Association study between obsessive–compulsive disorder and serotonergic candidate genes

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Abstract

Background: To date, research examining the relationship between serotonergic genes and obsessive–compulsive disorder (OCD) has yielded conflicting results. The purpose of this study is to investigate the association between four serotonergic polymorphisms (STin2 VNTR and 5-HTTLPR of the SLC6A4 gene, and A-1438G (rs6311) and T102C (rs6313) of the HTR2A gene) and OCD.

Methods: 99 OCD patients, 456 non-OCD psychiatric patients, and 420 healthy controls from a homogeneous Spanish Caucasian population were genotyped using standard methods.

Results: All groups showed Hardy–Weinberg equilibrium for the analyzed genetic variability. A-1438G and T102C polymorphisms were in complete linkage disequilibrium. OCD patients showed an excess of STin2.12 carriers (12/12, 12/10, and 12/9 genotypes) compared with healthy controls (χ² (1)=7.21, corrected p=0.021; OR=3.38, 95% CI=1.32–8.62) and non-OCD psychiatric patients (χ² (1)=6.70, corrected p=0.030; OR=3.24, 95% CI=1.27–8.26). However, no differences were found between non-OCD patients and healthy controls (χ² (1)=0.05, corrected p=0.81; OR=1.04, 95% CI=0.72–1.51). No significant differences were found with respect to A-1438G and 5-HTTLPR polymorphisms.

Conclusions: Our data provide supporting evidence of an association between the STin2 VNTR polymorphism of the SLC6A4 gene and OCD.

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Keywords: Genetic association; Obsessive–compulsive disorder; Polymorphisms; Serotonin transporter; Serotonin 2A receptor

1. Introduction

Obsessive–compulsive disorder (OCD) is a common and severe psychiatric disorder associated with high levels of morbidity and disability (Murray and Lopez, 1996; Bobes et al., 2001). Based on population surveys, the lifetime prevalence of OCD in adults is estimated at around 1–4% (Angst et al., 2004; Kessler et al., 2005). Epidemiological genetic studies suggest a genetic component in the etiology of this disorder (McGuffin and Mawson, 1980; Nestadt et al., 2000; Hudziak et al., 2004).
Several pieces of evidence support a role of the serotonin system in the pathophysiology of OCD, since potent serotonin reuptake inhibitors (SRI) such as clomipramine and other selective SRI have been shown to be efficacious in the treatment of OCD patients (Leonard et al., 1989; Rosenbaum et al., 2005). The serotonin transporter (5-HTT) is a prime target for SRI, and postsynaptic HTR2A receptor activation may be important for improvement in OCD after treatment with SRI (Greenberg et al., 1998).

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). Most studies regarding the 5-HTTLPR polymorphism have considered it functionally biallelic, even though other genetic variations were known (Hu et al., 2006). However, Hu et al. (2006) have reported that the 5-HTTLPR polymorphism is functionally triallelic (resulting from an A→G substitution in the L allele), and the L3 allele is similar to the S allele in its effect on gene expression, whereas the L2 allele is the highest-expressing allele. This polymorphism has been related to OCD in two family-based association studies (McDougle et al., 1998; Hu et al., 2006) but not in other family-based association studies (Camarena et al., 2001; Chabane et al., 2004; Walitza et al., 2004; Dickel et al., 2007), as well as in some case-control association studies (Bengel et al., 1999; Denys et al., 2006; Hu et al., 2006; Perez et al., 2006) but not others (Billett et al., 1997; Kinneer et al., 2000; Frisch et al., 2000; Camarena et al., 2001; Cavallini et al., 2002; Di Bella et al., 2002; Chabane et al., 2004; Meira-Lima et al., 2004; Kim et al., 2005). However, there have been two recent meta-analyses, one of them, including family-based association data, suggesting over-transmission of the L allele in OCD (Dickel et al., 2007), and the other, including case-control association studies, suggesting association of OCD with the homozygous S/S genotype (Lin, 2007). Another polymorphism of this gene, a 17 base pair (bp) variable number of tandem repeats (termed STin2 VNTR) involves different alleles that correspond to 12-, 10-, 9-, or 7-repeat units of 17 VNTR. The STin2.12 allele has been reported to be a transcriptional enhancer (MacKenzie and Quinn, 1999). This allele has been associated with OCD in two case-control association studies (Ohara et al., 1999; Baca-Garcia et al., 2007).

Finally, a rare polymorphism of this gene, located in exon 9, a mutation of isoleucine to valine at position 425 (Ile425Val), which increases the transport activity of the protein (Kilic et al., 2003), has been described. This rare variant has been associated with a transcriptional enhancer (MacKenzie and Quinn, 1999). The 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 (Spurlock et al., 1998; Kouzmenko et al., 1999; Arranz et al., 2000; Kusumi et al., 2002; Bray et al., 2004; Mata et al., 2004; Martinez-Barrondo et al., 2005; Saiz et al., 2007). One family-based association study reported association of the A-1438G polymorphism with tic disorder only in OCD subjects (Dickel et al., 2007). However, case-control association studies using these polymorphisms have produced conflicting results, with three positive findings (Enoch et al., 1998, 2001; Walitza et al., 2002) and several negative results (Nicolini et al., 1996; Frisch et al., 2000; Hemmings et al., 2003; Tot et al., 2003; Meira-Lima et al., 2004; Denys et al., 2006).

In spite of conflicting results, we believe that SLC6A4 and HTR2A gene polymorphisms are interesting candidates for increased risk of OCD. In this study, we endeavored to clarify the role of the serotonin system in a sample of OCD patients, non-OCD psychiatric controls, and healthy controls, all of Spanish Caucasian origin. We hypothesized that genetic variants of the SLC6A4 (STin2 VNTR and 5-HTTLPR) and HTR2A (A-1438G (rs6311) and T102C (rs6313)) genes would confer susceptibility to OCD.

2. Methods

2.1. Patient population

The total sample was composed of ninety-nine outpatients with OCD (mean age (SD)=37.1 (12.1) years; 46.5% males), four hundred and fifty-six psychiatric outpatients with psychiatric disorders other than OCD (12.3% unipolar depression, 17.1% bipolar disorder, 26.1% panic disorder, 19.7% alcoholism, 24.8% heroin dependence) (mean age (SD)=40.0 (12.2) years; 59% males), and four hundred and twenty unselected healthy controls (mean age (SD)=40.6 (11.3) years; 51.4% males), all from the region of Oviedo (Asturias, Northern Spain).

Presence or absence of psychiatric 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.

To minimize the problem of “population stratification,” all individuals were of Caucasian Spanish origin, shared similar sociodemographic profiles, and were comparable with respect to family geographic origin.

A written informed consent form 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 a statistical power of more than 86% 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
Table 1
Genotype frequencies of polymorphisms of the serotonin 2A gene and serotonin transporter gene

<table>
<thead>
<tr>
<th></th>
<th>A-1438G</th>
<th>STin2 VNTR</th>
<th>5-HTTLPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCD patients</td>
<td>A/A</td>
<td>17 (17.2%)</td>
<td>17 (17.2%)</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>57 (57.6%)</td>
<td>46 (46.5%)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>25 (25.3%)</td>
<td>46 (46.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 (5.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21 (21.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21 (21.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Others</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychiatric controls</td>
<td>A/A</td>
<td>104 (22.9%)</td>
<td>209 (45.8%)</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>228 (50.1%)</td>
<td>175 (38.4%)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>123 (27.0%)</td>
<td>66 (14.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 (1.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Others</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy controls</td>
<td>A/A</td>
<td>88 (21.0%)</td>
<td>174 (41.4%)</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>202 (48.1%)</td>
<td>177 (42.1%)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>130 (31.0%)</td>
<td>64 (15.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 (1.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Others</td>
</tr>
</tbody>
</table>

\( \chi^2 (df) \) values for A-1438G polymorphism

- Uncorrected: 4.35 (4), 11.57 (8), 1.10 (4)
- Corrected: 0.361, 0.171, 0.894
- Corrected \( \chi^2 \) linear by linear association (df)
- 2.11 (1), 4.88 (1), 0.03 (1)
- \( p \) values: 0.147, 0.027, 0.858

OCD: Obsessive–compulsive disorder; df: degrees of freedom; VNTR: variable number of tandem repeats.

The protocols (Miller et al., 1988). HTR2A and SLC6A4 gene polymorphisms were identified according to previously published methods (Martinez-Barrondo et al., 2005). 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 \( (\chi^2) \) tests. Odds ratios (OR) and their 95% confidence intervals were also calculated. The SPSS (version 14.0) and EPI Info (version 6) software packages were used for the statistical analyses. The GeneCounting Program (Curtis and Knight, 2003) was used to calculate the pairwise linkage disequilibrium (LD) between all pairs of markers, as well as to compare estimated haplotype frequencies in OCD patients and controls (non-OCD psychiatric patients plus healthy controls) and to test for differences with a likelihood ratio test (LRT). A Bonferroni correction coefficient of 3 (3 genetic markers were finally included in the study) was applied to \( p \) values to control for multiple comparisons.

3. Results

All groups showed Hardy–Weinberg equilibrium for the analyzed genetic variability. A-1438G and T102C polymorphisms were in complete LD (Cramer’s \( V=1.00, p=0.00 \)) in our population. Some degree of LD was found, independent of disease, between STin2 VNTR and 5-HTTLPR polymorphisms (Cramer’s \( V=0.280, p<0.00001; D^2=0.411; r^2=0.078 \)), but LD was not complete enough to warrant not genotyping both polymorphisms.

Since the A-1438G and T102C polymorphisms were in complete LD, the statistical analyses were performed on only the A-1438G polymorphism. Genotypic distribution for the STin2 VNTR, 5-HTTLPR, and A-1438G polymorphisms is summarized in Table 1. Non-significant differences were found with respect to 5-HTTLPR and A-1438G polymorphisms. However, there was a linear association between STin2 genotypes in the 3 samples (OCD, non-OCD patients, and healthy controls) \( (\chi^2 \) linear by linear association (df)=4.88 (1), \( p=0.027 \)). Thus the OCD patients had the highest frequency of the 12/12 genotype (48.5%) and the healthy controls had the lowest frequency (41.4%). On the other hand, OCD patients showed an excess of the 12/12 and 12/10 genotypes of the STin2 VNTR polymorphism when separately compared with healthy controls \( (\chi^2 (3)=8.71, \chi^2 (3)=8.41, \chi^2 (3)=8.41, \chi^2 (3)=8.41, \chi^2 (3)=8.41, \) uncorrected \( p=0.033, p=0.033, p=0.033, p=0.033, p=0.033, \) corrected \( p=0.114 \)). No differences in genotype frequencies were found between non-OCD patients and healthy controls \( (\chi^2 (3)=1.86, \chi^2 (3)=1.86, \chi^2 (3)=1.86, \chi^2 (3)=1.86, \chi^2 (3)=1.86, \) uncorrected \( p=0.603, p=0.603, p=0.603, p=0.603, p=0.603, \) corrected \( p=0.30 \)).

A haplotype analysis was performed only on SLC6A4 polymorphisms, as both HTR2A polymorphisms were in complete LD in our population. A total of 6 haplotypes were estimated by Genecounting to have non-zero frequencies in the entire control group (non-OCD psychiatric patients plus healthy controls) and 4 in the OCD group (no STin2.9+L or STin2.9+S subjects were found in this group). No apparent significant differences in the frequencies of these haplotypes were found between OCD patients and controls (LRT=6.62, \( df=5, p=0.251 \)).

Table 2
Stin2 VNTR allele 12 carriers versus non-carriers

<table>
<thead>
<tr>
<th>Allele 12 carriers</th>
<th>Allele 12 non-carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCD patients</td>
<td>94 (94.9%)</td>
</tr>
<tr>
<td>Psychiatric controls</td>
<td>389 (85.3%)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>356 (84.8%)</td>
</tr>
<tr>
<td>( \chi^2 (df) )</td>
<td>4.88 (1)</td>
</tr>
<tr>
<td>Uncorrected ( p )</td>
<td>0.026</td>
</tr>
<tr>
<td>Corrected ( p )</td>
<td>0.078</td>
</tr>
</tbody>
</table>

\( df \): Degrees of freedom.

\( a 12/12, 12/10, 12/9. \)

\( b 10/10 \) and 10/9.
4. Discussion

In the present study, we found an association between the STin2 VNTR polymorphism of the *SLC6A4* gene and OCD. We found an excess of STin2.12 carriers when comparing OCD patients with non-OCD psychiatric patients and healthy controls. No relationship was found between 5-HTTLPR and A-1438G (or T102C) polymorphisms and OCD. As expected, A-1438G and T102C polymorphisms were in complete LD in our population. A similar LD status has been observed not only in Spanish populations (Mata et al., 2004; Martinez-Barrondo et al., 2005; Saiz et al., 2007), but in other Caucasian and non-Caucasian populations (Spurlock et al., 1998; Kouzmenko et al., 1999; Arranz et al., 2000; Kasumi et al., 2002; Bray et al., 2004).

There are only two prior case-control association studies on the role of the STin2 VNTR polymorphism in OCD (Ohara et al., 1999; Baca-Garcia et al., 2007). Both found an association between the STin2.12 allele and OCD. On the other hand, in the present study, the genotypic frequencies of the STin2 VNTR polymorphism were similar to those reported by Baca-Garcia et al. (2007) in their Spanish Caucasian sample of OCD patients, non-OCD psychiatric patients, and healthy controls, but different from those found by Ohara et al. (1999) in a Japanese population, with higher rates of STin2.12 alleles in their study. However, prior studies (Abdolmaleky et al., 2004; Fan and Sklar, 2005) suggest significant heterogeneity between European and Asian populations with respect to *SLC6A4* and *HTR2A* polymorphisms.

The biological relevance of the STin2 VNTR polymorphism has still not been elucidated. In vitro data suggest that the STin2.12 allele acts as a more potent positive regulator of marker gene expression than the STin2.10 allele when transformed into embryonic stem cells (Fiskerstrand et al., 1999). In vivo data show that the STin2 VNTR polymorphism acts as a transcriptional regulator and has allele-dependent differential enhancer-like properties (STin2.12 seemed to be significantly stronger than the STin2.10 allele) within the rostral hindbrain, where the *SLC6A4* gene is known to be transcribed at the embryonic stage of development, which in turn may lead to aberrant serotonin neuron development (MacKenzie and Quinn, 1999). More recently, it has been suggested that individual repeat elements within the STin2 VNTR domain differ in their enhancer activity in an embryonic stem cell model (Lovejoy et al., 2003). On the other hand, Hranilovic et al. (2004) suggest a weak individual influence, but a possible combined effect of the 5-HTTLPR and STin2 VNTR polymorphisms on *SLC6A4* gene expression. However, in our sample, the haplotype analysis of both polymorphisms did not reveal any differences between cases and controls.

The 5-HTTLPR polymorphism has been extensively studied in OCD, but with inconclusive results. Several case-control association studies support our negative findings (Billet et al., 1997; Kinneer et al., 2000; Frisch et al., 2000; Camarena et al., 2001; Cavallini et al., 2002; Di Bella et al., 2002; Chabane et al., 2004; Meira-Lima et al., 2004; Kim et al., 2005). However, higher frequencies of the S/S genotype in OCD (Perez et al., 2006) or association of the S allele with OCD only in the female group (Denys et al., 2006) have been reported and these results are supported by case-control based meta-analytic evidence (Lin, 2007). Conversely, Bengel et al. (1999) report an excess of L/L carriers in OCD patients and Hu et al. (2006) report that patients with OCD are twice as likely to have the highest-expressing genotype, L/L. On the other hand, Baca-Garcia et al. (2005) described a linear association between 5-HTTLPR genotypes (L/L, L/S, and S/S) in three female samples (OCD, non-impulsive controls, and impulsive suicide attempters), the OCD female patients having the highest frequency of L/L genotype and the impulsive suicide attempters the lowest.

In relation to the *HTR2A* gene, negative results from case-control association studies have been previously reported regarding T102C (Nicolini et al., 1996; Frisch et al., 2000; Hemmings et al., 2003; Tot et al., 2003; Meira-Lima et al., 2004) and A-1438G (Denys et al., 2006) polymorphisms. However, other case-control studies report an association between the -1438A allele and female (Enoch et al., 2001), child and adolescent (Walitza et al., 2002), and adult (Enoch et al., 1998) OCD patients.

Discordant findings and failures to replicate candidate genes when studying complex genetic diseases have been attributed to several causes, mainly sample size, population stratification effect, disease heterogeneity, and symptomatology (Thomas and Witte, 2002).

In this study, we tried to mitigate the possibility of type I error using different methodological strategies: i) although the inclusion of only Caucasians of Spanish origin reduces stratification problems, we cannot conclude that this is a genetically homogeneous group. However, allele and genotypic frequencies of all studied polymorphisms were similar to those previously found in other samples of healthy controls of Caucasian Spanish origin (Virgos et al., 2001; Mata et al., 2004; Baca-Garcia et al., 2005; Baca-Garcia et al., 2007); ii) a fairly rigorous Bonferroni correction for multiple testing was applied across the *SLC6A4* gene, given that the two studied polymorphisms, at this gene, were found to be in some degree of LD. This makes our finding less likely to be a type I error. Nevertheless, it is possible that our study has insufficient statistical power to detect association due to other genes of minor effect size (type II error). However, the meta-analysis by Lin (2007) stated an OR=1.21 (95% CI=1.01–1.45) for the association between OCD and the S/S genotype of the 5-HTTLPR polymorphism, and the statistical power of our study is only 15% to detect genetic effects with an OR=1.21. On the other hand, regarding the 5-HTTLPR polymorphism, it is possible that unrecognized L compensated alleles in L/L and L/S genotypes could minimize differences between groups of subjects with different phenotypes. Finally, no specific psychometric tools were used to provide more detailed description of OCD symptoms and severity.

5. Conclusion

Our results replicate prior findings (Ohara et al., 1999; Baca-Garcia et al., 2007) and provide supporting evidence for an association between the STin2 VNTR polymorphism and OCD. Given the functionality of the aforesaid polymorphism and its association with OCD, it seems at least possible that it is a susceptibility variant for OCD. On the other hand, to date, there is no data analyzing the LD between the STin2 VNTR and the
triallelic 5-HTTLPR polymorphisms. However, given the existing LD between the STin2 VNTR and the biallelic 5-HTTLPR, it could be possible that the STin2 may be in LD with the triallelic 5-HTTLPR, which could be causing the association between the STin2 polymorphism and OCD. Nevertheless, the “real relevance” of this association is as yet unknown and further research is required, using larger samples sizes to increase the statistical power and representativeness of our findings, including specific assessment of OCD symptoms and severity as well as the triallelic 5-HTTLPR polymorphism.

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