

No direct association of serotonin transporter (STin2 VNTR) and receptor (HT 102T>C) gene variants in genetic susceptibility to migraine

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Abstract. We aimed to find out if the serotonin receptor (HT102T>C) and serotonin transporter (STin 2) polymorphisms play any role in genetic susceptibility of migraine. For the study, 217 migraine patients and 217 healthy controls (HC) were recruited and genotyping was carried out using the Polymerase Chain Reaction and Restriction Fragment Length polymorphism (PCR-RFLP) method. All results were Bonferroni corrected. We could not find any significant differences in the genotype or allele frequencies in case of HT 102 T>C polymorphism between migraine patients and healthy controls (P value = 0.224). No significant association was seen at allele and carrier levels. Sub-grouping the patients on the basis of gender or on basis of migraine type i.e. with or without aura also did not show any association. Similarly, no difference in genotype (P value = 0.236), allele (P value = 0.550) or carrier frequency (P value = 0.771) in STin 2 VNTR polymorphism was observed between migraine patients. However, HT 102 TC genotype was observed to interact significantly with the STin 2.10/10 genotype in enhancing risk of migraine, both with and without aura. In conclusion, the HT102 T>C receptor and the STin 2 VNTR transporter polymorphisms, did not individually confer any significant risk of migraine or its clinical subtypes but the two polymorphisms appear to synergistically influence susceptibility to migraine.

Serotonin transporter (STin2 VNTR) and receptor (HT 102T>C) polymorphisms; Migraine with aura (MA); Migraine without aura (MO); Genetic susceptibility

1. Introduction

Several dysfunctional genes in the serotonergic system are probable candidates to mediate susceptibility to migraine. Serotonin releasing agents and certain serotonin receptor agonists are shown to induce migraine-like symptoms. Also, it has been observed that there are changes in circulating levels of serotonin and its metabolites during the phase of migraine attack [1].

Platelet capacity to take up 5-hydroxytryptamine (5-HT) is diminished, while the release of stored 5-HT is increased during migraine attacks [2]. Lower plasma 5-HT and relatively higher levels of 5-hydroxyindoleacetic acid, a metabolite of 5-HT degradation, were reported in migraineurs [3]. 5-HT receptor agonists especially ergotamine derivatives and to a lesser extent triptans exhibit a relatively high affinity for 5-HT_{1A} receptor [4]. Moreover, several anti-migraine drugs are 5-HT₂ receptor antagonists and the 5-HT₂ receptor agonists m-CPP (meta-chlorophenylpiperazine) has been shown to induce a migraine attack [1]. So, the role of genes involved in serotonergic pathway as potential

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candidates in migraine pathogenesis cannot be ruled out.

The activity of serotonergic system is under the control of the many genes, of which one of the 5-HT related genes, the 5-HT_{2A} receptor gene containing the HT-102T>C polymorphism (rs6313) has been mapped to chromosomal position 13q14.1-14.2 [5]. The 5-HT_{2A} receptor is a post-synaptic G protein-linked receptor that activates phosphoinositide hydrolysis [6]. Till date, two studies have been performed to find the role of HT 102T>C polymorphism in migraine, in which one study did not find any association [7], whereas another study reported an association of the polymorphism with migraine accompanied with aura (MA) [8].

The human serotonin transporter protein is encoded by a single gene (the solute carrier member 6, family 4, SLC6A4) located on chromosome 17q11.1-17q12 [9]. A 17-bp VNTR polymorphism (STin2) has been identified in second intron of the gene (rs57098334). The polymorphism consists of a variable number of tandem repeats (VNTR), with 3 alleles (STin2*12, STin2*10 and STin2*9) [10]. Although the biological function of the 17-bp VNTR polymorphism is still not elucidated, a number of studies have explored association of the VNTR in migraine. Only a Turkish study had associated STin 2.10 allele to increased risk in migraine [10], whereas three other studies have shown negative association [11–13].

Since there is inconsistency in the literature on the association of HT 102T>C and STin 2 with migraine, we performed the present case-control study to find out if the HT102 T>C receptor and STin2 17 bp VNTR transporter polymorphisms play any role in genetic susceptibility to migraine in the studied population.

2. Subjects and methods

Migraine patients attending the out-patient Neurology clinic of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow (India) were interviewed for the presence of phenotypic variations like differences in age of onset, frequency and severity of attacks, environmental triggers, medication response and the presence or absence of various aura symptoms. All affected individuals were diagnosed as having either migraine with aura (MA) or without aura (MO) by an experienced clinical neurologist, using the criteria of HCCIHs [14], but because of small sample size, we have not used the patient's sub-groups for our analysis. The inclusion and exclusion criteria for the studied sub-

jects were same as reported in our previous study [15, 16]. We excluded patients with uncontrolled hypertension (blood pressure >160/95), peripheral vascular, ischemic bowel disease, history, symptoms, risk for any cardiovascular disease or patients with thyroid disorders. Finally 217 migraine patients, 84 having Migraine with aura (MA), 133 of Migraine without aura (MO) were recruited for the study. Normal control group (HC), 217 in number, comprised age and sex-matched healthy and normotensive volunteers free of any routine headache, neurological or vascular diseases. The study was approved by Institutional Ethical Committee. The demographic profile of the subjects under study is shown in Table 1. The blood samples for genotyping were taken with the understanding and written consent of each subject, and our study conforms with The Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (18 July 1964).

2.1. Laboratory protocol

3–4 ml of venous blood was taken from the subjects in EDTA vials. The genomic DNA was extracted from peripheral blood leucocytes pellet using the standard salting out method [17]. The quantitation of DNA was done by Nanodrop spectrophotometer (Thermo Scientific).

2.2. Genetic analysis

Genotyping of the polymorphisms was performed on DNA samples by polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis (RFLP). Genotypes were performed blinded to disease status, as samples were taken randomly for genetic analysis.

2.2.1. Genotyping of HT 102T>C polymorphism (rs6313)

PCR was performed in accordance to the conditions described by Erdal et al. [8]. The 342 bp PCR product was digested with 10 units of *MspI* (Fermentas, Maryland, USA), using enzyme buffer supplied by the manufacturer. After digestion, the 215 and 126 bp products were electrophoresed on a 15% Polyacrylamide Gel at 90 V for 60 minutes.

Table 1
Clinical characteristics and the demographic profile of the Subjects under study

Subjects	Age of onset (Years)		Frequency per day (Hours)		Frequency per month		Intensity		Unilaterality		Pulsatility		Photophobia		Phonophobia		Nausea		Vomiting	
	8-30	> 30	< 10	> 10	Episodic-5	6-daily	Mild-	Severe	Unilateral	Bilateral	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Migraine (Total)	63.1	32.3	54.8	41.5	57.1	37.8	11.5	86.6	41	55.8	91.7	4.6	59	37.8	90.8	6.5	69.1	28.1	49.8	48.4
Missing (%)	4.6		3.7		5.1		1.8		3.2		3.7		3.2		2.8		2.8		1.8	
MA	57.1	39.3	57.1	39.3	60.7	35.7	84.5	14.3	60.7	36.9	6.0	91.7	53.6	44.0	88.1	9.5	65.5	32.1	48.8	50.0
Missing (%)	3.6		3.6		3.6		1.2		2.4		2.4		2.4		2.4		2.4		1.2	
MO	66.9	27.8	53.8	42.4	54.9	39.1	9.8	88.0	52.6	43.6	3.8	91.7	62.4	33.8	92.5	4.5	71.4	25.6	50.4	47.4
Missing (%)	5.3		3.8		6.0		2.3		3.8		4.5		3.8		3.0		3.0		2.3	

MA, Migraine with aura; MO, Migraine without aura; TTH, Tension Type Headache; HC, Healthy controls.

Table 2
HT 102 T>C and STin 2 polymorphism genotype frequency distribution in studied subjects

HT-102 T>C						
Subjects	Genotype distribution N(%)			Allele distribution N#(%)		
	N	TT	CT	CC	T	C
Total						
Migraine	217	69 (31.8)	117 (53.9)	31 (14.3)	255(58.8)	179(41.2)
MA	84	30 (35.7)	41 (48.8)	13 (15.5)	101 (59.8)	68 (40.2)
MO	133	39 (29.3)	76 (57.1)	18 (13.5)	154 (57.9)	112 (42.1)
HC	217	81 (37.3)	110 (50.7)	26 (12.0)	272 (62.7)	162 (37.3)

HT-VNTR						
Subjects	Genotype distribution N(%)			Allele distribution N#(%)		
	N	ST12.12	ST12.10	ST10.10	ST12.12	ST10.10
Total						
Migraine	217	110 (50.7)	72 (33.2)	35 (16.1)	292 (67.3)	142 (32.7)
MA	84	40 (47.6)	30 (35.7)	14 (16.7)	110(65.5)	58(34.5)
MO	133	70 (52.6)	42 (31.6)	21 (15.8)	182(68.4)	84(31.6)
HC	217	109 (50.2)	84 (38.7)	24 (11.1)	302(69.6)	132(30.4)

N, Total number of subjects; Healthy Controls; T, Wild allele of HT-102 T>C; C, Variant allele of HT-102 T>C polymorphism; STin 2.12, Wild allele of HT VNTR; STin 2.10, Variant allele of HT-VNTR polymorphism.

2.2.2. Genotyping of STin 2, 17 bp VNTR polymorphism (rs57098334)

PCR amplification was done using the conditions described by Yilmaza M et al. [10] and the amplified products were run on 10% polyacrylamide gel. Alleles containing 10 and 12 repeats could be identified.

2.3. Statistical analysis

All other analyses were done using SPSS version 15 (SPSS, Chicago, IL, USA). The entire analysis was age and sex adjusted. χ^2 test was applied for the analysis of genotypic and allelic distributions. Logistic regression analysis was used to find out contribution of genetic polymorphisms to the risk of disease. Two tailed tests of significance were used throughout. The relative risk for different genotypes was estimated calculating the odds ratio (OR) and 95% confidence interval (CI). P values of < 0.05 were considered statistically significant. Additional statistic analyses using Bonferroni correction were applied to adjust for multiple comparisons. Since we have performed 4 tests in a sample per polymorphism (2 sub groups like MA and MO and 2 SNPs), a Bonferroni correction was applied for multiple comparisons with all novel associations, with a correction factor derived from the no of tests applied (P value after Bonferroni correction is depicted as Pcorr), to avoid chances of type 1 error. The coefficient of Bonferroni correction (Pcorr) in our analysis in case of recessive, dominant and genotype

model as well as at allelic level was taken as 4 for migraine patients throughout the analysis i.e. the corrected P values for comparison was taken as Pcorr for migraine patients = $0.05/4 = 0.0125$. Sample size and power was calculated using software QUANTO Version 1.0 (<http://hydra.usc.edu/gxe>), taking the design to be matched case-control and the hypothesis to be gene-only for each genetic marker. The significance level of our study was set at 0.0125 in a two-sided test after Bonferroni correction. In the inheritance model, we chose the log-additive one, which is the most suitable model in polygenic diseases. Migraine disease prevalence of 10% was assumed [18].

Setting the threshold for significance at 0.0125, a relative risk of 1.5, and assuming a minor allele frequency of 37.3% (in case of HT 102T>C), and 30.4% in case of STin 2, our study has power greater than 82% in case of HT 102T>C polymorphism and 85% in case of STin 2 polymorphism. Goodness of fit χ^2 -test was used to check if genotypes were in Hardy-Weinberg equilibrium. Gene-gene interaction for the polymorphisms was also performed using Logistic Regression analysis to examine the possibility of combined effects of the three polymorphisms in migraine susceptibility. Association was expressed as odds ratios (OR) or as risk estimates with 95% confidence intervals (CI). Statistical analysis was performed using SPSS software version 15.0 (SPSS, Chicago, IL, USA).

Table 3
Comparison of P value and Odds ratio associated with variant genotype and allele frequencies in studied subjects

Subjects	Genotype model (CC genotype)		Dominant model (CC+TC Vs CC)		Allele level (C allele)	
	P Value	OR(95% CI)	P Value	OR(95% CI)	P Value	OR(95% CI)
Migraine Vs HC	0.244	1.454 (0.755–2.729)	0.254	1.267 (0.844–1.902)	0.230	1.186 (0.898–1.567)
MA Vs HC	0.473	1.344 (0.600–3.011)	0.925	1.026 (0.599–1.760)	0.570	1.114 (0.767–1.620)
MO Vs HC	0.278	1.495 (0.723–3.091)	0.131	1.440 (0.897–2.310)	0.197	1.231 (0.898–1.689)

N, Total number of subjects; N[#], Total chromosome number; HC, Healthy Controls; T, Wild allele of HT-102 T>C; C, Variant allele of HT-102 T>C polymorphism; STin 2.12, Wild allele of HT VNTR; STin 2.10, Variant allele of HT-VNTR polymorphism.

3. Results

The genotype and allele frequencies of the studied subjects in HT 102 T>C polymorphism and STin2 polymorphisms are shown in Table 2. Both polymorphisms were in Hardy-Weinberg equilibrium in healthy controls (p value in case of HT102T>C polymorphism = 0.469, and STin2 = 0.452).

3.1. HT 102 T>C polymorphism

After applying Bonferroni correction, we did not find any significant differences in the frequencies of genotypes in case of HT 102 T>C polymorphism when migraine patients were compared with healthy controls (P value = 0.224). Similarly insignificant differences were observed at allele level (Migraine Vs HC, P value = 0.230). Also, no significant association was noticed in dominant model (Migraine Vs HC, P value = 0.254) (Table 3). In addition, no significant association was observed even after sub-grouping the migraine patients into MA and MO at genotype level, allele level or even in case of dominant model (Table 3). We also looked for association of genotypes with various migraine-related clinical variables like pain intensity, migraine frequency per month, pulsatility, unilaterality and photophobia but we could not find any significant association of the genotypes with these clinical variables (Table 1) as compared to HC (Data not shown).

3.2. STin2 17 bp VNTR polymorphism

Using STin2.12 as reference, no difference in genotype (P value = 0.236), allele frequencies (P value = 0.550) or STin2.10 allele carrier frequency (P value = 0.771) was observed between migraine patients and HC (Table 3). On sub-grouping total migraine patients on the basis of presence or absence of aura, again we did not find any difference in genotype (For MA, P value = 0.216; for MO, P value = 0.418), allele frequencies (For MA, P value = 0.335; for MO, P value = 0.891)

Table 4
HT102T>C* STin 2 gene-gene interaction analysis

Subjects	P value (OR; 95% CI)
	HT102T>C ¹ * STin 2 ² (Interaction)
Migraine Vs HC	0.005 (3.249; 1.416–7.457)
MA Vs HC	0.030 (3.057; 1.114–8.387)
MO Vs HC	0.008 (3.356; 1.375–8.193)

HT102T>C¹, CT genotype and * STin 2¹, STin 12/10 genotype; HT102T>C², CC genotype; STin 2², STin 2 10/10 genotype; OR- Odds ratio; CI-95% Confidence Interval.

or ST10 allele carrier frequency (For MA, P value = 0.772; for MO, P value = 0.516). Also, no significant association was noticed in dominant model (Migraine Vs HC, P value = 0.771, OR = 0.944, CI = 0.643–1.387) (Table 3). We could not find any significant association of the genotypes with these clinical variables as compared to HC (Data not shown).

3.3. Gene gene interaction analysis

Although we could not find any association of HT 102T>C and STin2 polymorphisms in migraine individuals, the HT 102 TC genotype was found to interact significantly to confer risk with the STin 2.10/10 genotype when migraine patients were compared to HC (P value = 0.005; OR = 3.249; CI = 1.416–7.457) (Table 4). The significant risk was observed both in migraine patients with (P value = 0.030; OR = 3.057; CI = 1.114–8.387) or without aura (P value = 0.008; OR = 3.356; CI = 1.375–8.193) (Table 4).

4. Discussion

We could not find any association of individual HT 102T>C or STin2 polymorphism in migraine, while interaction of the two polymorphisms showed significant risk. The HC group in both polymorphisms followed Hardy-Weinberg equilibrium in controls.

The variant C allele frequency of HT 102T>C polymorphism in HC (37.3%) matches with other stud-

ies [7]. One Indian study reported the HT102 C allele frequency to be 44%, although the number of controls in their study was larger [19].

In agreement to our study, another study has reported that 102 T>C polymorphism of the 5-HT_{2A} receptor does not play a significant role in migraine [7]. However, Erdal et al. from Turkey showed significant involvement of this polymorphism in determining migraine with aura [8]. It may be pointed out that the sample size in the Turkish study was very low and the variant allele frequency of their control population also differs from our results. However, we can not negate the ethnic variation between the populations to account for the difference.

The serotonin transporter intron-2 (STin 2) allele frequency (30.4%) in our control subjects matches with one Indian study from Kolkata [20]. Here also, we could not find any association of STin 2 VNTR polymorphism with migraine. Similarly, no association of STin 2 polymorphism with migraine was observed in the Austrian population [21]. However, the presence of STin 2.10 allele was shown to increase the risk of migraine in a Turkish population [10]. Two other studies on migraine, one from Hungary [11], and the other from Danish population [13] showed high prevalence of STin 2.12/12 genotype in migraineurs than in controls. Another study reported a trend toward significant effect of the 12-repeat allele as a risk factor for MA in a cohort of 44 Italian patients [12]. This controversy may have resulted from the coexistence of different psychosomatic factors in the migraineurs who live in different geographic regions, which may have an impact on the study results [13].

In the present study, the HT 102T>C and STin2 polymorphisms were not found to be individually associated with migraine susceptibility but HT 102 TC genotype was observed to interact significantly with the STin 2.10/10 genotype in enhancing risk of migraine in females. The reason for such synergistic interaction is not understood. However, in literature, there are several examples of similar interactions. Significant gene-gene interactions of serotonin system were observed between serotonin 2C receptor (5-HT_{2C}) and tryptophan hydroxylase 2 (TPH2), and among 5-HT_{2C}, serotonin transporter (5-HTT), monoamine oxidase A (MAOA) and TPH2 in conferring susceptibility to borderline personality disorders [22]. These studies suggest possible synergistic effect of genetic factors in influencing serotonergic neurotransmission on susceptibility to certain neurological disorders. But, due to our small sample size, we are unable to draw a strong conclusion about the synergistic role of the polymorphisms.

The TC genotype and C allele of 5-HT_{2A} receptor gene has been implicated in lower expression of receptors which are important not only for neurotransmission mechanisms but are also linked to cell signalling [23]. The functional significance of serotonin transporter intron-2 VNTR polymorphism (STin2) is not well known [10]. The VNTR is believed to act as a transcriptional regulator and has allele-dependent differential enhancer-like properties. STin 2.10 and STin 2.12- were found to differ in the strength of their transcriptional inducing abilities, where STin 2.12/12 seems to have stronger enhancer-like properties than the STin 2.10/10 and STin 2.10/12 forms [24]. Furthermore, the low-expressing 10-repeat allele was reported to act dominantly [25]. Therefore, the polymorphisms of the 5-HTT gene seem to be functional in modulating 5-HTT gene expression [25,26]. Also, platelets from individuals homozygous for the 12-repeat allele appear to have a lower affinity for serotonin uptake in comparison to those heterozygous for the 10-repeat/9-repeat allele [27]. Thus, based on ongoing discussion, lower levels of serotonin transporter in STin 2.10/10 along with HT 102 TC genotype may result in significantly higher risk of migraine.

Till date, various studies conducted on serotonin transporter and receptor polymorphisms have shown controversial results, suggesting that the effect of the disease causing allele of the polymorphisms would be smaller due to influences of other genes and environmental factors. Moreover, results of twin studies suggest approximately 50% heritability of migraine with multifactorial [28] polygenic inheritance, so the influence of other genes cannot be neglected.

In conclusion, although we did not find any significance of the HT receptor (HT 102T>C) and transporter (STin2 VNTR) gene polymorphisms in migraine, but their interaction may confer significant risk which stresses on the need to further explore this aspect in different cohorts with larger sample size.

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