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Polymorphisms in the glutamate decarboxylase 1 gene associated with heroin dependence

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ABSTRACT

The GAD1 gene encodes the 67-kDa glutamic acid decarboxylase isoform (GAD67), the rate-limiting enzyme responsible for γ-aminobutyric acid (GABA) biosynthesis from glutamic acid, and may be involved in the development of drug dependence. To identify markers contributing to the genetic susceptibility to heroin dependence, this study examined the potential association between heroin dependence and 15 single nucleotide polymorphisms (SNPs, rs1978340, rs3762556, rs3791878, rs3749034, rs11542313, rs2241165, rs2241164, rs769407, rs3749033, rs16858977, rs701492, rs16858988, rs4668331, rs7578661, rs769395) of GAD1 gene using the MassARRAY system. Participants included 370 heroin-dependent subjects and 389 healthy controls. The allelic or genotypic frequencies of the rs1978340 (promoter region), rs3791878 (promoter region), and rs11542313 (exon 3) polymorphisms in heroin addicts were significantly different from those in healthy controls. Strong linkage disequilibrium was observed in two blocks ($D' > 0.9$). Significantly more C-C-C-C-A haplotypes ($p = 0.0053$ after Bonferroni correction) and significantly fewer T-C-A-C-A haplotypes ($p = 0.0003$ after Bonferroni correction) were found in heroin dependent subjects. These findings point to a role for GAD1 polymorphism in heroin dependence among Han Chinese, and may be informative for future genetic or neurobiological studies on heroin dependence.

1. Introduction

Heroin addiction is a chronic, relapsing brain disease that is characterized by drug dependence, tolerance, compulsive seeking, and use despite harmful consequences. As with other types of substance abuse, genetic predisposition has been shown as a potential risk factor in heroin dependence [1]. Family, adoption, and twin studies have consistently demonstrated a substantial genetic influence on the development of drug addiction, with inherited risk estimates in the range of 40–60% [2,3]. Other studies have suggested that polymorphisms in the glutamic acid decarboxylase gene 1 (GAD1) may relate to addiction to drugs including heroin [4,5].

Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in the conversion of glutamate to GABA (11). Two isoforms of GAD have been identified, GAD1 and GAD2, which previously were called GAD67 and GAD65, respectively. GAD1 is involved in cytosolic GABA synthesis and is responsible for maintaining basal GABA levels, whereas GAD2 is predominately involved in synaptosomal GABA release, and can be rapidly activated when there is high demand for GABA [6]. Rodent studies suggest that knockout of GAD1 (which is important in maintaining GABA levels in the brain) is usually lethal, while knockout of GAD2 has no effect on brain GABA levels [4,7,8], suggesting that GAD1 is the primary rate-limiting enzyme regulating GABA levels under normal conditions. Thus, regulation of GAD1 expression may exert a more profound effect on GABA homeostasis and, possibly, be more sensitive to exogenous agents. Several studies have shown that chronic administration of drugs of abuse, such as alcohol [9], methamphetamine [10], cocaine [11], nicotine [12], and amphetamine [13] alters the activity of GAD1 in the brain, suggesting that the GAD1 gene is an excellent candidate for addiction disorders.

Alcohol dependence and heroin dependence have been associated with polymorphisms in GAD1. Loh et al. examined the association of nine single nucleotide polymorphisms (SNPs) for GAD1 and three SNPs for GAD2 with alcoholism in 140 alcoholic cases and 146 controls in Han Taiwanese [14]. They found evidence of an association of alcohol dependence (AD) with GAD1 but not GAD2. We are aware of only one published report examining the association of GAD1 with heroin dependence in humans. Levran et al. have performed a case–control association analysis of 1350 variants of 130 genes and found experiment-wise significant asso-

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ciation between SNP (rs2058725) in intron 5 and heroin dependence in African Americans [5]. Therefore, the role of GAD1 genes in drug dependence requires further study.

In view of the crucial role of the GAD1 in addiction disorders, as well as the controversy in genetic association studies, the present study investigated more loci in a large case–control sample of the same ethnic origin to verify the putative association between GAD1 polymorphisms and heroin dependence.

2. Materials and methods

2.1. Subjects

A total of 370 unrelated subjects with heroin dependence (aged 18 years and older; mean age of 36.6 ± 6.5) were recruited from the Methadone Maintenance Treatment (MMT) Program of the Xi'an Mental Health Center. Participants were daily or nearly daily users of heroin for a minimum of 1 year prior to assessment. Their addiction status was assessed by a psychiatrist from the Xi'an Mental Health Center, and each subject exhibited behaviors that fulfilled the DSM-IV diagnostic criteria for opioid dependence. The diagnosis of opioid addiction was based on DSM-IV criteria, medical history, urine test results, and interview responses. A case vignette was made to assist with diagnosis, using a semistructured interview with questions on (a) the age at initiation and duration of heroin use, (b) quantity of drug used over this period, (c) route of administration (nasal inhalation or injection), (d) whether other substances were used or abused, and (e) comorbidity for any other psychiatric disorder. Major central nervous system (CNS) diseases and psychoses were evaluated by a senior psychiatrist at the beginning of the methadone management program. Participants were excluded if they: met DSM-IV criteria for an additional Axis I disorder; had a history of alcohol, cigarette, amphetamine, barbiturate, benzodiazepine, or marijuana dependence according to DSM-IV; were taking other prescribed medications that could affect the central nervous system; had a history of seizures, hematological diseases, or severe liver or kidney impairment; or were pregnant. The study complied with the guidelines of our local Medical Ethical Committee, and all participants recruited in this study provided written informed consent.

In all, 389 healthy blood donors (mean age of 37.1 ± 5.8) were recruited at the First Hospital Affiliated to the Medical College of Xi'an Jiaotong University. Subjects who had substance abuse, participated in other studies, or suffered from chronic brain diseases were excluded. All participants were Han Chinese from Shanxi Province and not genetically related. Written informed consent was obtained from all participants. The study protocol was approved by the Ethical Committee of Xi'an Mental Health Center, Xi'an, China.

2.2. SNPs selection

SNPs in the promoter region, untranslated regions (UTRs), exons, and introns of GAD1 were systematically screened. Fifteen SNPs with minor allele frequencies (MAF) greater than 0.05 were selected from the GAD1 and nearby regions based on a review of published literature and a search of HapMap and dbSNP (public databases that contain information about the Han Chinese population). These SNPs were further analyzed in an association study. The positions of the SNPs in the GAD1 gene are shown in Fig. 1.

2.3. Genotyping

Three to five milliliters of peripheral blood were collected in tubes coated with EDTA. Genomic DNA was extracted from blood leukocytes using the EZNA™ Blood DNA Midi Kit (Omega Bio-Tek, Norcross, GA, USA), according to the manufacturer's protocol. SNP genotyping was performed using matrix assisted laser desorption ionization-time of flight (MALDI-TOF; MassARRAY system, Sequenom Inc., San Diego, CA, USA) mass spectrometry. Primers were designed using Sequenom software, and the extension reaction produced allele-specific products with masses differing by 30 Da, or approximately one single nucleotide. Primer extension and PCR were performed according to the manufacturer's instructions, using iPLEX enzyme (Sequenom) and HotStarTaq DNA polymerase (Qiagen, Hilden, Germany). The completed genotyping reactions were spotted onto a 384-well spectroCHIP (Sequenom) using the MassARRAY Nanodispenser (Sequenom) and the molecular weights of the products were determined using the matrix-assisted laser desorption/ionization time-of-flight mass spectrometer. Genotype calling was performed in real time using MassARRAY RT software version 3.0.0.4 and data analysis was performed using MassARRAY Typer software version 3.4 (Sequenom).

2.4. Statistical analysis

Allele and genotype frequencies for each individual polymorphism and Hardy–Weinberg equilibrium were evaluated by the Chi-square test. Associations between the case–control status and each polymorphism were assessed by the Fisher's Exact test or the Pearson Chi-square test. Unconditional logistic regression was used to calculate the odds ratio (OR) and 95% confidence interval (CI) of independent association between each locus and the presence of heroin dependence. Bonferroni correction was used in multiple testing, and the p-value was divided by the total number of loci or haplotypes. All statistical analyses were carried out using SPSS 11.5 software (SPSS Inc., Chicago, IL, USA). Pair-wise linkage disequilibrium (LD) statistics (D' and r^2) and haplotype frequency were computed using Haploview 4.0 to construct haplotype blocks.

3. Results

The distribution frequencies of 15 genotyped SNPs were in agreement with Hardy–Weinberg equilibrium. Linkage disequilibrium (LD) analyses of the patient and control data revealed that the

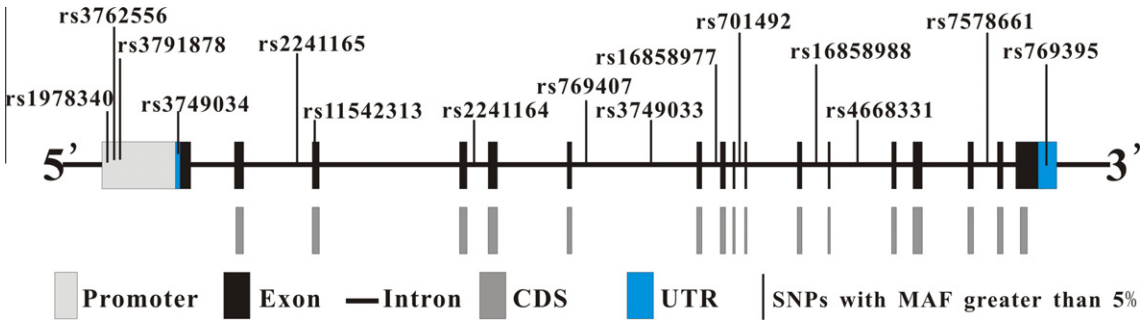

Fig. 1. Gene structure of human GAD1, showing the relative positions of the 15 SNPs used in our study.