Association study of serotonin 2A receptor (5-HT2A) and serotonin transporter (5-HTT) gene polymorphisms with schizophrenia

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Abstract

Objective: To investigate (i) the association between four serotonergic polymorphisms (A-1438G and T102C of the 5-HT2A receptor gene, and 5-HTT VNTR and 5-HTTLPR of the 5-HT transporter gene) and schizophrenia and (ii) the potential interaction of those polymorphisms in the development of schizophrenia.

Subjects and methods: 227 outpatients with schizophrenia (DSM-IV criteria) and 420 unrelated healthy controls from Asturias (Northern Spain) were genotyped using standard methods.

Results: Both groups showed Hardy–Weinberg equilibrium for the analyzed genetic variability. A-1438G and T102C polymorphisms are in complete linkage disequilibrium in our population. There was an apparent difference in the distribution of genotypes for the A-1438G (or T102C) polymorphisms (p=0.018, not significant after a Bonferroni correction). The 5-HT2A −1438A (or 102T) allele was significantly more frequent in patients than controls (0.53 and 0.45, respectively; corrected p=0.028, OR=1.39 (95% CI=1.11–1.75)). Genotype and allele distributions for 5-HTT polymorphisms were similar in both groups. However, assessment of the combined influence of 5-HT2A A-1438G and 5-HTTLPR polymorphisms demonstrated a significant effect (χ² (3)=11.51, p=0.009), whereby the combination of −1438A and 5-HTTLPR S alleles was associated with schizophrenia.

Conclusions: Our findings support a possible synergistic effect of genetic factors influencing serotonergic neurotransmission on susceptibility to schizophrenia.

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1. Introduction

Schizophrenia is one of the most severe psychiatric disorders, with a worldwide prevalence of approximately 1%. Genetic and environmental factors contribute to the clinical phenotype, but the etiology is still unknown, and several hypotheses have been formulated to explain its pathogenic mechanism (Karayiorgou and Gogos, 1997; Owen et al., 2005).

Recent evidence suggests a linkage between schizophrenia and markers on the long arm of chromosomes 13 and 17 (Badner and Gershon, 2002; Liu et al., 2003). It has been suggested that the serotonergic system may be related to the pathogenesis of schizophrenia due to its role in many physiologic processes such as mood, cognition, and perception. Alterations in the expression of serotonin receptors and transporter have been reported in the brain of patients with schizophrenia (Burnet et al., 1996; Hernandez and Sokolov, 1997) and may play a role in the differential action of second generation antipsychotics (SGA) as compared to first generation antipsychotics (Meltzer, 1999). In addition, different polymorphisms of the serotonergic system have been related to the prediction of response to SGA (Arranz and Kerwin, 2003). Furthermore, the effectiveness of clozapine
and the observation that serotonin modulates the activity of dopaminergic neurons constitute important evidence implying a role for serotonin (Arranz et al., 1998a). Both, the serotonin 2A (5-HT2A) receptor and the serotonin transporter (5-HTT) genes have been widely studied in this disorder. Nevertheless, association studies and even recent meta-analytic evidence have produced conflicting results (Abdolmaleky et al., 2004; Fan and Sklar, 2005; Li et al., 2006; Williams et al., 1997). However, we believe that 5-HT2A and 5-HTT polymorphisms are still interesting candidates for conferring an increased risk of schizophrenia.

In this study, we endeavored to clarify the role of the serotonin system in schizophrenia in a relatively large sample of patients with schizophrenia and controls, drawn from a homogeneous population from Northern Spain, and we hypothesized that genetic variants of the 5-HT2A receptor and 5-HTT genes, as well as their interactions, would confer susceptibility to schizophrenia. The aim of this study was to investigate the association between two single nucleotide polymorphisms (SNPs) of the 5-HT2A receptor gene (A-1438G and T102C), and two polymorphisms of the 5-HTT gene (5-HTT VNTR and 5-HTTLPR) and schizophrenia, as well as the potential interaction of the aforesaid polymorphisms in relation to schizophrenia risk.

2. Methods

2.1. Sample

Two hundred and twenty-seven unrelated outpatients with schizophrenia (mean age (SD) = 35.7 (11.7) years; males: 60.4%, mean age (SD): 34.7 (10.5); females: 39.6%, mean age (SD): 37.1 (13.3)), from the region of Oviedo (Asturias, Northern Spain; total population: 1 million) were enrolled in the study. All patients had a diagnosis of schizophrenia according to DSM-IV criteria (diagnoses were determined by experienced psychiatrists). The control group consisted of 420 unselected healthy individuals (mean age (SD)=40.6 (11.3) years, 51.4% males) from peripheral white blood cells obtained from each participant according to standard protocols (Miller et al., 1988). 5-HT2A and 5-HTT gene polymorphisms were identified according to previously published methods (Martínez-Barrondo et al., 2005).

2.2. Genotype characterization

Genotyping was performed blind to clinical data and in duplicate, with patients and controls analyzed side by side to eliminate errors in genotyping. Genomic DNA was extracted from peripheral white blood cells obtained from each participant according to standard protocols (Miller et al., 1988). 5-HT2A and 5-HTT gene polymorphisms were identified according to previously published methods (Martínez-Barrondo et al., 2005).

2.3. Statistical analysis

Observed frequencies were compared to those expected according to the Hardy–Weinberg equilibrium through a chi-square ($\chi^2$) test. Allele and genotype frequency comparisons between patients versus controls were performed by $\chi^2$ tests and odds ratio calculations. To control for possible bias linked to the unequal distribution of males and females in patient and control groups, logistic regressions were performed, including the interaction between gender and each polymorphism as covariates. To investigate the interaction between the polymorphisms, we stratified 5-HT2A typings across 5-HTT VNTR and 5-HTTLPR variants using ($\chi^2$) tests (Zhang et al., 2003). Power calculations were performed using the EPI Info (version 6) software package and SPSS (version 14.0) was used for the statistical analyses. The Genecounting Program (Curtis and Knight, 2003) was used to compare estimated haplotype frequencies in patients and controls and to test for differences with a likelihood ratio test (LRT), as well as to calculate the pairwise linkage disequilibrium (LD) between all pairs of markers. A Bonferroni correction coefficient of $7$ (for allelic and genotypewise comparisons of 3 markers and one LRT) was applied to all the reported $p$ values to control for multiple comparisons.

3. Results

Both groups (cases and controls) showed Hardy–Weinberg equilibrium for the analyzed genetic variability. As expected, A-1438G and T102C polymorphisms were in complete LD ($R$ values (Cramer’s $V$) = 1.00, $p = 0$) in our population. Some degree of LD was found, independent of disease, between 5-HTT VNTR and 5-HTTLPR polymorphisms ($R$ values (Cramer’s $V$) = 0.250, $p < 0.00001$), but LD was not complete enough to warrant not genotyping both polymorphisms.

Since the A-1438G and T102C polymorphisms were in complete LD, all statistical analyses were performed on only the A-1438G polymorphism. The distribution of the genotypes and the allelic frequencies for the A-1438G (or T102C),
5-HTT VNTR, and 5-HTTLPR polymorphisms in patients with schizophrenia and controls is summarized in Table 1. Although a difference was apparent in the distribution of genotypes for the A-1438G polymorphism, this was not statistically significant after adjustments for multiple comparisons. However, the −1438A allele was significantly more frequent in patients (0.53 in patients versus 0.45 in control subjects; \( \chi^2 (1) = 8.14 \), corrected \( p = 0.028 \), OR = 1.39 (95% CI = 1.11–1.75)). Logistic regression, including the interaction between gender and A-1438G polymorphism as covariate, confirms the previous univariate report (\( \chi^2 \) maximum likelihood = 13.24, \( df = 4 \), \( p = 0.010 \)) and showed no gender interaction (\( p \) for Wald \( \chi^2 = 0.060 \)). Genotype and allele distributions for 5-HTT VNTR and 5-HTTLPR showed no differences between patients and control subjects.

Haplotype analyses of 5-HT2A polymorphisms were not performed, as both polymorphisms were in complete LD in our population, independent of disease. With respect to 5-HTT haplotype comparisons between patients and controls, a total of 6 haplotypes were estimated by Genecounting to have non-zero frequencies. No apparent significant differences were found in the joint genotype frequencies for each individual haplotype. The 10-repeat 5-HTT VNTR and 5-HTTLPR S haplotype was more frequent in patients than in control subjects (15% and 10%, respectively; \( \chi^2 = 6.771 \), \( p = 0.009 \), not significant after Bonferroni correction).

The marginal association of the 10-repeat 5-HTT VNTR and 5-HTTLPR S haplotype with schizophrenia led us to test a possible interaction between 5-HT2A and the above mentioned polymorphisms. However, the \( \chi^2 \) test indicated a significant difference in the distribution of joint 5-HT2A and 5-HTTLPR genotypes between patients and controls (\( \chi^2 (3) = 11.51, p = 0.009 \)), reflecting a higher frequency of the 5-HT2A −1438A/5-HTTLPR SS combination compared with those of other combinations (Table 2). In order to verify this interaction, we separately assessed the subjects in the “high-risk” subgroups (with the 5-HT2A −1438A allele or the 5-HTTLPR SS genotype) and determined the effect of the other polymorphism. In each case, a significant effect was observed; the 464 subjects (patients = 174 and controls = 290) with the 5-HT2A −1438A allele showed a significant association of schizophrenia with the 5-HTTLPR SS genotype (\( \chi^2 (1) = 5.05, p = 0.025 \)), and the 152 subjects (patients = 59 and controls = 93) with the 5-HTTLPR SS genotype showed a significant association of schizophrenia with the 5-HT2A −1438A allele (\( \chi^2 (1) = 9.53, p = 0.002 \)). No interaction was observed between 5-HT2A and 5-HTT VNTR polymorphisms, nor when testing interactions among the three polymorphisms together (data not shown).

### Table 1
Genotype and allele frequencies of polymorphisms of the serotonin 2A receptor gene and serotonin transporter gene

<table>
<thead>
<tr>
<th>Genotype</th>
<th>A-1438G</th>
<th>SHTT VNTR</th>
<th>5-HTTLPR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient frequencies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[r (%)]</td>
<td>68 (30.0%) AA</td>
<td>97 (42.7%) 1212</td>
<td>64 (28.2%) LL</td>
</tr>
<tr>
<td>Control frequencies</td>
<td>106 (46.7%) AG</td>
<td>86 (37.9%) 1210</td>
<td>104 (45.8%) LS</td>
</tr>
<tr>
<td><strong>Control frequencies</strong></td>
<td>53 (23.3%) GG</td>
<td>42 (18.5%) 1010</td>
<td>59 (26.0%) SS</td>
</tr>
<tr>
<td>[r (%)]</td>
<td>88 (21.0%) AA</td>
<td>174 (41.5%) 1212</td>
<td>124 (29.5%) LL</td>
</tr>
<tr>
<td>Control frequencies</td>
<td>202 (48.0%) AG</td>
<td>177 (42.1%) 1210</td>
<td>203 (48.3%) LS</td>
</tr>
<tr>
<td><strong>Control frequencies</strong></td>
<td>130 (31.0%) GG</td>
<td>64 (15.2%) 1010</td>
<td>93 (22.2%) SS</td>
</tr>
<tr>
<td>[r (%)]</td>
<td>5 (0.01) LL</td>
<td>2 (0.9%) others</td>
<td></td>
</tr>
</tbody>
</table>

\( \chi^2 (df) = 0.03 (2) \)
Uncorrected \( p \) value = 0.18
Corrected \( p \) value = 0.012

### Table 2
Joint allele frequencies of 5-HT2A A-1438G and 5-HTTLPR polymorphisms in patients with schizophrenia and controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>5-HT2A −1438GG</th>
<th>5-HT2A −1438AA and −1438AG</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HTTLPR</td>
<td>5-HTTLPR</td>
<td>5-HTTLPR</td>
</tr>
<tr>
<td>LL and LS</td>
<td>SS</td>
<td>LL and LS</td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td>44 (19.4%)</td>
<td>9 (4.0%)</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>94 (22.4%)</td>
<td>36 (8.6%)</td>
</tr>
</tbody>
</table>

\( \chi^2 (3) = 11.51, p = 0.009 \).

4. Discussion

When assessing the distribution of the individual polymorphisms between patients and control subjects, two SNPs (A-1438G and T102C) were found to be associated with schizophrenia, suggesting a small to moderate trend toward susceptibility to schizophrenia. We have also reported no significant association of the 5-HTT VNTR and 5-HTTLPR polymorphisms with schizophrenia. However, there is an apparent synergistic interaction between 5-HT2A A-1438G and 5-HTTLPR polymorphisms in which the presence of the 5-HT2A −1438A allele and the absence of the 5-HTTLPR L allele is associated with schizophrenia.

Alleles −1438A and 102T (and alleles −1438G and 102C) were in complete LD in patients and controls. A similar LD status has been observed in other European populations (Arranz et al., 1998b; Martínez-Barrondo et al., 2005; Mata et al., 2004; Sáiz et al., 2001). Allele and genotypic frequencies of all studied polymorphisms were similar to those previously found in other samples of Spanish controls (Mata et al., 2004; Virgos et al., 2001), but different from that recently reported by Vaquero Lorenzo et al. (2006).

An excess of the A (T)-containing genotypes in patients with schizophrenia has been previously reported in case-control association studies (Tan et al., 2001; Tay et al., 1997). However,
other similar studies (Araga et al., 2002; Inayama et al., 1996; Joober et al., 1999; Vaquero Lorenzo et al., 2006; Williams et al., 1996) and two meta-analyses (Abdolmaleky et al., 2004; Williams et al., 1997) have reported a significant association of the C (G) allele and schizophrenia or negative findings (Chen et al., 2001; Mata et al., 2004; Serretti et al., 2000; Virgos et al., 2001; Zhang et al., 2004), and other recently published meta-analyses did not support an association between this polymorphism and schizophrenia (Li et al., 2006). 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).

Marginal association of the 10-repeat 5-HTT VNTR and 5-HTTLPR S haplotype was detected in our sample. However, the results would not be statistically significant if a Bonferroni correction was applied. The coincidence with previous findings (Fan and Sklar, 2005; Haider et al., 2002; Mata et al., 2004; Serretti et al., 2002) strengthens, at least in part, the hypothesis of a minor contribution of 5-HTT polymorphisms in the etiology of schizophrenia.

On the other hand, the present findings suggest that the interaction between variants of 5-HT2A and 5-HTTLPR polymorphisms might be involved in the etiology of schizophrenia. This view is supported by current theories of polygenic inheritance of schizophrenia, where each gene contributes only 2–10% of the total phenotypic variance (Comings, 1997). However, a previous report on a small Korean population sample (Pae et al., 2005) failed to support this finding. One possible explanation for this discrepancy is the fact that genotypic and allelic frequencies may differ between different ethnic groups. However, our reported allelic frequencies of both polymorphisms are different from those reported by Pae et al. (2005) in their Korean control sample (p < 0.05). Prior studies (Abdolmaleky et al., 2004; Fan and Sklar, 2005) suggest significant heterogeneity between European and Asian population with respect to 5-HT2A and 5-HTT polymorphisms. Another possible explanation may reside in the way of testing for the interaction between both polymorphisms, as Pae et al. (2005) used a logistic regression, whereas we looked at compound genotypes as proposed by Zang et al. (2003).

We use different methodological strategies in order to reduce the possibility of a type I error in the present study: i) a Bonferroni correction for multiple testing was applied, ii) the population stratification concern was mitigated by matching patients and controls for ethnicity and drawing from a homogeneous population, iii) we controlled for the unequal distribution of males and females in cases and controls by performing logistic regressions including the interaction between gender and each polymorphism as covariates. However, the fact that a significant difference (after Bonferroni correction) between cases and controls in the A-1438G 5-HT2A gene polymorphism was found only when testing for allelic frequencies (with a sample double the size of that used to assess genotypic frequencies) could lead to type I error and means that the findings should be interpreted cautiously and need further replication with independent samples.

The relevance of the possible association between A-1438G (or T102C) genotypes, as well as the synergistic effect of A-1438G and 5-HTTLPR polymorphisms, and schizophrenia is as yet unknown. Recent findings suggest that the A-1438G SNP might have functional effects on 5-HT2A receptor expression in the brain and could be responsible for the association of both A-1438G and the strongly linked T102C SNP with many neuropsychiatric phenotypes (Parsons et al., 2004). On the other hand, although the studied polymorphisms were not the disease-causing variants, they might demonstrate LD with a disease-causing variant present in these genes, leading to allelic association between marker and disease.

5. Conclusion

We have provided evidence that genetic variants in the promoter region of the 5-HT2A receptor gene seem to be associated with schizophrenia, and this effect is enhanced by 5-HTTLPR polymorphism. However, although interesting, these results should be interpreted with caution, since other studies have not found evidence for this association.

Acknowledgments

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References


