Drug and Alcohol Dependence 122 (2012) 100–104

Contents lists available at SciVerse ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

A haplotype analysis is consistent with the role of functional *HTR1B* variants in alcohol dependence

Verônica Contini, Guilherme P. Bertuzzi, Evelise R. Polina, Tábita Hunemeier, Elisa M. Hendler, Mara H. Hutz, Claiton H.D. Bau*

Departament of Genetics, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 3 November 2010
Received in revised form
13 September 2011
Accepted 17 September 2011
Available online 17 October 2011

Keywords:
Alcoholism
HTR1B gene
Association study
Haplotype analysis

ABSTRACT

Background: Animal and human studies have suggested that the serotonergic system plays an important role in alcohol consumption and abuse, mainly due to the serotonin receptor 1B (5-HT_{1B}) function in the mesolimbic reward pathway. Association studies between the *HTR1B* gene variants and alcoholism have found significant results. There is also evidence for a complex balancing regulation of the gene by two functional variants in the promoter region (rs11568817 and rs130058), which are in linkage disequilibrium.
Methods: The aim of this study is to investigate the role of the most relevant variants (rs11568817, rs130058, rs6296 and rs13212041) of the *HTR1B* gene in the susceptibility to alcohol dependence. The sample comprised 136 Brazilian alcoholics of European descendent and 237 controls.
Results: The results suggest an association between a functional variant of the gene (rs11568817) and alcohol dependence ($p = 0.001$). In addition, this association could also be confirmed in an independent sample using imputed data from a GWAS, where marginal significant association ($p = 0.03$, one-tailed) with the same allele was obtained. The pattern of distribution of haplotypes was significantly different between patients and controls ($p < 0.0001$), which is consistent with the role of the two functional variants of the promoter region.
Conclusion: In conclusion, our findings point to an association between functional variants in the promoter region of the *HTR1B* gene and alcohol dependence, supporting previous neurobiological evidences of the involvement of *HTR1B* variations in alcohol-related phenotypes.

1. Introduction

Alcohol dependence is a common and clinically heterogeneous disease, frequently comorbid with many other mental illnesses (Hartz and Bierut, 2010). It is widely accepted that both genetic and environmental factors influence the etiology of alcohol dependence, and several epidemiological studies have estimated the heritability as around 64% (Tyndale, 2003). Although the results of genome-wide association studies for alcoholism have not revealed strong findings (Edenberg et al., 2010; Treutlein et al., 2009), the candidate gene approach may still provide significant findings since the polymorphisms and haplotypes studied may be carefully chosen based on a hypothesis-oriented approach. Although there is strong evidence that alcohol metabolism-related genes play a part in alcohol dependence, these genes account for only a small proportion of the genetic variance (Hartz and Bierut, 2010; Huang, 2010; Ösby et al., 2010). Therefore, several efforts to identify other genetic variants associated with the susceptibility to alcoholism have been focusing on genes involved in the neurotransmitter regulation (Huang, 2010; McHugh et al., 2010).

The serotonergic system plays an important role in the rewarding and reinforcing properties of alcohol consumption, mainly due to the serotonin receptor 1B (5-HT_{1B}) (Koob, 2009). In rats, the overexpression of 5-HT_{1B} receptors on nucleus accumbens projection neurons is associated with increased ethanol consumption (Furay et al., 2010; Hoplight et al., 2006). In humans, a neuroimaging study suggested that alcoholics have increased 5-HT_{1B} receptors in the ventral striatum, including the globus pallidus and the nucleus accumbens, when compared with healthy control subjects (Hu et al., 2010). It is noteworthy that these brain regions are part of the mesolimbic reward pathway, which is relevant in alcohol consumption and abuse.

Most of the association studies between the serotonin receptor 1B gene (*HTR1B*) and alcohol dependence in humans focused

* Corresponding author at: Departamento de Genética, Instituto de Biociências, UFRGS, Caixa Postal: 15053, CEP: 91501-970, Porto Alegre, RS, Brazil.
Tel.: +55 51 3308 6718; fax: +55 51 3308 7311.
E-mail address: claiton.bau@ufrgs.br (C.H.D. Bau).

0376-8716/$ – see front matter © 2011 Published by Elsevier Ireland Ltd.
doi:10.1016/j.drugalcdep.2011.09.020

on a synonymous single nucleotide polymorphism (SNP), rs6296 (G861C), and the first association study reported an overrepresentation of the rs6296-C allele in antisocial alcoholic subjects (Lappalainen et al., 1998). Although there have been some replications since this initial study (Hasegawa et al., 2002; Soyka et al., 2004), other studies have provided inconsistent findings (Fehr et al., 2000; Hill et al., 2002; Huang et al., 2003). Huang et al. (1999) observed that the binding to 5-HT_{1B} in postmortem brain prefrontal cortex is slightly increased in subjects homozygous for the rs6296-G allele, but no direct functional effects of this polymorphism have been identified. It is possible that another variant in linkage disequilibrium (LD) with rs6296 is the underlying mechanism for the alteration in binding to 5-HT_{1B} associated with rs6296.

Although the human *HTR1B* is a short (1137pb) and intronless gene, it contains several polymorphisms in the coding sequence and surrounding 5′- and 3′-untranslated regions (UTRs) (Sanders et al., 2002). Sun et al. (2002) found an association between the rs130058 (A-161T) variant, located in the 5′UTR, and alcohol dependence in Taiwanese Han. Expression studies suggest that this variant affect the reporter gene activity (Sun et al., 2002) and point towards a complex balancing regulation of the *HTR1B* gene by two functional variants in the promoter region, rs130058 and rs11568817 (T-261G) (Duan et al., 2003). The linkage disequilibrium between rs130058, rs11568817 and rs6296 polymorphisms (Duan et al., 2003; Proudnikov et al., 2006) may explain the previous findings with the rs6296 variant.

Currently, there is evidence that at least one polymorphism in the 3′UTR of the *HTR1B* gene may also be a modulator of gene expression. Jensen et al. (2009) have identified a variant rs13212041 (A1997G) that seems to be a target for repression by microRNA-direct silencing. In this case, the inclusion of this polymorphism in haplotype studies could extend the understanding of the functional variation within the *HTR1B* gene. Two *HTR1B* haplotype association studies were performed in alcohol dependence, with negative results (Kranzler et al., 2002; Sinha et al., 2003). In both cases, three polymorphisms were included (rs11568817, rs6298 and rs6296) but rs130058 and rs13212041 were not included.

In this study, we investigated the possible role of a set of four *HTR1B* polymorphisms (rs11568817, rs130058, rs6296 and rs13212041) in the susceptibility to alcohol dependence. This is the largest representation of *HTR1B* genetic variation ever included in haplotype-based association studies in alcoholism.

2. Materials and methods

2.1. Subjects

The alcohol dependence sample is composed of 136 Brazilian males interviewed in an alcoholism treatment ward. The mean age is 41.15 (±9.78) and the mean educational level is 6.55 (±3.37) years of formal schooling. The diagnosis process followed the DSM-IV criteria (American Psychiatric Association, 2000), and the interview for alcohol dependence and lifetime comorbidities was performed with the Semi-Structured Assessment for the Genetics in Alcoholism (SSAGA; Bucholz et al., 1994). The treatment ward receives patients with a severe dependence and frequent comorbid psychopathology. Forty-seven (34.5%) patients presented major depressive disorder and 21 (15.4%) antisocial personality disorder. Twenty-one (15.4%) attempted suicide and 52 (38.2%) had suicide ideation. Illegal drug abuse (mostly marijuana, followed by cocaine) was present in 30 (22%) individuals and nicotine dependence in 121 (88.9%).

The control group for allele and genotype frequencies is composed of 237 Brazilian replacement blood donor males assessed in a blood bank. The mean age of this sample is 34.01 (±10.13). Exposure to alcohol was measured by the CAGE questionnaire (Ewing, 1984). This instrument is a combination of four questions for the screening (but not definitive diagnosis) of alcoholism. A total of two or more positive answers suggest alcohol abuse or dependence. Although 8% of the individuals sampled answered positively to two items, none of them was probable alcohol dependent considering their alcohol drinking patterns.

All individuals included in this study (patients and controls) are Brazilians of European descendent ascertained in Porto Alegre, the capital of Rio Grande do Sul, the Southernmost state of Brazil. The degree of African admixture in this European-derived population is smaller than in other Brazilian states and has been estimated as approximately 6% (Zembrzuski et al., 2006). Considering the lack of population structure among the European descendents from the state of Rio Grande do Sul (Zembrzuski et al., 2006), specifically in Porto Alegre (Santos et al., 2010), population stratification is unlikely (Hutchison et al., 2004). Accordingly, the allele frequencies of several genes studied in this control group are equivalent to those of other European control samples (Contini et al., 2006; Freire et al., 2005, 2006; Marques et al., 2006; Polina et al., 2009; Prestes et al., 2007a,b).

All subjects signed an informed consent approved by the Ethics Committees of the Hospital and the Federal University of Rio Grande do Sul.

2.2. Laboratory methods

The DNA was extracted from whole blood by an adaptation of the method of Lahiri and Nurnberger (1991). The *HTR1B* rs11568817, rs130058 and rs6296 polymorphisms were amplified using the polymerase chain reaction (PCR) conditions adapted from Guimaraes et al. (2009). The rs13212041 variant was genotyped using the Taqman SNP genotyping assays (Applied Biosystems), according the manufacturer's recommended protocol.

2.3. Statistical analyses

The characterization of the LD and the estimation of haplotypes comprising the *HTR1B* polymorphisms were performed with the MLOCUS program (Long et al., 1995; Long, 1999). The Haploview program was used to create a graphical representation of LD structure (Barrett et al., 2005). The analyses of Hardy–Weinberg equilibrium, differences in allele, genotype and haplotype frequencies between patients and controls and the presence of comorbidities were analyzed by the chi-square test. The haplotype analysis was restricted to haplotypes with frequency $\geq$0.05.

All analyses were conducted using SPSS version 12.0 software (SPSS Inc., USA). The Bonferroni procedure was applied considering the pattern of correlations between the variables included in the study, since independence between variables is an assumption for such corrections. The variance in the "outcome" variables could be ascribed to three main factors: (a) alcohol dependence case-control; (b) presence of internalizing comorbidities (major depressive disorder) and (c) presence of externalizing comorbidities (illegal drug abuse, nicotine dependence and antisocial personality disorder). Considering that all *HTR1B* polymorphisms are in strong LD, there were two genetic variables (genotypes and haplotypes). Therefore, the number of independent comparisons to correct for was 6 and the p value set at 0.008.

2.4. Replication analyses

Replication analyses were performed using imputed data from independent case-control sample (Treutlein et al., 2009) genotyping to Human-Hap 550 BeadChips (Illumina Inc, San Diego, California) (Frank et al., personal communication). Imputation analyses were conducted using PLINK version 1.0.7. with two SNP presenting high LD to rs11568817–rs9361235, $r^2 = 1.0$; and rs1213371, $r^2 = 0.86$ (http://www.broadinstitute.org/mpg/snap/, 1000 Genomes data). Imputation quality is sufficient: $r^2 = 0.95$. Reference panel for imputation was CEU population of HapMap Phase 3 and 1000 Genomes Project combined.

3. Results

Allele and genotype frequencies of the *HTR1B* polymorphisms in patients and controls are presented in Table 1. The genotype distributions among the samples did not deviate from the values expected according to Hardy–Weinberg equilibrium in any polymorphism analyzed (all $p > 0.2$). We observed a significant difference in the genotype and allele frequencies of the rs11568817 polymorphism between patients and controls (Genotype $\chi^2 = 14.33$, $p = 0.001$; allele $\chi^2 = 12.19$, $p < 0.0001$). The chi-square residual analysis revealed a higher frequency of the rs11568817-G allele among patients with alcohol dependence. No significant differences in allele and genotype frequencies between patients and controls were found in the other *HTR1B* polymorphisms (rs130058, rs6296 and rs13212041).

The haplotype analysis revealed that the rs11568817, rs130058, rs6296 and rs13212041 polymorphisms are in strong LD in patients and controls. The pairwise LD estimated for controls is in Fig. 1. Patients and controls presented significant differences in the estimated haplotype frequencies (Table 2). The chi-square residual