, healthy control.

Table 4. Genotypic frequencies of the two SNP loci in two groups

Group		AD	HC	*P*
Locus	Genotype	n (%)	n (%)	
rs6857715	CC	115 (0.211)	191 (0.223)	0.913
	CT	259 (0.475)	425 (0.496)	
	TT	171 (0.314)	240 (0.280)	
rs4425326	CC	54 (0.102)	89 (0.105)	0.902
	CT	215 (0.406)	352 (0.415)	
	TT	261 (0.492)	407 (0.480)	

AD, Alcohol dependence; HC, healthy control.

of interaction models were defined based on 1,000 permutations. All tests were two-tailed and *P*-value of 0.05 was considered statistically significant. Bonferroni correction was applied for all multiple tests.

Results

No subject showed any departure from Hardy-Weinberg equilibrium for either polymorphism (P > 0.05 of two loci, **Table 1**). Missing genotypic data was demonstrated in two loci as shown in **Table 2**, and the distributions of alleles and genotypes of two variations were as shown in **Tables 3** and **4**, respectively. There were no differences in the distributions of alleles and genotypes of the two SNPs between AD and HC groups (P > 0.05 of two loci). Linkage disequilibrium analysis revealed that rs4425326 and rs6857715 are located on one haplotype (D' = 0.972, r2 = 0.490), however, there was no difference in the distribution of this haplotype between AD and HC groups (P > 0.05).

Discussion

In this study, we investigated the distribution frequencies of two SNPs on the NPY2R gene in a large clinical sample of Chinese Han AD patients and HCs. No significant association was observed in the distributions of allelic and genotypic frequencies of distribution of either SNP between AD patients and HCs, and no possible haplotype or interaction of the loci associated with AD was found.

Both rs6857715 and rs4425326 are located on the upstream of NPY2R. As mentioned above, a previous study conducted with a Japanese sample suggested that rs4425326 may be related to smoking behavior in the elderly population [17], and rs6857715 is located 598 base pairs upstream from the transcription start site of the NPY2R gene [29]. No evidence of either variation associated with drug dependence (including nicotine or alcohol,) has been shown so far in any population, however. Although there is a G allele after the polymorphism in the DNA sequence on rs6857715 (C/T), we speculated that other genetic and epigenetic mechanisms may be involved in the regulation of NPY2R gene expression and structure.

The findings presented here may provide first-hand evidence that this locus is not associated with substance addiction in the Chinese population. Our results are significant for a number of reasons-for one, because women show higher risk for osteoporosis than men, women with a history of AD have a higher lifetime prevalence of fractures [30]; previous studies show that rs6857715 can be used to identify women at risk for osteoporosis [31] and AD and alcohol abuse, and additionally, to identify potential risk of morbidity, mortality, and decreased bone density in men [32]. The fact that rs6857715 may not be associated with AD in males is quite significant. We found that the distance between the two SNPs is almost 1.4 Mb, and identified them in one haplotype block in this study. Consequently, rs4425326 was not shown to be associated with AD.

Some limitations of the present study need to be noted. First, the age was not well-matched between HCs and AD patients, and some younger HCs may be susceptible to AD in the future. Second, only two polymorphisms of the

NPY2R gene were genotyped in this study, failing to fully account for all the genetic information of NPY2R. More variations of this gene, preferably tags and functional variations for the Chinese Han population, should be genotyped to further verify our conclusions. Third, the development of AD is a process of gene-gene/gene-environment interactions, and in this study, we did not consider the influence of environmental AD factors. In future, more environmental factors should be integrated into this research to add perspective to our understanding of AD development and improve the identification of notable genetic variants.

The present study is the first to discuss the role of two SNPs on the NPY2R gene in AD, and provides no evidence of the variations of NPY2R genes associated with AD in a Chinese sample. Our results suggest that the polymorphisms of the NPY2R gene are not associated with AD in this population, however, more variations on the NPY2R gene system and more samples need to be genotyped to pinpoint the NPY2R gene's role in AD.

Acknowledgements

This work was partly supported by the National Natural Science Foundation of China (81-100993, 81171787, 81300311, 81360212, 81571297, 81500519, 81541036, 81500665 and 81560229), the Opening Project of Zhejiang Provincial Top Key Discipline of Pharmaceutical Sciences, Zhejiang Provincial Natural Science Foundation (LY12H03001, LY1-2H15001 and LQ13H280002), Project of Medical Technology of Zhejiang Province (2013-KYA130, 2015KYB234, 2015KYA154, 2016-KYA166 and 2016KYB275), Public Project of Science and Technology of Wenzhou City (Y2-0140739 and Y20150094), Wenling Foundation of Science and Technology (2011-WLCB0109, 2014C311051 and 2015C312-055), Ningbo Natural Science Foundation (2015A610234), Xiangshan Science and Technology Project (2015C6005), and the Research Development Fund of Wenzhou Medical University (QTJ15001).

Disclosure of conflict of interest

None.

Abbreviations

NPY, Neuropeptide Y; CNS, central nervous system; SNP, single nucleotide polymorphism; AD, alcohol dependence; HC, healthy control.

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