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Association analysis of serotonin transporter gene polymorphism among the South-Indian migraineurs

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Abstract

Background: Migraine is a multifactorial neurological disorder characterized by frequent moderate to severe intensity headaches. The genetic variations in synaptic and post-receptor signalling proteins have direct effect on the process of serotonergic neurotransmission.

Methods: We aimed to investigate the genetic association of serotonin transporter (SERT) 5-hydroxytryptamine transporter-linked promoter region (5-HTTLPR) polymorphism and migraine risk in South-Indian population. A total of 304 subjects with migraine including with aura (MA) and without aura (MO) and 308 controls were included in the present study. The single nucleotide polymorphism (SNP) was detected using polymerase chain reaction (PCR) and confirmed by deoxyribonucleic acid (DNA) sequencing.

Results: The genotyping analysis revealed insignificant relationship with migraine subjects when compared with controls (P > 0.05). The minor 'S' allele showed no association with odds ratio (OR) = 1.23 [95% confidence interval (CI): 0.90-1.66], heterozygote with OR = 1.18 (95% CI: 0.82-1.69), and homozygote with OR = 1.51 (95% CI: 0.52-4.35).

Conclusion: Further clinical studies are required to validate the results of SERT 5-HTTLPR promoter polymorphism in diverse ethnic descents especially in Asian populations.

Introduction

Migraine is defined as a common neurovascular disorder characterized by frequent headaches, which occurs in two clinical subtypes such as migraine with aura (MA) and without aura (MO).[1] World Health Organization (WHO) classified migraine as the 3rd most frequent disease, and the 7th cause of reducing the quality of life by creating a medical and social problem.[2]

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Globally, migraine affects nearly 15% of the adults and it is 3 times more common in women than men due to interaction of enviro-genomic and hormonal imbalances. The reports from family-based association and twin studies infer that migraine accounts for nearly 46% heritability nature.[3] The migraine pathophysiology can be explained using the involvement of anatomical and cranial neurovascular structures. Further, the neurovascular scaffold incorporates three essential domains such as vascular (intracranial constriction and vasodilation), neural [cortical spreading depression (CSD)-like neural events], and nociceptive (neuropeptides and migraine mediators) domains, respectively.[4] The MA is mainly triggered by CSD, described as the wave of strong neuronal activity which gradually evolves above the cortex, and proceeded by events of neuronal inactivity. The signs and symptoms of headache occur primarily due to initiation of trigeminal vascular system.[5]

A genome-wide association study (GWAS) was conducted on 223773 subjects from the United Kingdom (UK) Biobank cohort. The genotyping was performed using bespoke Affymetrix UK Biobank chips. The results revealed 28 loci among which 14 loci had been previously found to be associated with migraine. This study also has identified nearly 3343 single nucleotide polymorphisms (SNPs) which reached the significance level ($P \leq 5 \times 10^{-8}$).[6] Clinical studies have documented the candidate genes influencing migraine pathogenesis; among the various genes, we have concentrated on serotonin transporter (SERT/also known as SLC6A4) gene in this study. The SERT gene is mapped on 17th chromosome at p-arm 11.2 region and encodes for integral membrane protein which transports the serotonin neurotransmitter from the synaptic spaces into the pre-synaptic neurons. The involvement of serotonin in migraine has been documented by Sicuteri's findings; they observed from the urine of patients an increase of 5-hydroxyindoleacetic acid (5-HIAA) during migraine attacks, which is the essential metabolite of serotonin.[7] The serotonin regulates various physiological activities through specific set of receptors such as the G-protein-coupled receptors that, in turn, activate intracellular messenger cascade which mediates the inhibitory or excitatory neurotransmission.[8]

The SERT system functions in the serotonergic transmission; 5-hydroxytryptamine transporter (5-HTT) might have a relationship with migraine. This gene harbors a 44 base pairs insertion/deletion functional SNP in the regulatory region which has two common allelic forms, such as the long (L) and short (S) variants.[9] The clinical studies on 5-HTT-linked promoter region (5-HTTLPR) short allele/variant have been shown to reduce the messenger ribonucleic acid (mRNA) transcription efficacy for the 5-HTT promoter region, which finally results in lower serotonin uptake and decreased gene expression.[10] Although 5-HTTLPR polymorphism has been previously studied in several ethnic descents, similar case-control study has not been performed in Indian population. Specific diseases are at risk for different racial groups because of genetic factors. In this case-control study, we have evaluated the role of 5-HTTLPR polymorphism as a risk factor in South-Indian migraineurs.

Materials and Methods

This case-control study enrolled 304 subjects with migraine from the Department of Neurology, Chettinad Super Speciality Hospital, Chettinad Health City, Tamil Nadu, India, from January to August 2018, and 308 healthy controls were recruited from the South-Indian population. The sample size of this case-control study was calculated using the G*Power analysis software. Power analysis is based on the formula used for sample size calculation: $N = p\,(1\text{-}p)\,(Z/E)^2$ with power value = 0.95.[11] All the study participants underwent a clinical assessment based on International Headache Society (IHS) guidelines[12] by a consultant neurologist (KS). The migraineurs were classified as having MA or MO. The healthy volunteers were recruited as healthy controls following a clinical examination by the general physician of Chettinad Super Speciality Hospital. Both the migraineurs and controls belonged to South-Indian population with Asian ethnic background. The subjects with cardiac illness, psychiatric disorders, and human immunodeficiency virus (HIV) seropositivity were excluded from the current study. A questionnaire was designed to obtain the demographic and clinical information from each study participant, after their physical examination. All the participants provided informed consent during the study enrolment.

Genetic examination of 5-HTTLPR polymorphism: Genomic deoxyribonucleic acid (DNA) of all the study participants was extracted from whole blood (4 ml) samples using salting-out method[13] with minor modifications. The DNA

samples were stored at -20 °C freezers (DW-HW138, Blue Star). The genotyping was performed as previously described[9] for the 5-HTTLPR polymorphism. The reaction was performed on ABI Veriti® Thermal Cycler (USA) in a total volume of 15 µl, including 5.0 µl of 10× polymerase chain reaction (PCR) buffer, 1.5 µl of deoxynucleoside triphosphates (dNTPs), 10 pmol/µl of each primer, 2 µl of genomic DNA, 0.6 µl of Taq DNA polymerase, and 5.9 µl of molecular biology grade water (Cat. no: ML024, HiMedia Laboratories). The PCR amplicons were analyzed along with 100 bp molecular weight marker (Dye Plus, Cat. no: 3422A, Takara) on 2% agarose gel electrophoresis. To identify the reproducibility of genotyping results, the selected samples were reconfirmed using Sanger sequencing methods.

The Pearson's chi-square statistics was used to test the association between 5-HTTLPR SNP and migraine. The genotypic frequency distribution among the healthy participants was determined by Hardy-Weinberg Equilibrium (HWE) ($P > 0.05$).[14] The genetic association of 5-HTTLPR SNP with migraine was examined by computing the odds ratio (OR) and with 95% confidence intervals (CIs).[15] The statistical analysis was performed by SPSS software (version 21, IBM Corporation, Armonk, NY, USA) and $P < 0.05$ was assumed as statistically significant. The genotypes were also further assessed based on dominant and recessive genetic models (LS + SS vs. LL and SS vs. LL + LS).

Results

Demographic analysis: The demographical and clinical features of migraineurs and controls are explained in table 1. The migraineurs were sub-classified into two groups according to MA (n = 132) and MO (n = 172), respectively. Among the study participants, mean age in subjects with migraine and controls was 35.54 ± 08.13 and 34.83 ± 06.44, respectively. Further, 71 subjects had family history of migraine and among women with positive family history were young when compared with men (Table 1).

Association of 5-HTTLPR SNP with migraine: The genotypic distributions of 5-HTTLPR polymorphism among the controls were under HWE, indicating good representation of the study participants. The genotypic and allelic frequencies along with the calculated data are explained in table 2. The genotypic frequencies of 5-HTTLPR polymorphism in migraineurs were 67.76%, 28.94%, and 3.28%, whereas in controls were 72.07%, 25.97%, and 1.94% for LL, LS, and SS, respectively.

Table 1. Demographic characteristics of patients with migraine and controls

Factors	Migraineurs (n = 304)	Controls (n = 308)
Men/women	106/198	97/211
Age (year) (mean ± SD)	35.54 ± 8.13	34.83 ± 6.44
Family history of migraine	71	Nil
Migraine type		
With aura	132	Nil
Without aura	172	
Pain severity		
Low	73	
Moderate	94	
Severe	137	
Location of headaches		
One side	93	
Both sides	107	
Entire head	104	Nil

SD: Standard deviation

Table 2. Allele frequencies and genotype distribution of serotonin transporter **(**SERT) gene polymorphism in patients with migraine and controls

Polymorphism	Frequencies	Migraineurs (n = 304)	Controls (n = 308)	HWE	OR	95% CI	χ^2	P*
		n (%)	n (%)					
5-HTTLPR	Allele							
	L	500 (82.23)	524 (85.06)	-		Reference	1.79	0.180
	S	108 (17.76)	92 (14.93)	-	1.23	0.90-1.66		
	Genotype							
	LL	206 (67.76)	222 (72.07)	0.69		Reference	0.87	0.199
	LS	88 (28.94)	80 (25.97)		1.18	0.82-1.69		
	SS	10 (3.28)	6 (1.94)		1.51	0.52-4.35	0.60	0.305
Genetic models								
Dominant	LS + SS vs. LL	-	-	-	0.58	0.20-1.62	1.08	0.216
Recessive	SS vs. LL + LS	-	-	-	0.81	0.57-1.15	1.35	0.141

*One-tailed test

5-HTTLPR: 5-hydroxytryptamine transporter-linked promoter region; HWE: Hardy-Weinberg Equilibrium; OR: Odds ratio; CI: Confidence interval; L: Long allele; S: Short allele; χ^2: Chi-square

The genotyping of SERT gene polymorphism revealed an insignificant relationship with migraine subjects when compared with controls (P > 0.050). The minor 'S' allele showed no association with OR = 1.23 (95% CI: 0.90-1.66), heterozygote with OR = 1.18 (95% CI: 0.82-1.69), and homozygote with OR = 1.51 (95% CI: 0.52-4.35), respectively. On stratification of migraine based on the clinical subtypes into MO and MA, the data showed an insignificant association with the healthy controls which are explained in table 3. The minor 'S' allele showed no association with OR = 0.73 (95% CI: 0.52-1.04) and OR = 0.92 (95% CI: 0.62-1.38) among the MO and MA subjects, respectively. Further, the analysis of dominant and recessive genetic models exhibited no significant difference among migraine cases and controls for SERT gene polymorphism. The nucleotide sequences (SERT gene) were submitted in National Center for Biotechnology Information (NCBI)-GenBank with accession numbers: MH614174, MH616089, and MH634423.

Discussion

Migraine is a neurological disorder characterized by headaches and symptoms like nausea, photophobia, vomiting, phonophobia, and sensory disturbances. The majority of migraine cases are reported in women, which suggests the potential involvement of female hormones in the pathophysiology of migraine.[16] This disorder has a strong genetic component; the familial risks of migraine are documented by population-based family studies and first-degree relatives of the probands possess twofold increased risk compared with general population.[17] Among various candidate genes in migraine, in this study, we have concentrated on SERT gene. Serotonin is distributed in neuronal tissues and various non-neuronal tissues of the gastrointestinal, renal, and cardiovascular system. The abnormal changes in gene transcription and expression levels, posttranslational modification, and intra-cellular trafficking in the constituents of serotonergic system produce gene products which could lead to the functional and structural modifications in neural circuits and provoke migraine.[18]

Based on our knowledge, this is the first Indian study investigating the relationship between serotonergic systems with genetic risk of migraine in our study population. The genotyping results of SLC6A45-HTTLPR polymorphism revealed an insignificant relationship with migraine. When comparing our study results with previously published studies from various ethnic descents, our results were similar with Asian and Spanish populations,[19] and dissimilar with Hungarian[20] population. Previous studies have reported a link between the presence of 'S' allele in 5-HTTLPR promoter polymorphism and reduced 5-hydroxytryptamine (5-HT) reuptake, leading to longer serotonergic function. However, the presence of 'L' allele leads to shorter serotonergic function due to fast reuptake of 5-HT.[21] Stratification of migraine based on the clinical subtypes into MO and MA also resulted in insignificant relationship. Several studies from the early 2000s have reported the insignificant association of 5-HTTLPR polymorphism with migraine pathogenesis.

Table 3. Distribution of serotonin transporter **(**SERT) gene polymorphism in migraine sub-groups and controls

Frequencies	**Controls (n = 308) [n (%)]**	**Patients with MO (n = 172) [n (%)]**	**OR**	**95% CI**	**P***
Allele					
L	524 (85.06)	278 (80.81)	Reference		-
S	92 (14.93)	66 (19.18)	0.73	0.52-1.04	0.054
Genotype					
LL	222 (72.07)	113 (65.69)	Reference		-
LS	80 (25.97)	52 (30.23)	0.78	0.51-1.18	0.148
SS	6 (1.94)	7 (4.06)	0.55	0.17-1.75	0.235
Frequencies	**Controls (n = 308) [n (%)]**	**Patients with MA (n = 132) [n (%)]**	**OR**	**95% CI**	**P***
Allele					
L	524 (85.06)	222 (84.09)	Reference		-
S	92 (14.93)	42 (15.90)	0.92	0.62-1.38	0.391
Genotype					
LL	222 (72.07)	93 (70.45)	Reference		-
LS	80 (25.97)	36 (27.27)	0.93	0.58-1.47	0.423
SS	6 (1.94)	3 (2.27)	0.90	0.21-3.80	0.574

*One-tailed test

OR: Odds ratio; CI: Confidence interval; MA: Migraine with aura; MO: Migraine without aura; L: Long allele; S: Short allele

A study in 2002, on Japanese population, reported no significant differences in the genotype and allele frequencies of 5-HTTLPR between patients with migraine and control subjects. However, the researchers observed that those with 'S' allele experienced significantly more frequent attacks than those with 'L' allele.[22] Similarly, study on Korean population revealed no significant association between 5-HTTLPR polymorphism and migraine.[23] Likewise, a study on Italian population revealed no difference in the allelic distribution between affected patients and control groups.[24] In concordance with the previous studies, a study on German population found no evidence for association of the 5-HTTLPR polymorphism with migraine attack frequency based on multinomial logistic regression analysis.[25] Various studies have investigated the association of other 5-HTT variants along with the 5-HTTLPR polymorphism in migraine, and studies have reported that the 5-HTT variable number tandem repeat (VNTR) STin2.12 allele or 12/12 genotype had an increased risk for migraine in the general population (STin2.12 allele: OR: 1.34, 95% CI: 1.09-1.64, P = 0.006; 12/12 genotype: OR: 1.55, 95% CI: 1.17-2.05, P = 0.002). However, no significant association was found between migraine and 5-HTTLPR.[26]

In contrast to studies that reported insignificant association, some studies on European population have identified borderline association with migraine. A case-control study on Hungarian population reported a borderline association (P = 0.049, OR = 1.45, 95% CI) between 5-HTTLPR 'S' allele and migraine.[27] Likewise, another study on Hungarian migraineurs reported a positive association between 'S' allele carriers and MO.[20] An early 2000 study on Italian population revealed significant association of the 5- HTTLPR polymorphism with migraine. In S/S carriers, the OR for MA risk was 2.60 (95% CI), and 2.14 (95% CI: 1.42-3.21) for MO risk.[9] In this regard, a systemic review and meta-analysis study on ten case-control studies revealed no overall association between the polymorphism and migraine for European and Asian populations. However, within the same study, it was observed that European women carrying the 'S' allele showed increased risk for migraine (dominant model: pooled OR = 2.02, 95% CI: 1.24-3.28). Furthermore, the study reported that the S/S genotype carried an increased risk of MA (recessive model: pooled OR: 1.41, 95% CI: 0.83-2.40).[28] The contrasting results observed in mentioned studies indicate the need for population and gender-based studies to understand the modifying effects of the polymorphism.

Although the specific polymorphism shows insignificant association to migraine, serotonin and other neurotransmitters such as glutamate, dopamine, and orexin appear to play an important role in the pathophysiology of migraine. Therefore, migraine susceptibility can be explained by the communication of several neurotransmitters, which are determined by the interaction of multiple genetic variations.[29] Our study has some limitations such as: i) stratification analysis based on gender and age was not performed, ii). Further analysis, including biochemical assays, may be required in the future to determine hormone levels. Therefore, future investigations will need to address the multifactorial aspects that lead to migraine.

Conclusion

The present case-control study reflects that SERT 5-HTTLPR polymorphism does not have an impact on developing migraine risk in South-Indian population. Due to the polygenic nature of this neurovascular disorder, the gene-gene, gene-nutrient, and gene-environmental interactions should be extensively identified. Further clinical studies combining the genetic, biochemical (hormonal), and electrophysiological characteristics are required to identify the migraine pathophysiology in our study population.

Conflict of Interests

The authors declare no conflict of interest in this study.

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