An evaluation of the association of serotonergic genes in patients with major depressive disorders in a tertiary care hospital in Coimbatore

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ABSTRACT
A multi-factorial illness that causes morbidity threatens life and affects the quality of life is the major depressive disorder (MDD). Serotonergic dysfunction is one of the most described theories of psychiatric symptoms. Several serotonergic genes have been reported in associated with MDD. Recent studies have suggested that serotonin transporter length polymorphic region (5HTTLPR), Tryptophan hydroxylase gene 1 (TPH1) and Tryptophan hydroxylase gene 2 (TPH2) are associated with MDD. In the present study, we have examined the possible correlation between serotonergic genes 5HTTLPR, TPH1 (A218C), and TPH2 (G703T) polymorphism in MDD patients in Coimbatore tertiary care hospital. Blood samples were collected from 245 major depressive disorder patients and 250 healthy volunteers belonging to the faculty of Karpagam College of Medical Science and Research. All the samples were analysed by PCR-RFLP method. The chi-square test has been used for comparing genotype and allele distribution of cases and controls. We observed the 5HTTLPR L/S genotype and allele frequencies (P=0.001 and p= 0.001) and TPH2 (G703T) (P=0.0002 and P= 0.005) TPH1 (A218C) (P=0.48 and P=0.43) polymorphisms with MDD. We also observed higher homozygous genotype frequencies of the short allele of 5HTTLPR, the G allele of TPH2 G703T polymorphism in patients. In short, our preliminary study suggests that the 5HTTLPR L/S and TPH2 G703T gene polymorphism were associated with the MDD. Serotonergic genes polymorphism in MDD patients and control groups were compared. Further clinical trials are to be carried out to find the exact mechanisms of the serotonergic system in MDD.

INTRODUCTION
MDD is a widespread psychiatric disorder and categorised as a mood disorder. The current understanding that depression is based on biological, psychological, genetic and social factors. Around 340 million people worldwide suffer from depression (Chiriță et al., 2015). According to estimates from the WHO depression would be the second leading cause of the problem after cardiac disease by 2020 (Reddy, 2010). A complete understanding of MDD remains elusive. Importance of genes for the responsiveness effect of depressive disorder is substantial data from families, twin and adoption
trials (Kendler et al., 2006). Many studies have indicated as serotonin is possible neurotransmitter involved in the etiology of depression (Cowen et al., 1989). A majority of serotonergic genes involved in neurotransmitter process (Anderson et al., 1990). Serotonin is a neurotransmitter synthesised from the basic tryptophan amino acid by the chronological action of TPH (Tryptophan hydroxylase), and it catalyses the rate-limiting step in the biosynthesis of serotonin neurotransmitter (Verbeek et al., 2013). Serotonin is one of the essential neurotransmitters in the central nervous system, and it maintains mood stability. Serotonin transporter (5HTT) is encoded by the SLC6A4 gene, which has a definite place in determining the duration and magnitude of serotonin neurotransmitter at the synaptic cleft. Many studies have reported that 5HTT gene is mainly involved in MDD 5HTT is located on chromosome 17q11.1-12 with 14 exons which span approximately 35Kb (Jéquier et al., 1967; Lesch et al., 1994). In the upstream 5’ serotonin transporter lipid polymorphic region (5HTTLPR), has been identified by Heils and co-workers, and it involves insertion/deletion of 44bp (Ramamoorthy et al., 1993). Its effect on two possible main alleles with a different length and potency, i.e., one long (L) allele with 16 repeats and one short (S) allele of 14 repeats (Gelernter et al., 1995). Several studies have indicated the relationship between the 5HTTL gene and major depressive disorder (Heils et al., 1996; Hariri et al., 2002). Hence, it is necessary to observe this functional 5HTTLPR variant as a candidate gene for genotype investigation in MDD (Goldman et al., 2010). This polymorphism is the most-studied genetic variant in psychiatric genetics to date among Indians and other countries. Still, there is no report in South India, specifically in Coimbatore.

We attempted to find out the genetic variation in MDD patients for better treatment. Tryptophan hydroxylase (TPH) is encoded by two genes: TPH1 and TPH2. TPH1 gene on chromosome 11p15.3-q14 is involved in serotonin synthesis in peripheral tissues (Uher and McGuffin, 2010). Numerous studies have demonstrated the association between single nucleotide polymorphism of the TPH1 gene and psychiatric disorder. The main polymorphism A218C is located in the intron 7 of the TPH1 gene. A218C allele is consistently associated with the increased liability of multiple ethnic groups with major depressive disorder (Walther and Bader, 2003; Sakowski et al., 2006). TPH2 gene, a newly identified isoform of TPH, regulates brain-specific 5HT synthesis. TPH2 plays a more significant role in the central serotonergic system than TPH1. TPH2 is located in the chromosome 12q21.1; this chromosome region is identified as a possible link associated with major depressive disorder. Previous studies have reported that single nucleotide polymorphism G703T in the TPH2 promoter region is associated with major depressive disorder (Wang et al., 2011; Latso et al., 2016). Until now, studies investigating the association of TPH1 and TPH2 variants with major depressive disorder have not yielded conclusive results. In this study, we analysed the strength of evidence for the association of 5HTTLPR, TPH1, and TPH2 polymorphism with MDD.

**MATERIALS AND METHODS**

**MDD Patients**

The Karpagam Faculty of Medical Science and Research (KFMSR), Coimbatore recruited 245 MDD patients (>18 years and both genders) who have met Diagnostic and Statistical criteria for Mental Disorders, text version IV (DSM-IV) Random selections were made for patients. A senior psychiatrist did the Mini-International Neuropsychiatric Interview 5.0 (M.I.N.I.) (Sheehan et al., 1998). This study ruled out the patients suffering from other psychiatric problems.

Healthy volunteers (Control) A total of 250 healthy volunteers were examined, and the criteria adopted for exclusion were similar to those of the patients. Healthy Controls were also rejected with first-degree families having DSM-IV Axis-1 psychiatric disorder. The KFMSR Ethics Committee accepted the research. All test subjects signed an informed consent agreement. The demographic data were collected from patients and Controls such as age, gender, marital status, education, employment, and income satisfaction. The demographic details of the patients and controls were provided Table 1.

**Genotyping**

Venous blood was immediately drawn and frozen in a fraction at -70 °C. Genomic DNA was extracted from the study groups by salt out technique (Suguna et al., 2014). The isolated DNA was stored at 4°C until analysis. The specific segment of DNA was amplified through Polymerase Chain Reaction (PCR) performed on an AB-applied Biosystem (VeriteeThero cycler) in a total amount of 20µl reaction. The Takara emerald GT PCR master mix kit composed of 2x buffer; DNTPs, enzymes, mgcl2. The polymorphism of SLC6A4 (5HTTLPR) gene was amplified to determine genotype only for the long allele(280bp) and short allele(230bp). The sequence of the 5HTTLPR PCR primers and cycling conditions are given Table 2. After migra-
Table 1: Demographic detail of patients and control subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (n=245)</th>
<th>Controls (250)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (Male/ Female)</td>
<td>142 (59.1%)/103 (42.9%)</td>
<td>158 (63.2%)/92(36.8%)</td>
<td></td>
</tr>
<tr>
<td>Age (Years)</td>
<td>39.09 ± 10.65</td>
<td>38.12 ± 10.35</td>
<td>0.305</td>
</tr>
<tr>
<td>Education (Years)</td>
<td>13.23 ± 3.52</td>
<td>13.89 ±4,27</td>
<td>0.063</td>
</tr>
<tr>
<td>Employment status (working/not working)</td>
<td>130 (54.1%)/ 115 (47.9%)</td>
<td>178 (71.2%)/72 (28.8%)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td>169 (70.4%)</td>
<td>190 (76%)</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>29 (12.0%)</td>
<td>2 (0.8%)</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>31 (12.9%)</td>
<td>54 (21.6%)</td>
<td></td>
</tr>
<tr>
<td>Unmarried</td>
<td>16 (6.6%)</td>
<td>4 (1.6%)</td>
<td></td>
</tr>
<tr>
<td>Income satisfaction</td>
<td>40 (16.6%)/205 (85.41)</td>
<td>72 (28.8)/178 (71.2%)</td>
<td></td>
</tr>
<tr>
<td>Yes/No</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The observation of 6 μl of PCR products on an agarose gel (2.5% depending on the predicted amplicon size) in 0.5X TAE, The gel was stained with ethidium bromide and amplified bands 230bp, and 280bp visualised under UV light. The gel image of the 5HTTLPR gene amplification given in Figure 1. The TPH-1 (rs180053) A218C have been amplified with the PCR primers, and cycling conditions were provided Table 2. The Amplified PCR product electrophoresed by 2% agarose and detected by UV illuminator. The PCR product were digested for 3 hours with restriction enzyme Nhel. The cleaved recognition sites C Allele (338) and A Allele (171+167) were analysed by 2.5% electrophoresis and viewed by UV illuminator. The amplified TPH1 A218C gel image is given in Figure 2. PCR was carried out with TPH-2 gene, primers and conditions are given in Table 2. PCR amplified product 309 were digested with Xap restriction enzyme cleaved G allele (218bp) and T(197+21bp) allele and were analysed by 3% agarose electrophoresis and viewed by UV illuminator. The amplified product of TPH2T703G were shown in Figure 3.

Statistical Analysis

To calculate the Descriptive statistics, all data analyses were performed using SPSS Software. Chi-square test carried out all comparisons of the genotype and allele frequencies between cases and Controls. The allele impact was estimated with odds ratios (OR) and confidence intervals at 95 % (CI). For all statistical tests, a p-value <0.05 was considered significant.

RESULTS AND DISCUSSION

In this study, we have included 245 primary depressive patients and 250 healthy volunteers of both genders aged above 18 years. The observation of the Baseline characteristics shows that the number of divorced people is higher in the group of patients than in Controls. In the patients group, 70% are married and 29% divorced, and also most of the patients are not satisfied with their income (85.4%). Among MDD patients, it was found that the unem-
Table 2: Primers used for the PCR amplification of the marker gene

<table>
<thead>
<tr>
<th>Markers</th>
<th>Primer</th>
<th>Sequence</th>
<th>Fragment size</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPH-1 (rs180053) A218C</td>
<td>F</td>
<td>5'-AATGGCATCTACCTATGGGTTCC-3'</td>
<td>338bp</td>
<td>Annealing 58°C for 15sec</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5'-CTTTATTTTCTCCATGGGACTCA-3'</td>
<td></td>
<td>Nhel</td>
</tr>
<tr>
<td>TPH-2 (rs4570625) G703T</td>
<td>F</td>
<td>5'-TTTCCATGATTTCCAGTAGAG-3'</td>
<td>309bp</td>
<td>Annealing 55°C for 15sec</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5'-AAGCTTTTTCTGACCTGACAAAT-3'</td>
<td></td>
<td>Xap</td>
</tr>
<tr>
<td>5HTT SLC6A4(5HTTLPR)</td>
<td>F</td>
<td>5'GGCGTTGCCGCGCTCTGAATGCC-3', Short allele</td>
<td>230bp, long allele 280bp</td>
<td>Annealing 64°C 15sec</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5'CAGGGGAGATCCTGGAGAGGT-3'</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 3: TPH2 Gene Amplification

Employment rate is higher than that in controls Table 1. An analysis of genotypes shows that 128 of the 245 patients (52%) have an SS genotype, 83 patients an SL genotype (33.8%) and 34 patients (14%) an LL genotype, indicating that a more significant number of patients have SS genotype compared to the Control group.

An application of the chi-square analysis reveals that the association of 5HTTLPR, genotype and allelic frequency with MDD is statistically significant (P=0.001 and p=0.001) as compared to Control. The results of 5HTTLPR gene polymorphism also show that the genotype carrying the S allele of 5HTTLPR is associated with major depressive disorder Table 2.

Observation of TPH1 (A218C) gene polymorphism shows that there is no statistically significant (P=0.43 and p=0.48) association of genotype and allelic frequency with MDD patients as compared to control Table 3. Similarly, observation of TPH2 (G703T) gene polymorphism shows that in MDD patients group 132 of 245 (53.8%) possess GG genotype, 89 GT genotype (36.6%) and 24 (9.9%) had TT genotype, which shows that a higher number of patients have GG genotype as compared to control. The TPH2 (G703T) polymorphism results show that the association of TPH2 (G703T) genotype and allelic frequency with MDD, is statistically significant (P=0.005 and p=0.0002) as compared to control. Observation of the results of TPH2 (G703T) gene polymorphism also shows that the genotype carrying the G allele of TPH2 (G703T) is associated with major depressive disorder Table 3.

In the present study, we assessed the demographic characteristic of the MDD patients and those of Control according to polymorphism of 5HTTLPR, TPH1 and TPH2 as depicted in Table 1. The frequency of divorcee and unemployment was observed in higher level among MDD patients than in Control group Table 1. This is the first report on investigation of this polymorphism (5HTTLPR(S/L), TPH1(A218C) and TPH2(G703T)) and its relationship. The result of our study shows that the genotype carrying the S allele of 5HTTLPR and G allele of TPH2 (G703T) is significantly associated with MDD, lower significant effect and weaker credibility for TPH1(A218C) gene when compared to healthy volunteers. Previous studies have shown that the polymorphism of 5HTTLPR S allele in Asia and the European population is more frequent and less prevalent in sub-Saharan Africans. Many previous reports have suggested that the S allele can be susceptible to major depressive disorder. TRH2 is found in brain-derived serotonergic neurons that are specifically...
Table 3: Allele and genotypic frequency of each SNP between cases and Controls

<table>
<thead>
<tr>
<th>Marker</th>
<th>Groups</th>
<th>Genotype frequency</th>
<th>P-value</th>
<th>Allelic frequency</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SS</td>
<td>SL</td>
<td>LL</td>
<td></td>
</tr>
<tr>
<td>5HTTLPR</td>
<td>Cases</td>
<td>128</td>
<td>83</td>
<td>34</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>79</td>
<td>123</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>TPH1</td>
<td>Cases</td>
<td>AA</td>
<td>AC</td>
<td>CC</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>72</td>
<td>122</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>TPH2</td>
<td>Cases</td>
<td>GG</td>
<td>GT</td>
<td>TT</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>92</td>
<td>119</td>
<td>39</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: Cl, Confidence interval, rpt, repeat

*odds=1.7497, Cl=1.3481-2.2709, Z-Statistics=4.202 (Bonferroni’s corrected value<0.0001)

*odds=1.0976, Cl=0.8546-1.4096, Z statistics = 0.730, p=0.4654

*odds=1.6752, Cl=1.2831-2.1872, Z statistics = 3.793 (Bonferroni’s corrected value<0.0001)

expressed raphe nuclei. There are signs of the biological function of rs4570625, and its correlations with other psychiatric disorders have also been identified (Martínez-Idárraga et al., 2017). Yoon and Kim have shown in their study the association between rs4570625 and suicide attempts in depressed patients (Zhang et al., 2005; Han et al., 2017). The TPH1 gene may participate differentially in the regulation of serotonin turnover rate in the central nervous system (Kim et al., 2009). Therefore, changes in the TPH gene may contribute to the predisposition to low serotonergic neurotransmission. Some reports have also shown the association of TPH1 gene with major depressive disorder (Gizatullin et al., 2006; Bellivier et al., 1998) while others haven’t (Du et al., 2000; Geijer et al., 2000). This study concludes that in tertiary care hospitals of Coimbatore, among MDD patients, the 5HTTLPR (S allele) short allele and TPH2 G allele variations are associated with MDD, and TPH1(A218C) gene polymorphism is not associated with MDD.

CONCLUSIONS

Major Depressive Disorder is probably caused by various genetic and environmental factors worldwide. The present study concludes that samples collected from MDD patients at Tertiary Care Hospitals in Coimbatore show 5HTTLPR (S allele) short allele and TPH2 G allele variations in more MDD patients when compared to the Control groups because of the significant association with MDD through the serotonergic genes except for TPH1(A218C). The presence serotonergic genes such as 5HTTLPR and TPH2 (G703) is found to create depression among the study population, and we need to be investigated further their mechanism. This will help to improve the clinical care and anti-depression treatment of depressed patients significantly.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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