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# Association of serotonin transporter (SLC6A4) & receptor (5HTR1A, 5HTR2A) polymorphisms with response to treatment with escitalopram in patients with major depressive disorder : A preliminary study

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***Background & objectives*: Genetic factors have potential of predicting response to antidepressants in patients with major depressive disorder (MDD). In this study, an attempt was made to find an association between response to escitalopram in patients with MDD, and serotonin transporter (*SLC6A4*) and receptor (*5HTR1A*, *5HTR2A*) polymorphisms.**

***Methods*: Fifty five patients diagnosed as suffering from MDD, were selected for the study. The patients were treated with escitalopram over a period of 6-8 wk. Severity of depression, response to treatment and side effects were assessed using standardised instruments. Genetic variations from *HTR1A* (rs6295), *HTR2A (rs6311 and rs6313)* and *SLC6A4* (44 base-pair insertion/deletion at *5-HTTLPR*) were genotyped. The genetic data of the responders and non-responders were compared to assess the role of genetic variants in therapeutic outcome.**

***Results*: Thirty six (65.5%) patients responded to treatment, and 19 (34.5%) had complete remission. No association was observed for genotype and allelic frequencies of single nucleotide polymorphisms (SNPs) among remitter/non-remitter and responder/non-responder groups, and six most common side-effects, except memory loss which was significantly associated with rs6311 (*P*=0.03).**

***Interpretation & conclusions*: No significant association was found between the SNPs analysed and response to escitalopram in patients with MDD though a significant association was seen between the side effect of memory loss and rs6311. Studies with larger sample are required to find out genetic basis of antidepressant response in Indian patients.**

**Key words** Antidepressant response - pharmacogenetics - serotonin receptor polymorphism (*HTR1A*, *HTR2A*) - serotonin transporter (*SLC6A4*) polymorphism

Genetic factors may serve as important predictors of drug response in major depressive disorder (MDD). In the last few years, due to its central role in the pathophysiology of depression, genes and their variants related to the serotonin (5-hydroxytryptamine, 5-HT) pathway, the serotonin transporter gene promoter polymorphism region (*5-HTTLPR*) has been the most studied genetic factors in association with antidepressant response[1]. The polymorphism consists of an insertion/deletion of 44 base-pair variations which have potential to influence the gene expression. The long L allele has been shown to have twice the expression in the basal state than the short S allele[2]. Other genes studied in the serotonin pathway include *TPH1, HTR1A, HTR1B, HTR2A, HTR2C, HTR3A* and *HTR6*. The findings, however, remain equivocal even today[1].

Several reports in Caucasians have shown a positive association between L allele and better response[1,3,4], but others failed to find an association[5]. A study from Japan[6] reported a better response in Japanese Asian population with SS genotype, but another study[7] from the same ethnicity found a better response with LL genotype. Another group of investigators[8] could not find any significant association. A study on association of serotonin receptor (*HTR1A* and *HTR2A*) polymorphisms with therapeutic outcome in patients with depression has shown inconsistent findings[9].

Most of the studies on pharmacogenetics of depression have been conducted in Caucasian, Japanese and Chinese populations. An Indian study[10] from Kashmir reported significant differences in response to escitalopram in patients with depression with homozygous L-allele group and in those having S- allele. The study, however, did not give any details about the ethnicity of the sample. There is a lack of pharmacogenetic studies on antidepressant response in diverse ethnicities of Indian population, which is a crucial factor affecting the gene drug response relationship. This preliminary study was conducted to examine association of serotonin transporter (*SLC6A4*) and receptor (*HTR1A*, *HTR2A*) polymorphisms with response to treatment with escitalopram in an ethnically homogeneous north Indian population with MDD.

## Material & Methods

This study was conducted in the outpatient setting at a tertiary care hospital (All India Institute of Medical Sciences, AIIMS) in New Delhi, India, from August 2010 to July 2011. The patients of north Indian ethnicity, in age group 18-65 yr, suffering from major depressive disorder, and off all psychotropic drugs (antidepressants, antipsychotics, anxiolytics, *etc.*) for a minimum period of one week (for fluoxetine, the minimum period was five weeks) were included[11]. Patients with severe medical diseases, on medications known to produce mood symptoms, pregnant and breast-feeding women and patients with recent suicide attempt or history of hypersensitivity to escitalopram were excluded. Past history of manic episodes, psychotic disorder, severe personality disorder and any substance dependence except nicotine, and history of mania in first or second degree biological relative were other exclusion criteria for the study. The north Indian ethnicity was defined as born in north India to parents of north Indian origin as per recommendations of the Indian Genome Variation Consortium[12], as hailing from States of Punjab, Haryana, Uttar Pradesh and Delhi. Sample was of convenience.

The patients were assessed on Mini International Neuropsychiatric Interview (MINI)[13] to diagnose MDD as per American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, 4th edition-Text Revision (DSM-IV-TR)[14]. A written informed consent was taken from patients. Socio-demographic and basic clinical information was collected on a semi-structured proforma designed for the study. Birth places of patient and his/her parents were noted to ascertain ethnicity. Severity of depression was assessed on Montgomery Asberg Depression Rating Scale (MADRS)[15] and Clinical Global Impressions Scale (CGI)[16]. Those having a minimum score of 22 on the MADRS and at least moderately ill in clinical severity item on CGI were included in the study to rule out sub-syndromal states. UKU Side Effect Rating Scale (Udvalg for Kliniske Undersoegelser: Committee for Clinical Investigation), a standard objective method to assess side effects in such studies[17] was used to assess the side effects. All adverse events were recorded, irrespective of the degree of severity and degree of association except those whose association was not possible.

At the time of first assessment, blood sample (10 ml) was collected for genotyping and treatment was started with tablet escitalopram (10 mg) once daily in morning, as per standard treatment practices. The patients continued their follow up in outpatient service. Dose could be increased to 15-20 mg per day by treating psychiatrist, if required as per clinical indication.

During the study period, no other psychotropic drugs except anxiolytics, sedatives and hypnotics were allowed (mostly low dose benzodiazepines as per our standard clinical practice). However, medications for general medical condition, if any, were allowed. The patients did not receive any form of psychotherapy during this period.

All patients were followed up after six to eight weeks of initiation of therapy, as per recommendations of Serretti *et al*[18] for pharmacogenetic studies of depression. An early follow up after two weeks was not considered as it would have picked up placebo response. At the time of second assessment, patients were considered compliant if drugs were taken on at least 80 per cent of days. Compliance was checked by self-report as well as from the family members (caregivers), besides the empty strips. On follow up visits, patients were assessed on MADRS, CGI and UKU Side Effect Rating scale, and were classified as responder (shown improvement in symptoms), if there was at least 50 per cent reduction in initial MADRS score and remitters (recovery from the episode of depression), if they scored ≤ 7 in MADRS scale[19].

Genotyping was conducted at the Institute of Genomics and Integrative Biology (IGIB), Delhi, India. Four single nucleotide polymorphisms (SNPs) namely, rs6311, rs6313, rs6295 and *5-HTTLPR* 44 base-pair insertion deletion were selected for testing, as these are part of the most probable candidate genes, and their biological relevance is supported by extensive literature. DNA was extracted using modified salting out procedure wherein blood cells were lysed, followed by lysis of nuclear membrane, digestion of nuclear proteins, saturation with sodium chloride and extraction of deoxyribonucleic acid (DNA) in ethanol[20]. After DNA extraction, samples were processed for genotyping of SNP. Three SNPs, rs6311, rs6313 and rs6295 were genotyped by the SNP genotyping platform, the SEQUENOME™- where primer extension reaction followed by Matrix assisted Laser desorption/Ionization time-of-flight (MALDI-TOF) mass spectrometry (Sequenom Inc., San Diego, USA) was used. Genotyping of 44bases insertion/deletion was performed by DNA fragment analysis method using ABI-3130 genetic analyzer (Applied Biosystems, CA, USA). The individuals performing genetic analysis were blind to clinical data. The study protocol was approved by the institute's ethics committee.

The data were analyzed using SPSS (version 17) (SPSS, Chicago, Illinos, USA) and PLINK software (version 1.07, PLINK, MA, USA).

## Results

Eighty five patients of north Indian ethnicity were approached for inclusion in the study. Two patients did not give consent and two were later excluded from the study due to change in the diagnosis. Of the 81 patients, 55 (67.9%) were compliant and completed the study. Amongst the rest 26, patients, 16 did not come for follow up, six changed treatment due to inadequate response and four were not compliant with medications. Of the 55 compliant patients, 36 (65.5%) were responders and 19 (34.5%) remitters.

The mean age of the patients was 35±10.3 yr with 32 (58%) being males. Majority (80%) of them were married and were Hindus. Twenty nine per cent of the patients had received less than 10 yr of formal education, whereas about one third had been to college; 25 per cent patients were in paid employment, 25 per cent belonged to labour class or small businesses, and about one third were homemakers. No significant differences were observed between the responder and non responder groups on various socio-demographic variables.

Mean duration of index episode was 13.36 months. Mean score on MADRS was 28.3 ± 5.0 at baseline, and about three-fourth patients were moderately ill on CGI scale. A shorter duration of depressive episode was significantly associated with response ($P$<0.05). Fifty eight per cent of the patients (n=36) had no side effects, interfering with daily life and 6 (11%) had mild side-effects. Dose was increased to 15 and 20 mg/day in 17 (31%) and 12 (22%) of the patients, respectively during follow up. Almost all our patients received low dose of benzodiazepines (maximum of 1 mg of clonazepam per day) as per standard prescribing practices. Table I summarises the clinical details of the patients.

All genotype and allele frequencies in the study were in Hardy-Weinberg equilibrium. The study sample constituted SNPs with both homozygous and heterozygous allelic variants. However, for *5-HTTLPR* 44base-pair insertion/deletion (S/L) polymorphism, L allele was not detected in homozygous condition though it presented as heterozygous. The observed allelic frequencies in 55 patients were 0.3 for L allele and 0.7 for S allele. Frequencies of S allele were found

**Table I.** Distribution of clinical variables across the responder and non-responder groups

| Clinical variables | | Non-responder (n=19) | Responder (n=36) | Total (n=55) |
|---|---|---|---|---|
| Age of onset of depression (yr) | | 33.4 ± 11.9 | 32.2 ± 9.5 | 32.6 ± 10.3 |
| Duration of current episode* | Upto 6 months | 9 (47.4) | 27 (75.0) | 36 (65.5) |
| | 6 month-2yr | 6 (31.6) | 8 (22.2) | 14 (25.5) |
| | > 2 yr | 4 (21.1) | 1 (2.8) | 5 (9.1) |
| Number of past depressive episode | No past episode | 16 (84.2) | 26 (72.3) | 42 (76.4) |
| | One or more episode | 3 (15.8) | 10 (27.8) | 13 (23.6) |
| Patients having family history of depression in first degree relative | | 4 (21.1) | 6 (16.7) | 10 (18.2) |
| Initial MADRS score | | 28.3 ± 5.7 | 28.2 ± 4.6 | 28.3 ± 5.0 |
| Initial CGI score | Moderately ill | 12 (63.2) | 30 (83.3) | 42 (76.4) |
| | Markedly ill | 7 (36.8) | 6 (16.7) | 13 (23.6) |
| Side effects | Tension | 8 (42.1) | 9 (25.0) | 17(30.9) |
| | Palpitation | 4 (21.1) | 5 (13.9) | 9 (16.4) |
| | Decreased sleep | 5 (26.3) | 4 (11.1) | 9 (16.4) |
| | Memory loss | 5 (26.3) | 4 (11.1) | 9 (16.4) |
| | Increased sleep | 3 (15.8) | 4 (11.1) | 7 (12.7) |
| | Decreased sexual desire | 3 (15.8) | 3 (8.3) | 6 (10.9) |

Numbers in parentheses denote percentage; MADRS, Montgomery-Asberg depression rating scale; CGI, clinical global impressions
*$P<0.05$

to be 0.88 and 0.82 in responder and non-responder groups, respectively (Table II).

Linkage disequilibrium (LD) analysis was performed for rs6311 and rs6313 variants of *HTR2A*. A moderate LD was found among these SNPs ($D'=0.84$ and $r^2=0.71$). There were no significant differences for constructed haplotypes among responders and non-responders (Table III). Similar results were obtained for remitter/non-remitter groups and across side-effects except memory loss which was found to be significantly associated with rs6311 ($P=0.03$).

## Discussion

We attempted to find an association of serotonin transporter (*SLC6A4*) and serotonin receptor (*5HTR1A, 5HTR2A*) polymorphisms with response to treatment with escitalopram in patients with MDD. We studied four SNPs, namely rs6311, rs6313, rs6295 and *5-HTTLPR* 44 base-pair insertion deletion. No significant association was found between the SNPs analysed and response to escitalopram, though a significant association was observed between the side effect of memory loss with rs6311. Our finding of no significant association between the SNPs tested with response to escitalopram was similar to that of a larger study like Sequenced Treatment to Relieve Depression (STAR*D)[5]. However, another study[8] has shown an association between SNPs and antidepressant response. The discrepant findings could be due to allele frequency differences in populations of diverse ethnicities. Our patients were predominantly from four northern States and had *S* allele frequency of 0.7, and there were no *LL* genotype. It is difficult to conclude, whether this is true allele frequency or a distorted value due to a small sample size.

A substantial number of our patients reported side effect of tension (31%) and memory disturbance (16%), which is unusual for escitalopram. This could be because both probable and possible side effects were included in listing as given in the UKU scale. It is possible that anxiety and difficulties in concentration, which are also symptoms of depression, were labelled as side effects by patients. The finding of association of the side effect of memory loss with rs6311 needs further investigation.

The study had a few limitations like a sample size of 55, which might not be considered adequate for a genetic study. Our study was a preliminary study. There is a need for studies with larger homogeneous

**Table II.** Distribution of genotype and allele frequencies between the responder and non-responder groups

| SNP | Genotypic distribution | | | Allelic distribution | | | |
|---|---|---|---|---|---|---|---|
| | Responder (n=36) | Non-responder (n=19) | *P* value | Responder (2n=72) | Non-responder (2n=38) | *P* value | Odds ratio |
| | Count (frequency) | Count (frequency) | | Count (frequency) | Count (frequency) | | |
| rs6311 | TT / TC / CC | TT / TC / CC | 0.94 | T / C | T / C | 0.74 | 1.143 |
| | 6 (0.17) / 16 (0.44) / 14 (0.39) | 4 (0.21) / 8 (0.42) / 7 (0.37) | | 28 (0.39) / 44 (0.61) | 16 (0.42) / 22 (0.58) | | |
| rs6313 | TT / TC / CC | TT / TC / CC | 0.71 | T / C | T / C | 0.46 | 1.349 |
| | 6 (0.17) / 15 (0.41) / 15 (0.42) | 5 (0.26) / 7 (0.37) / 7 (0.37) | | 27 (0.38) / 45 (0.63) | 17 (0.45) / 21 (0.55) | | |
| 44ins/del | LL / LS / SS | LL / LS / SS | 0.37 | L / S | L / S | 0.40 | 1.581 |
| | 0 (0) / 9 (0.25) / 27 (0.75) | 0 (0) / 7 (0.37) / 12 (0.63) | | 9 (0.13) / 63 (0.88) | 7 (0.18) / 31 (0.82) | | |
| rs6295 | CC / CG / GG | CC / CG / GG | 0.80 | C / G | C / G | 0.87 | 1.064 |
| | 7 (0.19) / 19 (0.53) / 10 (0.28) | 3 (0.16) / 12 (0.63) / 4 (0.21) | | 33 (0.46) / 39 (0.54) | 18(0.47) / 20 (0.53) | | |

**Table III.** Distribution of haplotypes between the responder and non-responder groups

| SNP | Haplotype | Responders | Non-responders | *P* value |
|---|---|---|---|---|
| rs6311\|rs6313 | TT | 0.35 (25) | 0.39 (15) | 0.62 |
| rs6311\|rs6313 | CT | 0.03 (2) | 0.05 (2) | 0.50 |
| rs6311\|rs6313 | TC | 0.04 (3) | 0.03 (1) | 0.68 |
| rs6311\|rs6313 | CC | 0.58 (42) | 0.52 (20) | 0.56 |

Numbers in parentheses denote number of chromosomes

sample size from India with a larger pool of SNPs which could unravel the genetic basis of response to antidepressants.

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