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LETTER TO THE EDITOR

Serotonin transporter gene polymorphism (5-HTTLPR) and emotional response to auditory hallucinations in schizophrenia

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The serotonin transporter (5-HTT) has a crucial function in the regulation of serotonin (5-HT) reuptake in presynaptic neurons. 5-HT is a major modulator of emotional behaviour and circadian rhythms. In addition to its neurotransmitter role, it is also an important regulator of morphogenetic activities during early brain development as well as during adult neurogenesis and plasticity (Murphy et al., 2001).

In humans, transcriptional activity of the *5-HTT* gene is modulated by a polymorphic repetitive element (5-HTTLPR), generated by a 44-bp deletion, located upstream of the transcriptional start site with two principal alleles, a 484-bp, 14 repeats, denoted as short (*s*) and a 528-bp, 16 repeats, denoted as long (*l*). It has been established that, the *s* allele has lower transcriptional activity than the *l* allele and restricts 5-HTT availability (Lesch et al., 1996). Multiple lines of evidence suggest that the 5-HTT gene-linked polymorphic region (5-HTTLPR) is related with several psychiatric pathologies particularly anxiety-related traits (Lesch et al., 1996).

Recently, the anxiety provoked by auditory hallucinations (AH) has become an important object of study. For example, some studies have pointed out that the main effect of antipsychotic medication and cognitive therapy would be to reduce the anxiety triggered by voices rather than the experience of the voices per se (Shergill et al., 1998). Although some studies have centred on 5-HTTLPR in schizophrenia, results have been contradictory as far as hallucination is concerned (Golimbet et al., 2004; Malhotra et al., 1998). To our knowledge, there are no genetic studies about the emotional response to AH.

We used a two-step approach: first, a comparison between schizophrenic patients and healthy controls in order to evaluate differences in the 5-HTTLPR allele frequency and investigate the association of 5-HTTLPR with the diagnosis of schizophrenia; secondly, we focused on the possible involvement of 5-HTTLPR in the emotional response to AH.

A total of 158 patients meeting DSM-IV criteria for chronic schizophrenia (109 males and 49 females), with a clinical history of AH and 138 blood donors as controls were investigated. These subjects were of a similar ethnic group from Valencia, Spain. Subjects with a psychiatric history or presence of perceptual abnormalities were not considered as controls. Neither group showed significant differences in sex and age. Although the excess of males in our sample is a limitation of our study, sex was not a significant variable in our exploratory analyses (data not shown).

Every patient was on antipsychotic treatment at the time of evaluation: 23.4% of patients ($n=37$) were treated with first-generation antipsychotics, 32.3% of subjects ($n=51$) were treated with second-generation antipsychotics and 44.3% ($n=70$) were given combined treatment (first- and second-generation antipsychotics). Diagnosis was made by medical file review using the Item Group Checklist of the Schedules for Clinical Assessment in Neuropsychiatry and was further confirmed by two of the authors (J.S., E.J.A.). Individuals were aged 38.4 ± 11.6 yr (mean $\pm$ S.D.) and were in-patients and outpatients at the Psychiatric Unit of the Valencia University Hospital.

AH were assessed using the Psychotic Symptom Rating Scale (PSYRATS) for auditory hallucinations (Haddock et al., 1999). This is a standardized scale for the evaluation of 11 different parameters for AH: frequency, duration, location, loudness, belief re-origin, amount of negative content, degree of negative content, amount of distress, intensity of distress, disruption and grade of control. The study was approved by the Ethical Committee of the Medical Faculty, University of Valencia.

Genomic DNA was extracted from the peripheral blood of patients with schizophrenia and controls according to the standard procedure. 5-HTTLPR genotype was determined by PCR as previously described (Bayle et al., 2003) with few modifications. The amplified products were then analysed on 2% agarose gel stained with ethidium bromide. We

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Table 1. Differences in genotype distribution of 5-HTTLPR related with dimensions of auditory hallucinations in the PSYRATS scale

	l/l ($n=34$)	l/s ($n=76$)	s/s ($n=48$)	χ^{2a}	p
Total score	15.1 (16.3)	16.4 (15.0)	22.7 (15.3)	6.5	0.04*
Frequency	1.5 (1.7)	1.7 (1.7)	2.1 (1.6)	3.1	0.22
Duration	1.3 (1.6)	1.6 (1.7)	2.1 (1.7)	4.9	0.09
Location	1.5 (1.6)	1.5 (1.7)	2.1 (1.7)	5.4	0.07
Loudness	1.2 (1.3)	1.3 (1.2)	1.8 (1.4)	5.7	0.06
Belief re-origin	1.2 (1.5)	1.7 (1.7)	2.2 (1.7)	6.2	0.04*
Amount of negative content	1.3 (1.7)	1.5 (1.6)	2.0 (1.7)	4.1	0.13
Degree of negative content	1.3 (1.7)	1.6 (1.6)	1.9 (1.6)	2.5	0.28
Amount of distress	1.3 (1.6)	1.2 (1.5)	2.0 (1.7)	7.3	0.03*
Intensity of distress	1.3 (1.7)	1.2 (1.4)	2.0 (1.7)	7.4	0.02*
Disruption	1.4 (1.6)	1.4 (1.5)	2.1 (1.4)	6.2	0.04*
Grade of control	1.8 (1.9)	1.8 (1.8)	2.5 (1.8)	5.5	0.06

PSYRATS, Psychotic Symptom Rating Scale.
[a] Kruskal–Wallis test; * $p<0.05$. Standard deviations in parentheses.

observed 14- and 16-repeat fragments corresponding to *s* and *l* alleles.

Allelic distribution in patients with schizophrenia and controls was then compared using a contingency χ^2 test and χ^2 test for Hardy–Weinberg equilibrium.

While the *s* and *l* allele frequency in the control group is representative of European populations, patients with schizophrenia exhibited a higher frequency of the *s* allele (43.8% vs. 54.4% respectively, $z=2.57$, $p<0.01$). Both populations (patients and controls) met the Hardy–Weinberg equilibrium ($\chi^2=$ 0.00041 and 0.0143 respectively). The relationship between the polymorphism and the clinical measurements of AH is shown in Table 1. We found significant associations between the *s* allele and total PSYRATS score and also with the following items: belief re-origin, amount of distress, intensity of distress, and disruption.

The excess of the short allele among schizophrenic patients could be a true positive association but also a sample bias since all of them had suffered from AH and our data support a role for this allele in the pathogenesis of AH.

Studies on the role of the 5-HTTLPR in schizophrenia have so far been inconsistent. Whereas Malhotra et al. (1998) reported a relationship between the l/l genotype and hallucinations in a cohort of neuroleptic-free patients, Golimbet et al. (2004) could not replicate this finding but, in contrast, found an association between the s/s genotype and depressive symptoms. Similarly, our data strongly suggest that patients carrying the *s* allele experienced enhanced emotional responses to AH. Significant PSYRATS items in our data, particularly the amount of distress, the intensity of distress and disruption in life are key elements of a negative emotional response to these phenomena. The link between the *s* allele and various syndromal dimensions associated with schizophrenia is further supported by Bayle et al. (2003), who concluded that the occurrence of the *s* allele is a risk factor for violent suicidal behaviour. Moreover, a recent study provided evidence of a 5-HTTLPR-related differential excitability of the amygdala to emotional stimuli (Hariri et al., 2002).

Furthermore, it has been remarked on the necessity of studying the neurobiological bases of AH, not as an on–off phenomenon but as a multidimensional one. Our data gives a further step in this approach by suggesting a molecular genetics basis for the emotional response to AH.

Along with cognitive approaches we think that AH can be considered as a stressful event for the patients. Inconsistent findings among studies with this polymorphism could be explained by different gene–enviroment interactions (Lesch, 2004). Caspi et al. (2003) found a gene × environment interaction in which individuals with the *s* allele were more prone to depression in response to life events. Taken together, these findings further support the notion of an interaction between allelic variation of 5-HTT function and emotional response to AH in patients with psychoses.

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Statement of Interest

None.

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