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ORIGINAL ARTICLE

rs2651899 variant is associated with risk for migraine without aura from North Indian population

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Abstract

Recently a GWAS study had identified 38 genomic variants commonly found in humans that influence migraine risk. For further replicate these findings, we selected two SNPs; rs2651899 on chromosome 1p36.32 in *PRDM16* gene and rs10166942 on chromosome 2q37.1 close to *TRPM8* gene for their associations with migraine in the North Indian population as much work has not been done on these variants before from this population. In this case–control association study, 300 unrelated subjects, including 150 migraineurs (43 migraine with aura and 107 migraine without aura) and 150 healthy controls were selected to collect genomic DNA. Polymerase chain reaction and restriction-fragment-length polymorphism methods were performed for genotyping of these variants. Univariate and multivariate analyses were done to find the association of different genotypes and alleles of these SNPs with migraine and its subgroups. We found a statistically significant difference in migraineurs with control for *PRDM16* rs2651899 polymorphism at genotypic ($p < 0.05$), allelic ($p = 0.022$; OR 1.462; 95% CI 1.058–2.022) and for dominant model ($p = 0.011$; OR 1.957; 95% CI 1.169–3.276). A similar trend was observed both on subgroup and gender analysis in migraine without aura (MO) and females respectively for rs2651899 variant. For the other SNP (rs10166942), statistically non-significant differences were reported in the allelic/genotypic frequencies between migraineurs and controls as $p > 0.05$. However, on subgroup analysis we found statistically significant differences at genotypic ($p < 0.05$) and dominant models in migraine with aura (MA) and in males with that of entire controls. But no significant association was found at allelic level in both subgroup and gender analysis for rs10166942. This research study showed that rs2651899 is a potential genetic marker for migraine susceptibility in MO and female subgroup at both genotypic and allelic level in the North Indian population and found that rs10166942 variant may be a potential marker for MA and male subgroup. Further work with large sample size is required for these SNPs to understand their functional mechanisms and to strengthen our results.

Keywords Migraine · Single nucleotide polymorphism · *PRDM16* · *TRPM8*

Introduction

Migraine is one of the most commonly widespread headache disorders which affects almost 15% of adult population worldwide and is more frequent in women [1]. Migraine is divided into two main sub groups clinically based on the presence or absence of an aura: migraine with aura (MA) and migraine without aura (MO) and nearly one-third of migraine patients experience an aura prior or during headache attacks [2]. Migraine is often accompanied by various symptoms such as unilateral headache, nausea, vomiting, photophobia and phonophobia [3]. Migraine not only affects the quality of life of a patient but can also cause big financial costs to the society due to reduced productivity and decrease in working hours. [4] Linkage analysis and genome-wide

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association studies have revealed many susceptible single nucleotide polymorphisms (SNPs) contributing to migraine [5–7].

Various genes responsible for migraine have been identified so far, but with a little proof that these identified genes can have a major role in common form of migraine. Mixed positive and negative types of results were often found in candidate gene association studies and were further failed to replicate [8]. Genome-wide association study (GWAS) of migraine revealed three SNPs in or near *LRP1, TRPM8* and *PRDM16* genes [9]. Rs2651899 is a SNP on chromosome 1p36.32 in the first intron of gene *PRDM16* (PR domain containing 16). This gene codes for a zinc finger transcription factor that controls the differentiation of brown adipose tissue along with repression of transforming growth factor beta (*TGF-β*) signalling [10, 11] but the role of which is still unclear in migraine. However, this gene has recently been shown a relationship with non-syndromic left ventricular non-compaction cardiomyopathy and dilated cardiomyopathy, a cardiac disease [12]. *PRDM16* gene can be used as a candidate marker in future studies by knowing the comorbidity between cardiovascular diseases and migraine, More case control studies with reasonable number of individuals from different geographical regions are needed to explore the relationship of this SNP with migraine [13].

However, rs10166942 is a SNP on chromosome 2q37 and located close to gene *TRPM8* (Transient receptor potential cation channel of subfamily M, member 8) at the transcription start site. *TRPM8* gene codes for a protein that acts as a sensor for cold and cold-induced burning pain and is reported to be primarily expressed in brain particularly in sensory neurons and the dorsal root ganglion. It has been investigated that *TRPM8* may act as a target in various models of animals with neuropathic pain [14, 15]. As both migraine and neuropathic pain have certain similar features [16], a study on the role of *TRPM8* gene in migraine and a link between both these pain syndromes can be an interesting topic for research scientists. Finding of these SNPs in various global populations is very important to build an association among them.

In this research study, we investigated two SNPs i.e. rs2651899 and rs10166942 for their association between migraine patients and healthy controls from North Indian population.

Materials and methods

Patients' enrolment

We selected 150 unrelated migraine subjects (43 with MA and 107 with MO) from ESIC Medical College & Hospital, Faridabad. Diagnosis of migraine was confirmed after the results of strict neurological examination following the guidelines of the International Classification of Headache disorders, 3rd edition [2]. A complete demographic and medical history questionnaire was obtained from all subjects. The migraine patients with age < 50 years and having at least one attack per month for last 3 months were included. Subjects having neurological disorders, comorbid disorders and non-migrainous headaches were excluded from this study. Further secondary cases of migraine with post head injury were also excluded from this study. A total of 150 healthy controls (HC) from volunteers, matched for age with patients, were also selected from same geographical areas for this study. We obtained informed consent in written from all subjects and further this study was approved by Ethics Committees from JMI, New Delhi and from that of ESIC Medical College & Hospital, Faridabad.

Genomic DNA extraction

After getting prior consent from all subjects, 5 ml of venous blood was taken in EDTA vials and stored at − 20 °C till further use. From these EDTA-treated whole-blood samples genomic DNA was extracted by salting out method [17] as well as with whole genomic DNA extraction kit (from Bangalore Genei). Quantification of extracted DNA was further done by Nanodrop spectrophotometer (from BioLab).

Genotyping

Genotyping of rs2651899 and rs10166942 by using primers (Table 1) from An et al. [18] was done by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. PCR amplification was done in a final volume of 25 µl containing 3 µl 10× PCR buffer containing 50 mM of $MgCl_2$, 1 µl of each 10 pmol forward and reverse primers, 1 µl 10 mM dNTPs, 0.5 µl DNA template and 1 µl of 3 U/µl Taq DNA polymerase. PCR was performed with conditions as an initial denaturation step of 5 min at 94 °C, then 40 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for rs2651899 and 53 °C for rs10166942 for 30 s respectively, and extension at 72 °C for 1 min 30 s followed by a final extension at 72 °C for 10 min in thermal cycler (from Eppendorf). Final PCR products were checked on gel

Table 1 Primer sequences used in this study for polymorphism

SNP	Primer	Primer sequence
rs2651899	Forward	5′-GCTTTGAGTTCTGCGGATG-3′
	Reverse	5′-CGATCTGGATTGCACCTG-3′
rs10166942	Forward	5′-CTACTTACTACCTAACACTTGGC-3′
	Reverse	5′-CTGAAAGGAAGGATAGGGTTG-3′

electrophoresis using 2% agarose with ethidium bromide staining.

Different restriction enzymes used in RFLP for rs2651899 and rs10166942 were *AvaI* and *MseI* (Fermentas, USA) respectively. The digestion mixture made for each PCR product was having a final volume of 20 µl with 2 µl restriction buffer, 0.1 µl restriction enzyme and 10 µl PCR product. This mixture was then incubated overnight at 37 °C with *AvaI* and at 65 °C with *MseI*. Digested PCR products were then checked on 3% agarose gel stained with ethidium bromide. rs2651899 PCR products of 273 bp were digested into 192 bp and 81 bp for T allele by *AvaI* enzyme and rs10166942 PCR products of 180 bp were cut into 107 bp and 73 bp fragments for T allele by *MseI* enzyme at 65 °C. The expected results obtained during PCR and by restriction digestion of these SNPs are given in additional file 1 showing Figs. 1–4.

Statistical analysis

Chi-squared test was used to calculate the genotypic and allelic frequencies of these SNPs and to find out their relationship with Hardy–Weinberg equilibrium. Numbers and percentages were chosen to represent the categorical variables. Whereas continuous variables were represented as means ± standard deviation (SD) and evaluated between subgroups by t-Test. SPSS 24.0 was used to do statistical analysis and value of $p < 0.05$ was considered statistically significant for this study. The association of both polymorphisms with migraine was checked by dominant models. For both rs2651899 and rs10166942 with minor alleles as T, dominant model can be CC versus CT+TT and recessive model is TT versus CT+CC. For mutant allele carriers the risk of migraine was taken as odds ratios (OR) and 95% confidence intervals (CI) between groups. Power of this study was calculated using Quanto version 1.2.4. The prevalence of 12% was assumed for migraine and significance level was set at 0.05. Assuming the minor allele frequency as 36.3% (rs10166942) and 39.3% (rs2651899) in control groups and taking the sample size 150. This study achieved 59.3% and 64.4% powers for rs10166942 and rs2651899 respectively.

Results

Characteristics of patients

The 300 subjects taken in this study comprised of 150 in migraine group and 150 in the control group. From 200 subjects included in this study (100 migraineurs and 100 controls), we have already reported association of *MTHFR* gene polymorphism [19]. Mean age of the 150 migraine patients, having 110 females and 40 males was 35.28 ± 6.6 while that of 150 controls including 60 males and 90 females was 34.45 ± 7.6 years. In 150 migraine patients, 28.6% were diagnosed with MA and 71.3% had MO. We could not find any statistically difference in terms of age as $p = 0.35$ on comparing migraine patients and healthy controls. But showed a statistically significant difference in sex ($p = 0.014$) because most of the cases were female (migraine and control groups having 73 and 60% females, respectively). The information regarding demographic and clinical features of the migraineurs was recorded in Table 2.

Genome profiling of *PRDM16* rs2651899 polymorphism

The genotypes of rs2651899 in migraine patients ($p = 0.014$) did not follow Hardy–Weinberg equilibrium (HWE) but followed for controls ($p = 0.273$) as checked by chi-squared

Table 2 Demographic and clinical features of migraineurs

Demographic features	Cases (N = 150)	%
Sex		
Male	40	26.7
Female	110	73.3
Age (years)	35.28 ± 6.6	
Migraine subgroup		
MA	43	28.7
MO	107	71.3
Unilateral pain		
Yes	105	70.0
No	45	30.0
Pulsating pain		
Yes	90	60.0
No	60	40.0
Nausea		
Yes	118	78.7
No	32	21.3
Vomiting		
Yes	115	76.7
No	35	23.3
Photophobia		
Yes	120	80.0
No	30	20.0
Phonophobia		
Yes	125	83.3
No	25	16.7
Aggrevation by physical activity		
Yes	136	90.7
No	14	9.3
Family history		
Yes	23	15.3
No	127	84.7

goodness of fit test. We found a statistically significant difference in migraineurs with control for *PRDM16* rs2651899 gene polymorphism at genotypic ($p < 0.05$), allelic ($p = 0.022$; OR 1.462; 95% CI 1.058–2.022) and for dominant model ($p = 0.011$; OR 1.957; 95% CI 1.169–3.276). The percentage of frequencies of CT and TT genotypes is higher in migraine against control (60 vs. 52% and 18.7 vs. 13.3% respectively) and also the percentage of minor T allele frequency is more in migraine on comparing with control (48.7 vs. 39.3%). By logistic regression analysis, adjusted for age and sex, we found statistically significant difference at genotypic level as $p < 0.05$. This association indicated a significant risk factor of migraine for T allele in patients as OR 1.462 as shown in Table 3. On subgroup analysis, similar trend was observed in MO on comparing with control at genotypic ($p < 0.01$), allelic ($p = 0.007$; OR 1.631; 95% CI 1.145–2.325) and dominant model ($p = 0.001$; OR 2.809; 95% CI 1.514–5.211) as in Table 4. But no such association was found at any level in MA on comparing with controls as $p > 0.05$ as reported in Table 5. However, we found statistically significant difference at genotypic level ($p < 0.05$) on comparing MA with MO subgroup analysis in migraine patients as shown in Table 6. The frequency of CT genotype in MO (65.4%) is higher than that of MA (46.5%) or control (52%). Moreover, the results of gender analysis for this polymorphism showed a statistically significant difference at genotypic ($p < 0.05$), allelic ($p = 0.009$; OR 0.625; 95% CI 0.440–0.889) and dominant models ($p = 0.017$; OR 0.498; 95% CI 0.282–0.881) by comparing female migraine patients with entire control. The percentages of frequencies of CT and TT genotypes were 56.4 and 22.7% in females as compared with 52 and 13.3% in controls. However the frequency of T allele was 50.9% in females on comparison with 39.3% in controls as shown in Table 7. But no such significant association was found in male subgroup analysis with controls at any model for this SNP ($p > 0.05$).

Genome profiling of *TRPM8* rs10166942 polymorphism

The genotypes of rs10166942 in migraine patients ($p = 0.450$) followed HWE but controls ($p = 0.011$) were in Hardy–Weinberg disequilibrium (DHW) according to chi squared goodness of fit test. For both at allelic and dominant levels, no statistically significant difference was observed for this polymorphism in migraineurs with control as $p > 0.05$. However, the frequencies of CC and CT genotypes were 35.3 and 50.7% on comparing with 45.3 and 36.7% in controls

Table 3 Genotype and allele frequencies of two SNPs in migraine patients vs. controls

SNP	Model	Genotype/allele	Migraine N = 150 (%)	Control N = 150 (%)	p Value	Univariate analysis OR (95% CI)	Multivariate analysis[#] OR (95% CI)	p Value[#]
rs2658199[a]	Genotype	CC	32 (21.3)	52 (34.7)	0.030*			
		CT	90 (60)	78 (52)				
		TT	28 (18.7)	20 (13.3)				
	Dominant	CT vz. CC			0.021*	1.875 (1.099–3.200)	1.727 (1.003–2.974)	0.049*
		TT vz. CC			0.026*	2.275 (1.104–4.689)	2.164 (1.042–4.495)	0.039*
		TT+CT	118 (78.7)	98 (65.3)	0.011*	1.957 (1.169–3.276)		
		CC	32 (21.3)	52 (34.6)				
	Allele	T	146 (48.7)	118 (39.3)	0.022*	1.462 (1.058–2.022)		
		C	154 (51.3)	182 (60.7)				
rs10166942[b]	Genotype	CC	53 (35.3)	68 (45.3)	0.050			
		CT	76 (50.7)	55 (36.7)				
		TT	21 (14)	27 (18)				
		CT vz. CC			0.025*	1.773 (1.076–2.921)	2.000 (1.191–3.357)	0.009**
		TT vz. CC			0.995	0.998 (0.509–1.958)	1.076 (0.542–2.136)	0.834
	Dominant	TT+CT	97 (64.7)	82 (54.7)	0.078	1.518 (0.954–2.414)		
		CC	53 (35.3)	68 (45.3)				
	Allele	T	118 (39.3)	109 (36.3)	0.448	1.136 (0.817–1.581)		
		C	182 (60.7)	191 (63.7)				

Data presented as number (percentage), *OR* odds ratio, *CI* Confidence Interval

*$p < 0.05$, **$p < 0.01$, statistically significant, migraine patients compared with controls by genotypes and alleles

[#]Adjusted for age and sex

[a]Power = 0.654, [b]power = 0.582

Table 4 Comparison of SNP genotypes and alleles between MO and controls

SNP	Model	Genotype/allele	MO N = 107 (%)	Control N = 150 (%)	p Value	Univariate analysis OR (95% CI)	Multivariate analysis[#] OR (95% CI)	p Value[#]
rs2651899[a]	Genotype	CC	17 (15.9)	52 (34.6)	0.003**			
		CT	70 (65.4)	78 (52)				
		TT	20 (18.7)	20 (13.3)				
		CT vz. CC			0.002**	2.745 (1.454–5.183)	2.456 (1.285–4.694)	0.007*
		TT vz. CC			0.008**	3.059 (1.338–6.994)	2.938 (1.264–6.829)	0.012*
	Dominant	TT+CT	90 (84.1)	98 (65.3)	0.001**	2.809 (1.514–5.211)		
		CC	17 (15.9)	52 (34.6)				
	Allele	T	110 (51.4)	118 (39.3)	0.007**	1.631 (1.145–2.325)		
		C	104 (48.6)	182 (60.7)				
rs10166942[b]	Genotype	CC	45 (42)	68 (45.3)	0.772			
		CT	44 (41.1)	55 (36.7)				
		TT	18 (16.8)	27 (18)				
		CT vz. CC			0..497	1.209 (0.700–2.089)	1.349 (0.763–2.385)	0.303
		TT vz. CC			0.984	1.007 (0.498–2.040)	2.126 (0.917–4.931)	0.079
	Dominant	TT+CT	62 (57.9)	82 (54.7)	0.602	1.143 (0.693–1.885)		
		CC	45 (42)	68 (45.3)				
	Allele	T	80 (37.4)	109 (36.3)	0.808	1.046 (0.727–1.505)		
		C	134 (62.6)	191 (63.7)				

Data presented as number (percentage), *OR* odds ratio, *CI* Confidence Interval

*p < 0.05, **p < 0.01, statistically significant, MO compared with controls by genotypes and alleles

[#]Adjusted for age and sex

[a]Power = 0.870, [b]power = 0.091

which resulted in p = 0.009 in logistic regression analysis by adjusting for age and sex as covariates as in Table 3. The frequency of CT genotype in MA (74.4%) is higher than that of MO (41.1%) or control (36.7%) as shown in Tables 4 and 5. Hence on sub group analysis, we found a statistically significant difference in percentages of frequencies at genotypic (p < 0.001) and dominant model of MA (p = 0.002; OR 3.628; 95% CI 1.578–8.342) with that of control as in Table 5. But no such association was reported at allelic level for this SNP. We even found no such association at any level in MO subgroup of migraine on comparing with controls. However we found statistically significant differences at genotypic (p = 0.001) as well as at dominant models (p = 0.008) on comparing MA with MO for this SNP as in Table 6. On gender analysis, the percentages of frequencies of CC and CT genotypes in males were 25 and 70% on comparing with 45.3 and 36.7% in controls. Hence we found a statistically significant differences at genotypic (p < 0.05) and at dominant models in males (p = 0.023; OR 0.402; 95% CI 0.183–0.881) on comparing with that of entire control as in Table 7. No such association was found at allelic level in both subgroup and gender analysis as p > 0.05 for this SNP. We did not found any statistically significant difference in female subgroup on comparing with entire controls at any level for rs10166942 (p > 0.05). Reason behind variations in p values (mostly < 0.001) for this SNP could be the small sample size as well as that this SNP was in DHW in control group.

Discussion

There is a strong need of replication of different variants associated with migraine in different populations and ethnic groups so as to provide a framework for unravelling the genetic cause of this complex disorder. Therefore in this study we conducted a replication study for rs2651899 and rs10166942 polymorphisms associated with .migraine GWAS.

In this study, we found that the *PRDM16* rs2651899 SNP may be a potential genetic risk factor for migraine patients. Also in the subgroup and gender analysis, a significant correlation was observed in MO and females on comparing with control. Our findings were similar to that of Chasman et al. [9] that also showed a strong association of rs2651899 polymorphism with migraine. However none of the SNPs specifically associated with migraine subtypes and features in their study which was contradicted with our results as we found

Table 5 Comparison of SNP genotypes and alleles between MA and Controls

SNP	Model	Genotype/allele	MA 43 (%)	Control 150 (%)	p Value	Univariate analysis OR (95% CI)	Multivariate analysis[#] OR (95% CI)	p Value[#]
rs2651899[a]	Genotype	CC	15 (34.9)	52 (34.6)	0.661			
		CT	20 (46.5)	78 (52)				
		TT	8 (18.6)	20 (13.3)				
		CT vz. CC			0.760	0.889 (0.417–1.893)	0.808 (0.373–1.750)	0.589
		TT vz. CC			0.522	1.387 (0.510–3.774)	1.354 (0.493–3.718)	0.556
	Dominant	TT+CT	28 (65.1)	98 (65.3)	0.979	0.991 (0.486–2.018)		
		CC	15 (34.9)	52 (34.6)				
	Allele	T	36 (41.9)	118 (39.3)	0.673	1.111 (0.682–1.807)		
		C	50 (58.1)	182 (60.7)				
rs10166942[b]	Genotype	CC	8 (18.6)	68 (45.3)	< 0.001**			
		CT	32 (74.4)	55 (36.7)				
		TT	3 (7)	27 (18)				
		CT vz. CC			< 0.00i**	4.945 (2.109–11.598)	4.798 (2.012–11.445)	< 0.001*
		TT vz. CC			0.936	0.944 (0.233–3.829)	0.897 (0.215–0.736)	0.881
	Dominant	TT+CT	35 (81.4)	82 (54.7)	0.002**	3.628 (1.578–8.342)		
		CC	8 (18.6)	68 (45.3)				
	Allele	T	38 (44.2)	109 (36.3)	0.187	1.387 (0.853–2.256)		
		C	48 (55.8)	191 (63.7)				

Data presented as number (percentage), *OR* odds ratio, *CI* Confidence Interval

*$p < 0.05$, **$p < 0.01$, statistically significant, MA compared with controls by genotypes and alleles

[#]Adjusted for age and sex

[a]Power = 0.117, [b]Power = 0.988

statistically significant differences at genotypic and allelic levels in MO and females. But they reported that the three variants: rs2651899 (*PRDM16*), rs10166942 (*TRMP8*), and rs11172113 (*LRP1*) showed the maximum associations with migraine having $p < 5 \times 10^{-6}$ but none of the three variants specifically associated with migraine subtypes. In this subgroup analysis, we found no correlation with MA, the reason behind could be the small number of MA patients (only 43) as compared with MO (n = 107) and control (n = 150). As females were accounted for a big proportion of this study (> 72%), on comparing females with control, we found the similar results but for the male subgroup this correlation did not exist. Further studies with large number of MA and male patients are needed to validate these results to find an association of genetic variations of this polymorphism.

An et al. [18] reported a potential role of rs2658199 variant in Chinese MO migraine susceptibility and found a significant difference in allelic distribution of this SNP between migraine without aura (MO) and control (p = 0.049). These findings were similar to our study as we also found a statistical significant difference of frequencies of allelic distribution in rs2658199 between MO and control (p = 0.007). But they reported that gender did not play any role which was in contrast with our results as we found statistically significant difference in frequencies both at genotypic as well as allelic level in females on sub group analysis. Reason behind this may be the small sample size in our study which further needs to be verified with large population. Similar results were reported by Fan et al. [20] that revealed the association of migraine with the minor allele of rs2651899 (p = 0.005, OR 1.382, 95% CI 1.100–1.736) and confirmed the role of *PRDM16* to migraine susceptibility in Chinese Han population. Ran et al. [21] also confirmed similar results with our study and revealed that the minor allele T of rs2651899 was more common in cases than controls and this association gave an odds ratio of 0.68 with 95% CI of 0.79–0.94 indicating that the minor allele T causes a potential risk of migraine. However, Ghosh et al. also reported a protective effect of *PRDM16* rs2651899 SNP on migraine and MO susceptibility at both genotypic and allelic levels (p = 0.041; OR 0.758; 95% CI 0.591–0.972) which strengthened our results [22].

For variant rs10166942, we found no association with migraine on comparing with control at genotypic and allelic levels in this study (p > 0.05) although in subgroup analysis we found a statistically significant difference at genotypic level in MA and male subgroup as p < 0.05. But no such association was found at allelic level in migraine or its

Table 6 Comparison of SNP genotypes and alleles between MA and MO subgroups of Migraine patients

SNP	Model	Genotype/allele	MA 43 (%)	MO 107 (%)	p Value	Univariate analysis OR (95% CI)	Multivariate analysis[#] OR (95% CI)	p Value[#]
rs2651899[a]	Genotype	CC	15 (34.9)	17 (15.9)	0.030*			
		CT	20 (46.5)	70 (65.4)				
		TT	8 (18.6)	20 (18.7)				
		CT vz. CC			0.010*	3.088 (1.315–7.253)	3.316 (1.387–7.930)	0.007*
		TT vz. CC			0.149	2.206 (0.753–6.459)	2.098 (0.704–6.246)	
	Dominant	TT+CT	28 (65.1)	90 (84.1)	0.012*	0.353 (0.156–0.796)		0.183
		CC	15 (34.9)	17 (15.9)				
	Allele	T	36 (41.9)	110 (51.4)	0.136	0.681 (0.411–1.128)		
		C	50 (58.1)	104 (48.6)				
rs10166942[b]	Genotype	CC	8 (18.6)	45 (42)	0.001**			
		CT	32 (74.4)	44 (41.1)				
		TT	3 (7)	18 (16.8)				
		CT vz. CC			0.002**	4.091 (1.698–9.854)	2.180 (0.821–5.789)	0.118
		TT vz. CC			0.930	0.938 (0.223–3.937)	0.018 (0.001–0.288)	0.004**
	Dominant	TT+CT	35 (81.4)	62 (57.9)	0.008**	3.175 (1.346–7.494)		
		CC	8 (18.6)	45 (42)				
	Allele	T	38 (44.2)	80 (37.4)	0.276	1.326 (0.798–2.203)		
		C	48 (55.8)	134 (62.6)				

Data presented as number (percentage), *OR* odds ratio, *CI* Confidence Interval

*p < 0.05, **p < 0.01, statistically significant, MA compared with MO by genotypes and alleles

[#]Adjusted for age and sex

[a]Power = 0.660, [b]power = 0.934

subgroups on comparison with entire control. As reported earlier that this SNP was out of HWE in control group, findings in this study needs to be checked further with larger sample size. SNP rs10166942 was replicated in two different studies by Fan et al. [19] and An et al. [18] and they also reported statistically non-significant differences in allelic or genotypic frequencies between migraine patients and controls and further supported our study. Our findings were contradictory with Ghosh et al. who revealed that *TRPM8* rs10166942 SNP did not show any significant effect on migraine susceptibility in the North Indian population [21]. But in our study we found statistically significant results for MA and male subgroups of migraine. Similar results with our study were shown by Esserlind et al. [23] that reported a significant association of SNP rs10166942 near *TRPM8* and rs11172113 in *LRP1* gene with migraine. Sintas et al. [24] also reported a nominal association of the variants rs2651899 (allelic model, P = 0.0056) and rs10166942 (recessive, P = 0.0442) with migraine with aura (MA) and reported minor alleles C and T in rs2651899 and rs10166942 respectively as associated risk alleles.

These three genome-wide significant associated SNPs (rs2651899, rs10166942 and rs11172113) were previously confirmed by meta-analysis to confer the risk of migraine. Reason for this discrepancy from the original GWAS from our study may be that actual risk variants may differ in North Indian population and further different environmental conditions may affect the genes to different extent and hence modulating the extent of association as this disorder is polygenic and multi-factorial in nature. In this study, we did not check the relationships of these SNPS with migraine-associated characteristics. Further the small number of migraine patients also limits the strength of our findings. An even larger scale case–control study in North Indian population is needed to confirm the association of these SNPs to migraine susceptibility.

Conclusion

We replicated the association of two variants i.e. rs2658199 and rs10166942 form migraineurs including both migraine with aura and without aura form North Indian population and found that variant rs2658199 is significantly associated with migraine without aura and with female subgroup. We also reported that rs10166942 variant may be a potential marker for MA and male subgroup. These findings

Table 7 Comparison of SNP genotypes and alleles between gender subgroups of Migraine and Controls

SNP	Genotypic/ allelic models	Control 150 (%)	Female migraine 110 (%)	p Value	Univariate analysis OR (95% CI)	Male migraine 40 (%)	p Value	Multivariate analysis OR (95% CI)
rs2651899[a]	CC	52 (34.6)	23 (20.9)	0.022*		9 (22.5)	0.125	
	CT	78 (52)	62 (56.4)			28 (70)		
	TT	20 (13.3)	25 (22.7)			3 (7.5)		
	CT vz. CC			0.053	1.797 (0.993–3.253)		0.085	2.074 (0.905–4.751)
				0.025*[#]	2.013 (1.092–3.779) [#]		0.842	0.867 (0.213–3.531)
	TT vz. CC			0.008**	2.826 (1.314–6.079)		0.025[#]	0.152 (0.029–0.788)[#]
				< 0.001[#]	6.931 (2.606–18.436) [#]		0.950	1.037 (0.329–3.274)[#]
	TT+CT	98 (65.3)	87 (79.1)	0.017*	0.498 (0.282–0.881)	31 (77.5)	0.147	0.547 (0.242–1.236)
	CC	52 (34.6)	23 (20.9)			9 (22.5)		
	T	118 (39.3)	112 (50.9)	0.009*	0.625 (0.440–0.889)	34 (42.5)	0.608	0.877 (0.532–1.447)
	C	182 (60.7)	108 (49.1)			46 (57.5)		
rs10166942[b]	CC	68 (45.3)	43 (39.1)	0.506		10 (25)	0.001**	
	CT	55 (36.7)	48 (43.6)			28 (70)		
	TT	27 (18)	19 (17.3)			2 (5)		
	CT vz. CC			0.246	1.380 (0.801–2.377)		0.002**	3.462 (0.104–2.451)
				0.069[#]	1.772 (0.957–3.280)[#]		0.097[#]	2.208 (0.868–5.617)[#]
	TT vz. CC			0.765	1.113 (0.553–2.241)		0.396	0.504 (0.104–2.451)
				0.177[#]	1.773 (0.772–4.074)[#]		0.103[#]	0.249 (0.047–1.323)[#]
	TT+CT	82 (54.7)	67 (60.9)	0.315	0.774 (0.469–1.276)	30 (75)	0.023*	0.402 (0.183–0.881)
	CC	68 (45.3)	43 (39.1)			10 (25)		
	T	109 (36.3)	86 (39)	0.521	0.889 (0.621–1.273)	32 (40)	0.547	0.856 (0.516–1.419)
	C	191 (63.7)	134 (61)			48 (60)		

Data presented as number (percentage), *OR* odds ratio, *CI* Confidence Interval

*$p < 0.05$, statistically significant, female migraine patients compared with controls by genotypes and alleles

**$p < 0.05$, statistically significant, male migraine patients compared with controls by genotypes and alleles

[a]Power = 0.428 for males and 0.698 for females

[b]Power = 0.165 for females and 0.950 for males

[#]Multivariate analysis; [#]Adjusted for age and sex

strengthen the previous association of these variants with migraine pathology and provide new targets for migraine mechanisms and drug therapies.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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