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Differential role of serotonergic polymorphisms in alcohol and heroin dependence

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ABSTRACT

Background: Twin studies suggest that genetic factors account for 40–60% of the variance in alcohol dependence. It has been stated that different drug dependencies may have unique genetic influences. Alterations in serotonin availability and function can affect drinking behaviour. This study aimed to investigate whether three serotonergic polymorphisms (HTR2A A-1438G (rs6311), and SCL6A4 5-HTTLPR and STin2 VNTR) were associated with alcohol dependence, and, whether the serotonergic polymorphisms played a similar role in conferring vulnerability in alcohol and heroin dependence.
Methods: 165 alcohol dependent patients, 113 heroin dependent patients, and 420 healthy controls from a homogeneous Spanish Caucasian population were genotyped using standard methods.
Results: Genotypic frequencies of the A-1438G, 5-HTTLPR, and STin2 VNTR polymorphisms did not differ significantly across the three groups. None of the three polymorphisms contributed to distinguishing alcoholic patients from healthy controls. There was an excess of −1438G and 5-HTTLPR L carriers in alcoholic patients in comparison to the heroin dependent group (OR (95% CI) = 1.98 (1.13–3.45) and 1.92 (1.07–3.44), respectively). The A-1438G and 5-HTTLPR polymorphisms also interacted in distinguishing alcohol from heroin dependent patients (Wald (df) = 10.21 (4), $p = 0.037$). The association of −1438A/G with alcohol dependence was especially pronounced in the presence of 5-HTTLPR S/S, less evident with 5-HTTLPR L/S and not present with 5-HTTLPR L/L. SCL6A4 polymorphism haplotypes were similarly distributed in all three groups.
Conclusions: Our data do not support a role of serotonergic polymorphisms in alcohol dependence but suggest a differential genetic background to alcohol and heroin dependence.

1. Introduction

It is accepted that alcohol dependence is influenced by environmental and genetic factors (Edenberg and Foroud, 2006). Twin epidemiological studies show that genetic factors account for about 40–60% of the overall variance in alcohol dependence (Dick and Beirut, 2006). However, predisposition to addiction may be due both to genetic variants that are common to all addictions and to those specific to a particular addiction (Kreek et al., 2005).

Alterations in serotonin (5-HT) availability and function can affect drinking behaviour (Johnson and Ait-Daoud, 2000). The serotonin 2A receptor (HTR2A) antagonists have been shown to reduce alcohol intake (Overstreet et al., 1997), and recent data suggest a lower HTR2A receptor binding in the prefrontal cortex (PFC) of alcohol dependent patients with a positive family history of alcoholism (Underwood et al., 2008). The serotonin 2A receptor (HTR2A) gene is located on chromosome 13q14-q21. Two polymorphisms of this gene, T102C (rs6313) and A-1438G (rs6311), have been described as being in complete linkage disequilibrium in different populations (Saiz et al.,

Abbreviations: bp, Base pair; CI, confidence interval; df, Degrees of freedom; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders-IV; EuropASI, European addiction severity index; HTR2A, serotonin receptor type 2A; 5-HT, serotonin; 5-HTT, serotonin transporter; 5-HTTLPR, serotonin transporter promoter polymorphism; IPDE–CIE 10, International Personality Disorders Examination–CIE 10; LD, linkage disequilibrium; LRT, likelihood ratio test; MINI, Mini-International Neuropsychiatric Interview; OR, odds ratio; PFC, prefrontal cortex; SD, standard deviation; SERT, serotonin transporter gene; SLC6A4, serotonin transporter gene; SPSS, Statistical Package for Social Sciences; STin2 VNTR, serotonin transporter intron 2 variable number of tandem repeats polymorphism; χ^2, Chi-square test.

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2008a). Recent findings suggest that the A-1438G polymorphism might have functional effects on expression of the HTR2A receptor in the brain (Parsons et al., 2004; Myers et al., 2007). On the other hand, the 102T allele has been suggested to be associated with alterations in the amount of HTR2A receptor concentration in the PFC (Turecki et al., 1999; Underwood et al., 2008). Association between these polymorphisms and alcoholism has been reported in two prior case-control association studies (Nakamura et al., 1999; Hwu and Chen, 2000).

One of the most important mechanisms related to the control of the synaptic 5-HT concentration is the functionality of the 5-HT transporter (5-HTT). The 5-HTT gene (also known as SLC6A4 or SERT) is mapped on chromosome 17q11.1-q12. A functional polymorphism of this gene (5-HTTLPR) involving two common L (44-base pair insertion) and S (deletion) alleles is related to the differential expression of 5-HTT binding sites in cell lines (Lesch et al., 1996). Several case-control association studies and even two meta-analyses (Gorwood et al., 2004; Feinn et al., 2005) suggest an association between the low activity variant (S allele) and alcohol dependence (Sander et al., 1997; Konishi et al., 2004a,b) or the risk of relapse in abstinent alcohol dependent patients (Pinto et al., 2008). Nevertheless, other studies have described an association of the long allele of this polymorphism with alcohol dependence (Heinz et al., 2000; Hu et al., 2005; Kweon et al., 2005; Bleich et al., 2007; Gokturk et al., 2008; Johnson et al., 2008). Several negative studies have also been published (Gorwood et al., 2000; Thompson et al., 2000; Kranzler et al., 2002; Foley et al., 2004; Choi et al., 2006; Köhnke et al., 2006; Dick et al., 2007; Mokrović et al., 2008; Samochowiec et al., 2008).

Another functional polymorphism of the SLC6A4 is a 17 base pair (bp) variable number of tandem repeats (termed STin2 VNTR), located in intron 2, involving two major alleles (termed STin2.10 and STin2.12) that correspond to 10- or 12-repeat units of 17 VNTR. The STin2 polymorphism appears to modulate the gene's transcription in an allele-dependent manner (Hranilovic et al., 2004). The 10/10 genotype and STin2.10 allele has been associated with alcoholism in one case-control association study (Mokrović et al., 2008), but not in another (Thompson et al., 2000).

Finally, linkage studies of alcohol dependence have identified several chromosomal regions, including chromosomes 13 and 17 (Agrawal et al., 2008; Gelernter et al., 2009).

On the other hand, prior studies also suggest a possible contribution of the above mentioned polymorphisms towards susceptibility to heroin dependence (Tan et al., 1999; Gerra et al., 2004; Saiz et al., 2008b), as well as, a linkage between heroin dependence and markers on the long arm of chromosome 17 (Gelernter et al., 2006; Glatt et al., 2006).

Finally, associations have been suggested between aggressive and impulsive behaviours and genes related to the serotonergic system (Preuss et al., 2001; Giegling et al., 2006; Brezo et al., 2008). However, high levels of impulsivity and aggressiveness have been also related to substance abuse and dependence (Cuomo et al., 2008).

In this study, we aimed to investigate first whether three serotonergic polymorphisms (HTR2A A-1438G (rs6311), and SCL6A4 5-HTTLPR and STin2 VNTR) were associated with alcohol dependence, and second, whether the serotonergic polymorphisms played a similar role in conferring vulnerability in alcohol and heroin dependence.

2. Methods

2.1. Patient population

The total sample was composed of one hundred and sixty-five unrelated alcohol dependent outpatients (mean age (SD) = 47.78 (9.08) years; 84.8% males), one hundred and thirteen unrelated heroin dependent outpatients (mean age (SD) = 31.65 (6.30) years; 88.5% males), and four hundred and twenty unselected healthy controls (mean age (SD) = 40.6 (11.3) years; 51.4% males), all from the North of Spain. All individuals were of Caucasian Spanish origin.

Alcohol dependent patients were consecutively admitted to an outpatient detoxification unit with a diagnosis of alcohol dependence. Only active drinkers with an alcohol intake of at least 210 g/week for men and 140 g/week for women during the past month were enrolled. Alcoholic patients with a current diagnosis of dependence or abuse of other substances except nicotine were excluded from the study (Florez et al., 2008). Enrollment of heroin dependent patients has been previously described by Saiz et al. (2008b). Severity of the addiction was measured using the European Addiction Severity Index (EuropASI) (Kokkevi and Hartgers, 1995). Only alcohol or heroin dependent patients without DSM-IV, Axis I diagnoses were included in the study.

Absence of Axis I diagnoses was determined by experienced psychiatrists based on DSM-IV criteria and clinical records. In addition, the Spanish version of the Mini-International Neuropsychiatric Interview (MINI, DSM-IV criteria) was used as a diagnostic interview in the two groups of patients, as well as in the healthy control group. Only healthy controls without a history of drug or alcohol abuse or dependence and without a personal or first-degree family history of psychiatric disorders were enrolled in the study.

Written informed consent was obtained from all subjects included in the study. The study was subject to and in compliance with Spanish national legislation, was conducted according to the provisions of the World Medical Association Declaration of Helsinki, and received institutional approval (World Medical Association, 1989).

This clinical sample had at least 80% statistical power to detect genetic effects with an odds ratio of 2, assuming that the frequency of minor alleles was at least 35% in our sample of healthy controls. Similar frequencies have been previously described in other Spanish Caucasian samples (Mata et al., 2004).

2.2. Genotyping

Briefly, genomic DNA was extracted from peripheral white blood cells obtained from each participant according to standard protocols (Miller et al., 1988). HTR2A and SLC6A4 gene polymorphisms were identified according to previously published methods (Florez et al., 2008). Patients and controls were analyzed side by side to eliminate errors in genotyping. The genotypes were determined by researchers who were blind to patient information.

2.3. Data analysis

The genotype and allele distribution as well as the presence of Hardy–Weinberg equilibrium were tested by chi-square (χ^2) tests. A Bonferroni correction coefficient of 3 (3 genetic markers were under study) was applied to p values to control for multiple comparisons. To assess whether the polymorphisms distinguished alcohol dependent patients from either healthy controls or heroin dependent patients, two series of logistic regression models were estimated. First models were estimated with only one polymorphism as the independent variable. Genotypes with similar associations with the dependent variable were merged in order to form more efficient models. Polymorphisms with crude associations beyond the 5% level of statistical significance were entered into a multivariate logistic regression model in order to assess whether each polymorphism independently distinguished alcohol dependent patients from the other group. Whether the multivariate model distinguished alcohol dependent patients from the other group was assessed by the likelihood ratio test with Bonferroni adjustment (i.e. $p<0.25$ for a model with two independent variables and $p<0.17$ for a model with three independent variables). In addition, models were estimated to investigate whether two-way interactions between genetic variants were associated with alcohol dependence. SPSS versions 15.0 and 16.0