

## **Polymorphic Variations in 5-HT<sub>2A</sub>, 5-HTT and DISC 1 in first episode schizophrenia patients**

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### **Introduction**

Schizophrenia is a severe psychotic disorder characteristically marked by a retreat from reality with delusion formation, hallucinations, emotional disharmony, and regressive behaviour. Affecting approximately 1% of the worldwide population and is associated with a high rate of morbidity and mortality. Studies suggest that genetics, early environment, neurobiology and psychological and social processes are important contributory factors. Family, twin, and adoption studies provided strong evidence of a genetic contribution to the aetiology of schizophrenia. However, attempts to isolating specific genes that confer vulnerability to the disorder have thus far been only moderately succesful. Schizophrenia is a condition of complex inheritance, with several genes possibly interacting to generate risk for schizophrenia. Schizophrenia is a chronic disease without remission and the treatment requires the use of antipsychotic drugs. Antipsychotics have a multitarget profile without a clear mechanism of action. The aims of pharmacogenetic investigations are the identification of genes influencing response, validation of targets, prediction of treatment response, prediction of side-effects, identification of patients likely to respond to a particular treatment, and the selection of most beneficial treatment according to genetic profile. Identification of drug-target mutations with a direct influence on treatment outcome would constitute evidence of specific mediators of drug activity (1). Currently available antipsychotic drugs achieve a certain degree of clinical improvement in the treatment of psychosis in about 50% of schizophrenia patients (1-3). Antipsychotic treatment failure has substantial clinical and economic costs. Delay in finding an adequate treatment for psychosis has a detrimental effect on patients' prognosis and chances of recovery (4-5). These strong implications reinforce the need for pharmacogenetic research into factors affecting antipsychotic response and ways to predict clinical outcome.

## **Aim of study**

The standardisation of methods to genotype three polymorphism (5HT2A 102 T/C, 5HTTLPR and rs25531) and two microsatellite markers (D1S2709 and D1S 2833), in order to study these polymorphisms in schizophrenia patients. A sample of 200 Spanish Caucasian first episode schizophrenia patients and a sample of 340 healthy control subjects matched for ethnic origin were used in the study. These patients were evaluated at baseline, and after 3, 6 and 12 months after the start of the treatment to assess treatment response.

## **Methods 1.Selection of polymorphisms**

Polymorphisms were selected from previous reports which showed association with schizophrenia and treatment outcome.

### **1.1 Serotonin system**

Alterations of the serotonergic system have been implicated in depression, anxiety, eating disorders and negative symptoms of schizophrenia. Antipsychotic drugs, specially the atypical antipsychotics, show affinity for serotonin (5-HT) neurotransmitter receptors and the most extensively studied have been the 5HT2A and 5-HT2C. Many reports have associated a silent 5HT2A 102-T/C polymorphism with the response to clozapine and risperidone. This polymorphism is in nearly complete linkage disequilibrium (LD) with a -1438-G/A promoter polymorphism in Caucasian populations (1,6,7,8). The selective serotonin reuptake inhibitors (SSRIs) are the most widely prescribed antidepressants worldwide. Despite recent advances in antidepressant pharmacotherapy, response rates are variable and can be low as 60 % for the first drug administered. The underlying mechanism for this variation is complex, involving both environmental and genetics factors and their interactions. One possible genetic mechanism involves the serotonin transporter gene (SLC6A4) which encodes the serotonin transporter (5-HTT) protein. This protein initiates the antidepressant effect of SSRIs, which are drugs that are thought to act primarily by terminating serotonin reuptake by the presynaptic serotonergic neuron. Genetic variations in 5-HTT-LPR polymorphism have been also found to be associated with variations in antipsychotic drug response (Arranz et al., 2000 (9)). Most studies have focused on a single common polymorphism located in the promoter region of the SLC6A4. This insertion/deletion polymorphism, reported to be 43 bp in length, is commonly subdivided into S (short) and L (long) (10,11) alleles. The long allele has shown a higher protein transcription than the short allele (12). Recent reports have detected a single nucleotide polymorphism

(SNP), rs25531 A>G, near the promoter region of the SLC6A4 (13). This SNP subdivided the previous S and L polymorphism into LA, SA, LG and SG.

## **1.2 Disrupted-in –Schizophrenia-1 (DISC 1)**

Disrupted-in –Schizophrenia-1 (DISC-1) was identified as the sole gene in which a mutant truncation by a balanced traslocation (1; 11) (p42.1; q14.3) is cosegregated with schizophrenia in a large Scottish Family (14). The DISC1 locus has now been implicated by cytogenetics, linkage and association studies as a predisposing risk factor for neuropsychiatric illnesses, including schizophrenia, schizophrenia spectrum, bipolar, depression and autism spectrum disorders. Despite a few negative reports, the accumulating genetic evidence is strongly positive. We selected two microsatellite markers D1SC2709 and the D1S2833 which were found by Ekelund and colleagues. These two microsatellite markers in the DISC1 gene provided strong evidence for linkage to schizophrenia in a Finnish family (15,16).

## **2.Genotyping methods**

### **2.1 Genotyping by RFLP of 5-HT2A 102-T/C and the 5-HTT LPR & 25531 polymorphisms**

We standardised two polymerase chain reaction protocols followed by restriction fragment length polymorphism analyses (PCR-RFLP) for genotyping the 5-HT2A 102-T/C and the 5-HTT LPR & 25531 polymorphisms. DNA was amplified in 25uL containing 50 ng genomic DNA, 10mM dNTPs, 0.5 U of Taq polymerase, a final concentration of MgCl<sub>2</sub> of 3mM for the 5-HT2A 102-T/C and 1 mM for the 5-HTT LPR & 25531.

**Table 1** shows the final concentrations and the sequences of oligonucleotide primers for each polymorphism. Thermal cycling consisted of 35 cycles of 96 oC (35s), 60oC (60s) for the 5HT2A 102-T/C or 65.5oC (60s) for the 5-HTT LPR & 25531 and 72o (60s) each with a final extension step of 10 min at 72aC. Subsequently, 15 uL of PCR product were digested by Msp I at 37oC overnight. Finally the PCR and the digestion product were loaded onto an agarose gel and visualized by ethidium bromide (Table 1, **Figure 1 and 2**).

Polymorphism and oligonucleotide primer concentrations and sequences.	Amplicon and restriction fragment lengths of alleles	
	PCR product	Msp I
<u>5-HT2A 102-T/C</u> 400 nM of F: 5'- tct gct aca agt tct ggc tt - 3' 400 nM of R: 5'- ctg cag ctt ttt ctc tag gg - 3'	372bp	T- 372bp C- 156 + 216bp
<u>5-HTT LPR &amp; 25531</u> 200 nM of F: 5'-tcctccgcttggcgectcttcc- 3' 200 nM of R: 5'-tgggggttgaggggagatcctg- 3'	S -469bp  L-512bp	S <sub>A</sub> - 469 bp S <sub>G</sub> -402 + 67 bp  L <sub>A</sub> - 512 bp L <sub>G</sub> -402 + 110 bp
<b>Microsatellite of the DISC 1</b>		
Oligonucleotide primer concentrations and sequences	PCR product	
<u>D1S 2709</u> 200nM of F: 5'-FAM-tcataccacatatca gaatgtc- 3' 200nM of R: 5'-atcaatcagtatctaatagcac- 3'	191-197 bp	
<u>D1S 2833</u> 150nM of F: 5'-FAM- ttgggggtaattgttacatage c- 3' 150nM of R: 5' ttc tga gcc cca gat tga cac tg 3'	128-146 bp	

Table 1. Description of the primers concentrations, sequences and PCR products.

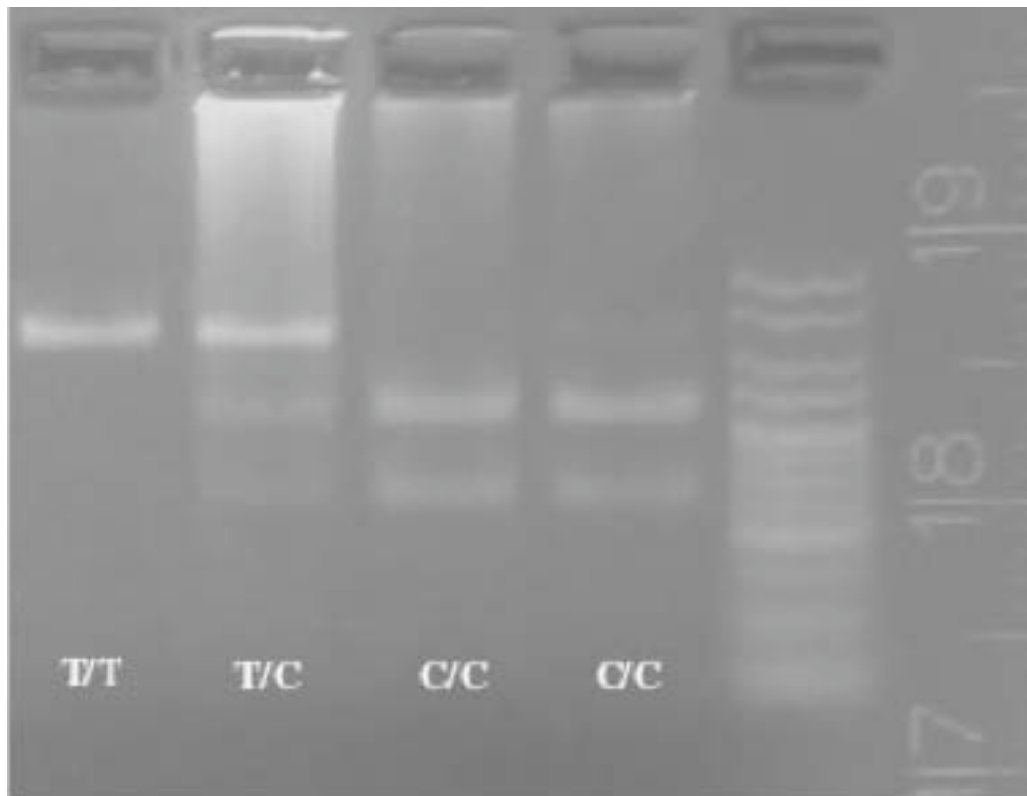


Figure 1. PCR product of the 5-HT2A 102-T/C. The picture shows the three possible genotypes T/T, T/C and C/C.

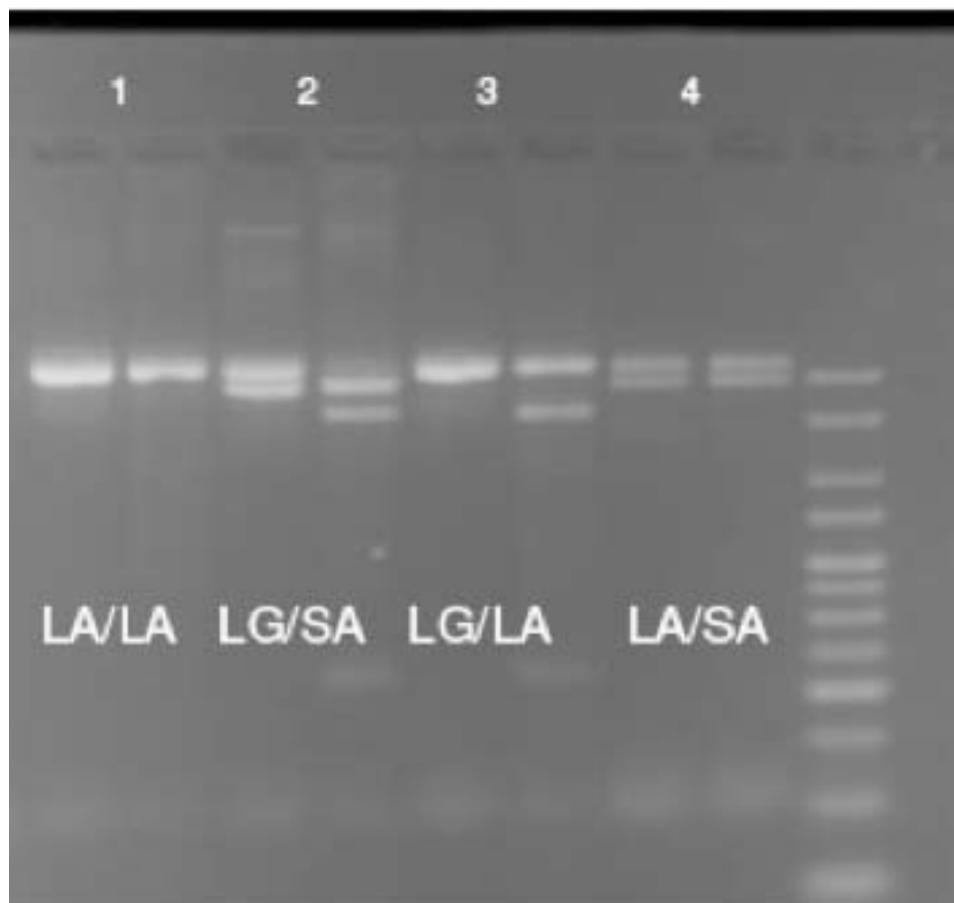


Figure 2. 5-HTT LPR & 25531 genotype. The first lane of each individual represent the PCR product, and the second one, the digestion product.

## 2.2 Automatic genotyping of D1S2709 and D1S2833 microsatellites

DNA was amplified in 25uL containing 2ng genomic DNA, 10mM dNTPs, 0.5 U of Taq polymerase, a final concentration of MgCl<sub>2</sub> of 2mM for the D1S 2709 and 1mM for the D1S 2833. Thermal cycling consisted of 35 cycles of 96oC (35s), 60oC(60s) for the D1S 2709 or 63oC (60s) for the D1S 2833 and 72oC (60s) each with a final extension step of 10 min at 72oC. The primer sequences and concentrations are shown in Table 1. Subsequently, the length of the PCR product was analyzed in the ABI prism® 3100 by capillary electrophoresis. Our electrophoregrams results showed a microsatellite size range between 191-197 bp for the D1S2709 and 128-146 bp for the D1S2833.

## Results

At the moment we have the results of the genotyping of 5-HT2A 102-T/C and the 5-HTT LPR & 25531 polymorphisms in controls and patients. However, we only have the first results for the DISC1 microsatellite genotyping and more tests should be carried out. Now we

have to check the sequence of the microsatellite polymorphism in order to identify the true length of the allele between the stutter products. The aim is to know the allele frequencies of these polymorphisms in our population and to study the association between each polymorphism and the treatment response. For this study we are going to use statistical analyses of frequency distribution and haplotype combinations.

## Conclusion

Pharmacogenetics findings constitute a clear advance towards a future tailoring of antipsychotic treatment to individual needs. However, further development of the field can be obtained by the application of pharmacogenomic strategies to the identification of novel factors influencing response.

## References

1. Arranz MJ and de Leon J. Pharmacogenetics and pharmacogenomics of schizophrenia: a review of last decade of research. *Mol Psychiatry*. 2007 Aug;12(8):707-47.
2. Miyamoto S, Duncan GE, Marx CE, Lieberman JA. Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol Psychiatry*. 2005 Jan;10(1):79-104.
3. Kerwin RW, Osborne S. Antipsychotic drugs. *Medicine* 2000; 28: 23-25.
4. Perkins DO, Gu H, Boteva K, Lieberman JA. Relationship between duration of untreated psychosis and outcome in first-episode schizophrenia: a critical review and meta-analysis. *Am J Psychiatry*. 2005 Oct;162(10):1785-804.
5. Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, Keefe RS, Davis SM, Davis CE, Lebowitz BD, Severe J, Hsiao JK; Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) Investigators Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N Engl J Med*. 2005 Sep 22;353(12):1209-23.
6. Arranz MJ, Munro J, Sham P, Kirov G, Murray RM, Collier DA, Kerwin RW. Meta-analysis of studies on genetic variation in 5-HT<sub>2A</sub> receptors and clozapine response. *Schizophr Res*. 1998 Jul 27;32(2):93-9.
7. Arranz MJ, Munro J, Owen MJ, Spurlock G, Sham PC, Zhao J, Kirov G, Collier DA, Kerwin RW. Evidence for association between polymorphisms in the promoter and coding regions of the 5-HT<sub>2A</sub> receptor gene and response to clozapine. *Mol Psychiatry*. 1998 Jan;3(1):61-6.
8. Lane HY, Chang YC, Chiu CC, Chen ML, Hsieh MH, Chang WH. Association of risperidone treatment response with a polymorphism in the 5-HT<sub>2A</sub> receptor gene. *Am J Psychiatry*. 2002 Sep;159(9):1593-5.
9. Arranz MJ, Bolonna AA, Munro J, Curtis CJ, Collier DA and Kerwin RW. 2000. The serotonin transporter and Clozapine response. *Molecular Psychiatry* 5:124-130.
10. Serretti A, Artioli. *Pharmacogenomics J* 2004; 4: 233-244.
11. Murphy DL, Lerner A, Rudnick G, Lesch KP. Serotonin transporter: gene, genetic disorders, and pharmacogenetics. *Mol Interv*. 2004 Apr;4(2):109-23
12. Lesch KP, Balling U, Gross J, Strauss K, Wolozin BL, Murphy DL, Riederer P. Organization of the human serotonin transporter gene. *J Neural Transm Gen Sect*. 1994;95(2):157-62
13. Kraft JB, Peters EJ, Slager SL, Jenkins GD, Reinalda MS, McGrath PJ, Hamilton SP. Analysis of association between the serotonin transporter and antidepressant response in a large clinical sample. *Biol Psychiatry*. 2007 Mar 15;61(6):734-42.
14. Millar JK, James R, Christie S, Taylor MS, Devon RS, Hogg G et al. Functional characterisation of DISC 1, a candidate susceptibility gene for major psychiatric illness. *Am J Med Genet* 2002; 114: 748.
15. Ekelund J, Lichtermann D, Hovatta I, Ellonen P, Suvisaari J, Terwilliger JD, et al . Genome-wide scan for schizophrenia in the Finnish population: evidence for a locus on chromosome 7q22. *Hum Mol Genet*. 2000 Apr 12;9(7):1049-57.
16. Ekelund J, Hovatta I, Parker A, Paunio T, Varilo T, Martin R, et al. Chromosome 1 loci in

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