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Incremental effect for antisocial personality disorder genetic risk combining 5-HTTLPR and 5-HTTVNTR polymorphisms

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ABSTRACT

As the serotonin transporter gene (*SLC6A4* or *5-HTT*) is a key regulator of central serotonergic activity, several association studies between Antisocial Personality Disorder (APD) and the *SLC6A4* polymorphisms have been conducted in the last decade. In the present study, the role of both 5-HTTLPR and 5-HTTVNTR polymorphisms of the *SLC6A4* gene in APD is investigated. A sample of 147 male inmates was analyzed. APD was assessed by Aluja's Antisocial Personality Disorder Scale, a measure that correlates 0.73 with the dimensional score of DSM-IV APD and 0.62 with factor II of the Psychopathy Checklist-Revised. Inmates presenting both 5-HTTLPR S/S + S/L and 5-HTTVNTR 12/12 had a higher risk of being classified in the APD group (Odds ratio = 3.48). The results also showed that the genotype and haplotype distribution was more dissimilar when extreme groups were compared with odds ratios up to 6.50. Our results supported that, in addition to the widely investigated 5-HTTLPR polymorphism, the 5-HTTVNTR polymorphism might be an interesting candidate for association studies with APD. Results also suggested that previous failures to replicate the association between serotonin transporter gene polymorphisms and APD, or similar phenotypes, could have been due to an under-representation of extremely high APD subjects in the samples analyzed.

1. Introduction

Antisocial Personality Disorder (APD) is a diagnosis applied to persons who routinely behave with little or no regard for the rights, safety or feelings of others. APD and its ultimate behavioral consequences, such as distinct types of aggression, violence and impulsivity, are associated with a decrease in serotonin (5-hydroxytryptamine, 5-HT) activity (Goodman and New, 2000; Lesch and Merschdorf, 2000). Specifically, a serotonergic reduction has been identified in highly impulsive aggressors (Däderman and Lidberg, 2002), mentally disordered violent offenders (Lidberg et al., 2000), and patients suffering from severe personality disorders of the high aggression/low impulse control-type, such as the borderline and antisocial ones (Reif and Lesch, 2003).

Following the release of serotonin, the serotonin transporter gene (*SLC6A4*; solute carrier family 6 [neurotransmitter transporter, serotonin], member 4), also known in the literature as *5-HTT* gene (5-HT transporter), plays a key role in controlling 5-HT availability in the synapse by regulating the reuptake of 5-HT (Lesch et al., 1994). The human *SLC6A4* has been mapped to chromosome 17q11.1-q12, and several common polymorphisms of this gene have been identified, including two variable number of tandem repeat (VNTR) polymorphisms located in the promoter region (serotonin transporter gene-linked polymorphic region; 5-HTTLPR) (Lesch et al., 1996) and intron 2 (termed Stin2 by Lesch et al., 1994 and 5-HTTVNTR by Ogilvie et al., 1996).

The 5-HTTLPR polymorphism is a variable number of tandem repeat (VNTR) with a repeat unit of 22 nucleotides. It has two common alleles, designated as short (S) and long (L) alleles (corresponding to zero and two repeats, respectively) and two rarer alleles designated as very long (vL) and extra long (xL) alleles (carrying more than two repeats) (Gelernter et al., 1999). Heils et al. (1996) and Sander et al. (1998) demonstrated that the low-activity *S* allele of the 5-HTTLPR polymorphism conferred susceptibility to alcohol dependency in subjects with dissocial personality disorder in a German population. This finding is further supported by the results of a later study of a Finnish sample, which found that the *S* allele is associated with an increased risk of early onset alcoholism, antisocial personality disorder (APD), and habitually violent behavior (Bennett et al., 2002). In a study of adoptees, it was demonstrated that male individuals with the *S* variant were more likely

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to have higher symptom counts for conduct disorder or aggression (Cadoret et al., 2003). However, not all studies have demonstrated this association between the *S* allele and aggression. In an American study, no association between antisocial alcoholism and the 5-HTTLPR polymorphism was demonstrated (Hill et al., 2002). Furthermore, a study of African-American cocaine-dependent individuals showed no evidence of an association between the 5-HTTLPR polymorphism and impulsive-aggressive traits (Patkar et al., 2002). Also, in a study with Chinese inmates, carriage of the *S* allele was associated with extremely violent criminal behavior, but not with APD (Liao et al., 2004).

The 5-HTTVNTR is a VNTR polymorphism with a repeat unit of 17 nucleotides, presenting two common alleles (corresponding to 10 and 12 repeat units) and two rarer alleles (corresponding to 11 and 9 repeat units) (Gelernter et al., 1999). This polymorphism has been the subject of considerably less research than the 5-HTTLPR. Recently, the 5-HTTVNTR polymorphism was also found to be significantly associated with Attention Deficit Hyperactivity Disorder (ADHD) in two studies, the frequency of allele 10 being higher in patients than in healthy controls (Zoroglu et al., 2002; Kent et al., 2002). In an article related to the topic of the present study, the results of Davidge et al. (2004) revealed a significantly reduced frequency of the 5-HTTVNTR 10 repeat allele in children displaying the high-aggression phenotype compared with normal controls.

The use of single polymorphic markers to explore the contribution of the *SLC6A4 locus* on APD could be more informative if we also incorporated the analysis of combined effects at both, genotype and haplotype levels. Recent studies on the structure of human genetic variation have revealed that the transmission of neighboring genetic variants is strongly influenced by the genomic architecture of their specific *loci*. In recent years, linkage disequilibrium (LD) patterns around the human genome have been investigated theoretically and empirically (Pritchard and Przeworski, 2001; Wall and Pritchard, 2003). Following an international initiative, the International HapMap Consortium has obtained a haplotype map of the human genome (Internacional HapMap Consortium, 2005; Thorisson et al., 2005) which is a useful tool to guide the design and analysis of genetic association studies.

In the present study, we investigated the role of both 5-HTTLPR and 5-HTTVNTR polymorphisms of the *SLC6A4* gene in APD in a sample of male inmates. APD is estimated to affect 3% of males and 1% of females in normal populations (Sutker et al., 1993). As these percentages are considerably higher among prison inmates, especially for males (Fazel and Danesh, 2002; Moran, 1999), the optimal target population to conduct a study of the genetic basis of APD is male inmates. Related to this point, taking into account the low prevalence of APD in the general population, the availability of a sufficient number of subjects with extreme scores in APD could only be expected to be reached in samples such as inmates.

As far as we know, no study has ever considered the possible role of the 5-HTTVNTR polymorphism in APD. Moreover, we wanted to investigate the combined effect of both polymorphisms. If indeed a certain allelic combination showed an effect, this would underline the functionality of single polymorphisms and the need to investigate both polymorphisms in clinical studies exploring potential influences on APD. In addition, we incorporate available information from HapMap on the transmission architecture of the *SLC6A4* genomic region, to be integrated in a model to better explain the results obtained. In this way, the study constitutes the first attempt to combine both single polymorphisms and haplotypes in order to explore APD differences.

2. Methods

2.1. Subjects

A group of 147 male inmates of the Spanish Penitentiary Centre of Ponent (Lleida) participated in the present study. The mean age was 33.31 (S.D.: 8.6). Exclusion criteria for participation in the study were as follows: 1) Non-Caucasian, 2) presence of psychotic or affective disorder diagnosed by the psychiatric staff of the penitentiary, 3) cognitive disability or language difficulties, and 4) being a relative of one of the participants in the study. Most (95.9%) of the sample was Caucasian Spanish, and the remainder (4.1%) had European Caucasian ancestors (three Portuguese, one Croatian, one French, and one Romanian). Before administering the protocol, we confirmed that all non-native Spanish subjects were sufficiently fluent in Spanish to comprehend the protocol. After information about the study was given, subjects who agreed to participate signed a voluntary consent document. No reward was given for participating in the study. Almost all participants (around 98%) were formally sentenced for one or more of the following crimes: robbery (more than 50% of the sample) with or without violence, murder, assault or threatening behavior, rape, trafficking in narcotics, fraud or domestic violence. The study complied with the Code of Ethics of the Official Scientific Medical Committee of Ethics from the University of Lleida and the "Arnau de Vilanova" University Hospital.

2.2. Measures

APD was assessed using Aluja's Antisocial Personality Disorder Scale (AAPDS[1]; Aluja, 1991). This scale has 47 items in a true–false answer format. The scale obtained a sensitivity of 88.88% and a specificity of 89.06% in relation to presence/absence of APD in an imprisoned criminal population (cut-off score ≥ 15; Aluja, 1991). The AAPDS correlates 0.73 ($P<0.001$) with APD dimensional scores computed using the Structured Clinical Interview for DSM-IV Axis II personality disorders (SCID-II; First et al., 1999), and 0.62 ($P<0.001$) with factor II of the Psychopathy Checklist Revised (PCL-R; Hare, 1991) in a later study by Moltó et al. (2000) carried out with prisoners. The internal alpha consistency of the AAPDS was 0.92 in the present inmate sample.

2.3. Genotyping

DNA from inmates was obtained from buccal snaps using the BuccalAmp DNA extraction kit (Epicentre, Madison, WI, USA). Polymerase Chain Reaction (PCR) protocols were followed to detect two polymorphisms of the *SLC6A4* gene, the insertion/deletion polymorphism 5-HTTLPR and the variable number of tandem repeats (VNTR) polymorphism 5-HTTVNTR. A schematic structure of the *SLC6A4* genomic region, with location of the analyzed polymorphisms, is presented in the upper panel of Fig. 1. PCR primers and methods were performed as described by Heils et al. (1996) for 5-HTTLPR and Lesch et al. (1994) for 5-HTTVNTR. Amplified fragments were resolved in a 12% acrylamide gel using the Mini-Protean equipment (BioRad Laboratories, El Prat de Llobregat, Spain). DNA bands were detected by ethidium bromide staining. Genotyping quality was assessed by twice genotyping randomly selected individuals by two independent technicians.

Only the two common 5-HTTLPR and 5-HTTVNTR alleles were observed in our sample. Following the literature, the 5-HTTLPR alleles were coded as large allele (LPR_L), corresponding to fragments of 528 bp (insertion allele) and short allele (LPR_S) corresponding to fragments of 484 bp (deletion allele) and the 5-HTTVNTR alleles were coded as 12 repeat allele (VNTR_12), corresponding to fragments of 300 bp and 10 repeat allele (VNTR_10) corresponding to fragment of 266 bp.

2.4. Linkage disequilibrium, haplotype and block structure of the SLC6A4 genomic region

We presented the analysis of public data from the HapMap Project of a genomic region of 60 kb surrounding the *SLC6A4 locus*. The LD structure and the haplotype block pattern were estimated from the CEPH dataset (Utah residents with ancestry from northern and western Europe; CEU). Results obtained are presented in the bottom half of Fig. 1.

Hardy–Weinberg equilibrium and pairwise linkage disequilibrium (D') were calculated using Haploview 3.32 software (http://www.broad.mit.edu/mpg/haploview). Block structure was considered for marker pairs showing $D'>0.8$, following the "solid-spine" block definition implemented in Haploview. D' values were plotted by Haploview facilities (Fig. 1). Haplotype frequencies were estimated and compared by using WHAP software (http://www.broad.mit.edu/personal/shaun/whap/). WHAP is an SNP haplotype analysis suite that performs a weighted logistic regression-based test of association in single marker and multi-marker designs under dominant and recessive models. The single marker analysis is a χ^2 test with 1 degree of freedom to derive the associated *P*-value. For the multi-marker analysis, each estimated haplotype included in the model and regression weights (β coefficients) was calculated to provide the relative contribution of each haplotype. The effect of each haplotype individually was estimated by the haplotype-specific option. Complementary analysis was performed by SPSS 14.0 software. Bonferroni correction was applied for multiple testing.

3. Results

According to the LD pattern of the *SLC6A4 locus* as derived from HapMap genotype data, the 5-HTTLPR and 5-HTTVNTR polymorphisms

[1] The AAPDS scale is available upon request from the second author. E-mail: aluja@pip.udl.es.