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Serotonin Transporter Gene Polymorphism is Associated with Antidepressant Response to Escitalopram in Multiethnic Malaysians with Major Depressive Disorder: A Preliminary Study

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Abstract

Objective: This study investigates the relationship between antidepressant response to escitalopram and polymorphism of the serotonin transporter gene promoter region (5-HTTLPR) in multiethnic Malaysian patients with Major Depressive Disorder. Methods: An eight-weeks prospective study of treatment response to escitalopram was conducted on 29 Malaysian patients with Major Depressive Disorder. The severity and improvement of depression were assessed with the Montgomery-Asberg Depression Rating Scale. Patients were also genotyped for long (L) and short (S) polymorphisms in 5-HTTLPR using polymerase chain reaction. Results: Response to escitalopram treatment was more frequent in patients with 5-HTTLPR SS genotype than in those with LL or LS genotypes (p = 0.04, OR = 10.0, 95% CI = 1.05-95.2). The favourable allelic variant for response was S allele (p <0.01, OR = 4.73, CI = 2.60-8.59). However, there was no difference in the adverse effects rates between the 5-HTTLPR genotype groups (p = 0.39, OR = 2.44, 95% CI = 0.41-14.75). Conclusion: Polymorphism of 5-HTTLPR was associated with antidepressant response to escitalopram treatment but not to its adverse effects in the Malaysian depressed patients.

Keywords: Serotonin Transporter Gene, Polymorphism, Psychopharmacogenetics, Pharmacogenetics

Introduction

Unipolar Major Depression could become the second leading factor in the disease burden by 2020¹. Pharmacologic treatment of mood disorders has reduced morbidity of depressive disorder and improved mental health for millions of individuals worldwide. Since the early 1950s antidepressant drugs have shown to improve well-being and increase the chance of good long term outcome. Unfortunately 30–40% of all patients do not respond sufficiently to the initial treatment and it takes up to 6 weeks for them to be identified². Efficient clinical predictors have not yet been established, but
there is some evidence suggesting that genetic factors play a substantial role in response to antidepressants\textsuperscript{3-5}.

The brain serotonin transporter (5-HTT) is the principal site of action of many antidepressants and its role is to re-uptake serotonin (5-HT) into the pre-synaptic neuron, which thus terminates the synaptic actions and recycles it into the neurotransmitter pool. A single gene encoding the human 5-HTT was identified and cloned, localized to chromosome 17q11.1–q12\textsuperscript{6}. The gene spans 31 kb and consists of 14 exons\textsuperscript{7}. Heils \textit{et al} (1996) reported a polymorphism in the transcriptional control region upstream of the 5-HTT coding sequence\textsuperscript{8}. It consists of a 43-bp insertion [long (L) allele] or deletion [short (S) allele] involving repeat elements 6 to 8. Lesch called it Serotonin Transporter-Linked Polymorphic Region (5-HTTLPR) and a study on lymphoblastoid cell lines found that the basal activity of the L variant was more than twice that of the S allele of the 5-HTT gene promoter\textsuperscript{7}. The "long/short" polymorphism in 5-HTTLPR has been proposed as a pharmacogenetic marker for antidepressant efficacy.

Even if some studies including Asian patients are discordant\textsuperscript{9-11}, many research groups have shown that homozygotes for the L variant have a better response to different SSRIs when compared to heterozygotes and homozygotes for the S variant\textsuperscript{12-20}. Some authors however, did not find such association\textsuperscript{21}.

Genetic differences among patients may contribute to differences in medication response and the severity of adverse effects. The profile of adverse events classically associated with SSRIs such as gastrointestinal disturbances, sexual dysfunction, headache and anxiety is linked directly to stimulation of several kinds of serotonin receptors\textsuperscript{22}. These treatment-emergent adverse effects may lead to early discontinuation rate as high as 30\%\textsuperscript{23}. The ability to identify patients at greater risk for particular adverse effects would allow clinicians to anticipate and perhaps add prophylaxes against these effects\textsuperscript{24}. In a study of 37 patients with depression who were receiving fluoxetine, those homozygous for the S allele of 5-HTTLPR showed a higher frequency of agitation and insomnia after treatment\textsuperscript{25}. Another study with Japanese patients found no association between the 5-HTTLPR polymorphism and fluvoxamine-induced nausea\textsuperscript{26}.

The Malaysian population is unique with three main ethnic groups consisting of Malay, Chinese and Indian. Pharmacogenomics study of treatment response to antidepressant has not been previously carried out in Malaysia. Hence this preliminary study was designed to determine whether antidepressant response to SSRI is associated with genetic polymorphisms of the 5-HTTLPR in the local population. The primary hypothesis of the study is that significant association exist between antidepressant response to escitalopram and genetic polymorphisms of 5-HTTLPR in Malaysian patients with major depression and that the 5-HTTLPR polymorphism is associated with adverse effects of escitalopram.

**Methods**

A prospective eight weeks study on unrelated depressed patients was conducted at the University Malaya Medical Center (UMMC). Sample size was determined based on the L and S allelic frequency of 5-HTTLPR polymorphism in Japanese and Korean populations as there was no such data of the local population. We presumed
that allelic frequency in our population will be closer to the Japanese and Korean populations rather than to the Caucasian population. The allele frequency of the 5-HTTLPR L variant is about 25% in Japanese and Korean populations\textsuperscript{9,10,27,28}. Based on the L and S allelic frequencies of 25 and 75 percents respectively, we need at least 36 patients to give 80% power to detect any significant association at the significance level of 5%. Patients were of 18 years old and above and the diagnosis was confirmed using Mini International Neuropsychiatric Interview (M.I.N.I.)\textsuperscript{29}, based on the diagnostic criteria for Major Depressive Episode in Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR). Patients were excluded from the study if they were pregnant, having psychotic features, suicidal, having other concomitant Axis I psychiatric disorders, having other significant medical conditions, such as seizures, head trauma with loss of consciousness or other neurological illness, or having alcohol or drug dependence. All patients were treated with escitalopram for eight weeks. Dosage for individual patient was titrated into the usual clinical range based on initial tolerability and clinical response. The antidepressant response was assessed at 2\textsuperscript{nd}, 4\textsuperscript{th}, 6\textsuperscript{th} and 8\textsuperscript{th} week using Montgomery-Asberg Depression Rating Scale (MADRS)\textsuperscript{30}. Responders were defined as patients with at least a 50% reduction in MADRS total scores. Benzodiazepine was not allowed during the study period as it may influence the outcome of the study. Adverse effects that were both enquired by the investigator and reported by the patients were documented. The compliance to treatment was determined by pill counting and verification from the relatives. Blood of each patient was sampled during first visit for genotyping. Ethical approval was obtained from the UMMC Medical Ethics Committee. The confidentiality of the participants was assured and the purpose of the study was explained to the participants. Written informed consent was obtained from all the participants.

**Genotyping**

Genomic DNA was extracted from the whole blood using QIAamp\textsuperscript{®} DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Patients were genotyped for 5-HTTLPR promoter region L/S variations. The insertion/deletion of the 5-HTTLPR polymorphism was amplified with forward primers 5’ GGC GTT GCC GCT CTG AAT GC 3’ and the reverse primer 5’ GAG GGA CTG AGC TGG ACA ACC AC 3’\textsuperscript{7}. The PCR reaction was carried out in a total volume of 15 µl containing 1.5 mM magnesium chloride, 1 X buffer, 5% of dimethylsulfoxide (DMSO), 200 mM of dATP, dTTP and dCTP each, 100 mM of dGTP and 7’-deaza-dGTP, 0.4 µM of each primer, 0.4 U of Taq polymerase and 50-100 ng of template DNA. Thermal cycling was carried out at 94 °C for 5 minutes (denaturation), followed by 35 cycles at 94 °C for 30 seconds, 69 °C for 30 seconds in series, and 72 °C for 1 minute before a final extension step at 72 °C for 10 min. The PCR products were visualized using 2.5% agarose gel electrophoresis containing ethidium bromide (EtBr) or GelRed\textsuperscript{TM}, the latter being used in the later part our study in the pre-cast agarose gel instead of the EtBr, due to its non-mutagenic and non-cytotoxic features. The gel was then visualized using 3Door MultiDoc-It Imaging Systems. Genotyping was performed blind to the clinical course of illness at the Pharmacogenomics Laboratory of the Department of Pharmacology.
**Statistical Analysis**

Means and standard deviations, ranges of continuous variables, and proportions of categorical variables are presented as descriptive statistics. The genotypes of 5-HTTLPR were classified into two groups: carriers of the L allele (LL and LS genotypes) and carriers of the SS genotype, the L allele having been reported to have a higher 5-HTT density and activity than the S allele\(^7,8\).

Data were analyzed based on two groups: LL/LS and SS, on account of the recessive nature of the S allele and the small sample size. Nonparametric statistics were used because of the small sample size. Categorical variables were compared by means of \(\chi^2\) tests. Continuous variables were compared by means of Mann–Whitney and Kruskall–Wallis tests when appropriate. Hardy–Weinberg equilibrium was tested by the \(\chi^2\) test. Results were considered significant at \(p<0.05\). All statistical analyses were performed using PASW Statistics version 18.

**Results**

A total of 29 patients were enrolled into the study which comprised of 20 females and 9 males. The patient characteristics are shown in Table 1, together with a tabulation of the LL/LS and SS genotype groups. The female to male ratio of 2:1 in our sample is consistent with previous epidemiological studies\(^31\). The ethnic distributions of Malays 41.4%, Chinese 37.9% and Indian 20.7% differ from other parts of Malaysia where Malays comprises 65% of the population followed by Chinese 26% and Indian 8%\(^32\). This is probably due to the site of the study which was in Kuala Lumpur which is a Chinese predominant area. However, depression affects all ethnic groups equally and this ethnic variation in our sample would not affect the generalization of our results to the Malaysian population. There was no statistically significant difference in the socio-demographic characteristics and baseline severity of MADRS scores between the 5-HTTLPR SS group LL/LS group.

| Table 1. Socio-demographic characteristics and baseline MADRS scores for the various genotype groups |
|---------------------------------|-----------------|-----------------|
| 5-HTTLPR SS (n=10) | 5-HTTLPR LL/LS (n=19) |
| Age, years (SD) | 40.9 (13.9) | 39.9 (14.2) |
| Gender | | |
| Male | 3 | 6 |
| Female | 7 | 13 |
| Ethnicity | | |
| Malay | 5 | 7 |
| Chinese | 4 | 7 |
| Indian | 1 | 5 |
| Marital status | | |
| Single | 2 | 7 |
| Married | 8 | 11 |
| Widow | 0 | 1 |
| Education | | |
| Primary | 2 | 6 |
With regard to the 5-HTTLPR genotypes, 7 out of 29 patients (24.1%) were carriers of the LL genotype, 12 (41.4%) were carriers of the heterozygous (LS genotype) and 10 (34.5%) were carriers of the SS genotype. No significant deviation from the Hardy–Weinberg equilibrium was detected ($p = 0.44$). The frequency of L and S allele was 44.8% and 55.2% respectively (Table 2). The L allele frequency was lower than that of the Caucasian population (57%)\(^7\), but is similar to that observed in some Asian populations\(^9,27\). The genotype distribution did not differ significantly between Malay and Chinese ($\chi^2=3.89; p=0.14$). However, the Indian ethnic group differs significantly from Malay and Chinese ($\chi^2=7.48; p=0.02$) by having LL genotype as the predominant group, which is more similar to the Caucasian population.

**Table 2. Genotype and Allele Distributions of Serotonin Transporter Gene Polymorphism among 3 ethnic groups**

<table>
<thead>
<tr>
<th>5-HTTLPR Polymorphism</th>
<th>No. (%) of study patients</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malay</td>
<td>Chinese</td>
<td>Indian</td>
</tr>
<tr>
<td>SS</td>
<td>5 (41.7)</td>
<td>4 (36.4)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>LS</td>
<td>4 (33.3)</td>
<td>7 (63.6)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>LL</td>
<td>3 (25.0)</td>
<td>0 (0)</td>
<td>4 (66.6)</td>
</tr>
<tr>
<td>S</td>
<td>58%</td>
<td>68%</td>
<td>25%</td>
</tr>
<tr>
<td>L</td>
<td>42%</td>
<td>32%</td>
<td>75%</td>
</tr>
</tbody>
</table>

More patients with the SS genotype responded to escitalopram treatment (90%), compared to patients with the LS and LL genotype (58.3% and 28.6% respectively). The S allele showed a higher response rate to escitalopram compared to the L allele (Table 3). The MADRS mean scores started showing statistically significant difference between the 2 genotype groups from 2\(^{nd}\) week onwards till 8\(^{th}\) week of study (Figure 1). There was no significant difference between the genotype groups in term of adverse effects rates.
Table 3. Genotype and Allele Distributions of Serotonin Transporter Gene Polymorphism in Responders and Non-responders to escitalopram

<table>
<thead>
<tr>
<th>5-HTTLPR</th>
<th>Responders n(%)</th>
<th>Non-responders n(%)</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>9 (50.0)</td>
<td>1 (9.0)</td>
<td>10.0 (1.05-95.2)</td>
<td>0.04*</td>
</tr>
<tr>
<td>LS</td>
<td>7 (38.9)</td>
<td>5 (45.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>2 (11.1)</td>
<td>5 (45.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.69</td>
<td>0.32</td>
<td>4.73 (2.60-8.59)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>L</td>
<td>0.31</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5-HTTLPR, serotonin-transporter-linked polymorphic region
*Statistical analysis was performed between SS and LS/LL.

Discussion

The results from our preliminary study showed that there is an association between the 5-HTTLPR polymorphism and antidepressant response to escitalopram in our mixed ethnic sample and that the favourable allelic variant for response was the S variant. Thus, our primary hypothesis was confirmed. The results are in line with studies done on other Asian populations\(^9,10,33\) but differ from that of the
Caucasian populations in which the L variant showed better response to antidepressant treatment with SSRIs. The reason for this ethnic difference remains unclear. However, there are some possible explanations as discussed below.

**Ethnic differences in 5-HTTLPR allele**

Functional influence of the 5-HTTLPR polymorphism is related to the transcription of the gene. The L and S variants of the promoter polymorphism have functional differences in modulating transcription of the 5-HTT gene as well as subsequent 5-HTT availability. The L variant of 5-HTTLPR is associated with more than twice that of the S variant in the transcriptional activity and serotonin uptake. These allele-specific functional differences have been confirmed in human tissues including the brain.

In our study, the overall L allele frequency is 45% (Table 2), which is lower than that of Caucasian populations. The L allele frequency is about 57% in Caucasian populations but only about 25% in the Japanese and Korean populations. If we assume that the L variant of 5-HTTLPR is the favourable allele for response to SSRIs based on studies on Caucasian patients and also the studies done by Heils et al (1996) and Lesch et al (1996), we might expect a low response rate in our patients because of lower L allele frequency in our population. However the overall response rate was 62.1% in our study. Also, several other clinical trials have shown a consistent finding that 60-70% of depressed patients respond to SSRIs regardless of ethnic groups. Therefore, other genetic explanations involving other genes should be sought.

**Effects of other genetic polymorphisms**

The mechanisms by which antidepressant agents exert their clinical effects are not yet fully understood, and studies that focus on single candidate genes may not identify novel genetic information of clinical importance. Up to now, pharmacogenomics studies have focused on candidate genes implicated in the mechanisms of antidepressant drug action or in the pharmacokinetics of such drugs. It may not be possible to explain the different antidepressant response by analyzing only one polymorphism. There are several other genes which may also affect antidepressant response. For example, a second polymorphism in the 5-HTT gene occurring in intron 2 (STin2) is also linked to the 5-HTT gene expression and antidepressant response. In their study, both 5-HTTLPR and STin2 variant alleles were shown to contribute to the efficacy of SSRI.

Another possible explanation could be the presence of a new single nucleotide polymorphism (SNP) and one such SNP is the (A>G) in the sixth repeat of the 5-HTTLPR. Hu reported that a SNP (rs25531, A/G) in the L variant of 5-HTTLPR may have functional significance: The more common L_A allele is associated with higher basal activity, whereas the less common L_G allele has transcriptional activity similar to the S allele. These investigators suggest that in tests of association the L_G alleles should be analyzed along with the S alleles.

Different ethnic populations may have other functional polymorphisms which may also affect the antidepressant response to SSRIs. A variation in the ATP-binding cassette B1 gene (ABCB1) coding for a P glycoprotein that determines brain tissue penetration of...
many antidepressant drugs may predict clinical outcome in patients treated with substrates of this blood brain barrier regulation molecule. Several studies reported that variants of a gene coding for FKBP5, a co-chaperone involved in stress hormone signaling, and variants for serotonin receptor 5-HT2A are predictive of treatment response. Further associations have been reported for the glutamatergic receptor gene GRIK4, the enzymatic gene phosphodiesterase 11A (PDE11A), inflammation-related genes (CD3E, PRKCH, PSMD9, and STAT3), and urocortin-3 gene (UCN3) expressing a ligand of the corticotropin-releasing factor receptor (CRF2). Newer approaches that include whole gene sequencing or entire single nucleotide polymorphism (SNP) analyses are needed to identify other responsible functional loci. Polymorphisms of cytochrome P450 enzymes responsible for the metabolism of the SSRIs may also affect clinical response. Furthermore, treatment response is not only determined by genetic makeup but also by clinical features such as course of illness, comorbid anxiety, age at disease onset, current age, and sex.

Adverse effects and 5-HTTLPR polymorphism

Our preliminary study did not find significant association between adverse effect of escitalopram and 5-HTTLPR polymorphism. However, small sample size could explain why there was no significant association. Furthermore, there were patients who defaulted and were not contactable to give their reason of default. The possibility of these patients experiencing adverse effects cannot be ruled out.

Limitations and Recommendations

This was a preliminary study with limitations. The study was under power because of the small sample size. There were several reasons why the sample size was small. Firstly, since the UMMC is a referral hospital for the district, many depressed patients who came to the UMMC Psychiatric Clinic were already on various antidepressants prescribed by the referring general practitioners and these patients were therefore excluded from the study. Other reasons for the small sample size include patient refusal to participate, suicidal patients and patients with psychotic features. In view of the possible effect of ethnicity on antidepressant response, adequate subject numbers should be included to allow for sample stratification into the different ethnic groups in future studies.

The second limitation was the absence of a placebo control group. Without a placebo group to compare to, we were unable to determine whether the response to treatment is due to the escitalopram effect, or non-specific response, or a combination of both.

The lack of serum drug level monitoring to monitor patient’s compliance to treatment was another limitation. Even though we did pill counting it was not possible to determine whether patients were compliant or not to their medication. Failure to comply will affect the clinical response hence affecting the outcome of the study. For the next stage of the study, we plan to carry out drug level monitoring to check on patient compliance and to exclude the non-compliant from the study.

The monitoring for adverse effects is now being carried out in this on-going study. Patients may not have been able to remember all the adverse effects that they had experienced, especially if the adverse effects were transient. Therefore a
systematic assessment using rating scale is now being done.

In summary, our study showed that 5-HTTLPR polymorphism is associated with antidepressant response to escitalopram treatment in mixed ethnic depressed patients in Malaysia. Further research is needed to confirm this preliminary result.

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