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Original article

PRDM16, LRP1 and TRPM8 genetic polymorphisms are risk factor for Pakistani migraine patients

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ABSTRACT

Background: Migraine is a chronic neurovascular condition characterized by recurring attacks of pulsating headaches. Genome-wide association studies (GWAS) identified many potential loci associated with migraine. To check the association of polymorphisms of *PRDM16* (rs2651899), *LRP1* (rs11172113), and *TRPM8* (rs10166942) with migraine, the first time a case-control study was conducted in understudied Pakistani population.

Methods: The study included 127 migraine patients (21 in migraine with aura and 106 with migraine without aura group) and 120 healthy control subjects from different areas of Punjab, Pakistan. Blood samples were collected from all the participants, and DNA was isolated from the lymphocytes by the modified organic method. Sanger's sequencing was done for *PRDM16* (rs2651899), *LRP1* (rs11172113), and *TRPM8* (rs10166942) in all the samples to check the genotype. Logistic regression analysis was done using SPSS 20.0 to check the association of these SNPs with migraine susceptibility.

Results: We found statistically significant differences between case and control group for *PRDM16* (rs2651899) at genotypic level ($p < 0.001$), allelic level ($p < 0.001$; OR 3.088; 95% CI 2.082–4.579) and for dominant model ($p < 0.001$; OR 5.437; 95% CI 3.112–9.498). The major findings of this study suggested that *PRDM16* rs2651899 is strongly associated with migraine in overall and subgroup analysis of genotypes. *LRP1* (rs11172113) showed significant association with migraine except in subgroup comparison. A similar trend of association was found for *TRPM8* (rs10166942) however, significant association was found only at the allelic level but no significant difference was seen at the genotypic level between case and control. One novel mutation c.67 + 4436_67 + 4438delA was also identified in the current study near *LRP1* (rs11172113) polymorphic site.

Conclusion: In this first-ever replication report from Pakistan, *PRDM16* (rs2651899) was found as a potential genetic marker in migraine susceptibility while *LRP1* (rs11172113) and *TRPM8* (rs10166942) showed partial association in subgroup analysis.

1. Introduction

Migraine is a chronic neurovascular condition characterized by recurring attacks of pulsating headache linked with nausea, vomiting, phonophobia, photophobia, and other visual disturbances (Vos et al., 2017). Clinical diagnosis of this common yet debilitating disorder is made in concordance with the guidelines of the International Classification of Headache Disorders (ICHD-II) (Olesen and Steiner, 2004). According to the International Headache Society (IHS), there are seven subgroups of migraine, including migraine with aura, migraine without aura, retinal migraine, chronic migraine, childhood periodic syndrome, probable migraine, and abdominal migraine (GASPARINI, 2014). The most prevalent subtypes of this disease are migraine with aura (MA), usually known as classic migraine and, migraine without aura (MO) or common migraine. In MA, the patient experiences focal neurological symptoms with varied effects on vision and sensory systems preceding or accompanying the headache phase (Stovner et al., 2007).

Migraine is considered the third highly prevalent disorder affecting 1 billion people worldwide and the seventh major cause

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of disability across the world (Herekar et al., 2017). According to a survey, the 1-year prevalence of migraine in Pakistan is 22.7% being more common in the age group of 40–49 years (Ashina et al., 2021). Despite the substantial burden posed by this disorder, it is underdiagnosed in the majority of the population (Francis, 2016).

Migraine etiology involves environmental as well as genetic factors. Inflammatory mechanisms, neurological and cardiovascular impairments have been considered as the underlying causes in the pathophysiology of migraine (Mulder et al., 2003; Pietrobon and Moskowitz, 2013). Strong genetic predisposition has been identified by population-based studies estimating heritability from 34 to 57% in migraine (Mulder et al., 2003). Genetic susceptibility in migraine was further confirmed by several twins' studies and familial aggregation analyses (Stewart et al., 1997). Genome-wide association studies (GWAS) have unearthed multiple single nucleotide polymorphisms (SNPs) linked to migraine susceptibility that further explain the genetic nature of this complex disabling disorder (Gormley et al., 2016). Different genome-wide *meta*-analyses identified more than 142 SNPs associated with migraine at 12 loci (Anttila et al., 2013).

In a GWAS, 3 loci have been discovered in or near transient receptor potential cation channel subfamily M member 8 (*TRPM8)*, PR/SET domain 16 (*PRDM16*), and LDL receptor-related protein 1 (*LRP1)* genes that are strongly associated with migraine (Chasman et al., 2011). Cold-induced burning pain and cold sensor are primarily encoded by *TRPM8* (Mueller-Tribbensee et al., 2015). It is largely expressed in dorsal root ganglion and sensory neurons (Peier et al., 2002). rs10166942 being close to the *TRPM8* transcription site has been a prime focus of the neuropathic pain models. As migraine and neuropathic pain disorders share some attributes, so a pathophysiological link of *TRPM8* could be present between both these pain syndromes (Biondi, 2006).

LRP1 is expressed in the brain, vasculature and, many other tissues of the body where it is responsible for modulating synaptic transmission. It also serves as an important sensor of the extracellular environment. rs11172113 of *LRP1* plays a crucial role in modulating neuronal glutamate signaling (Lillis et al., 2008).

The potential role of *PRDM16* is still inexact in migraine pathophysiology. Initially, it was found associated with acute myeloid leukemia (Secker-Walker et al., 1995) and subsequently, its transcriptional role in brown fat development was also delineated (Kajimura et al., 2008). Variants of both *LRP1* and *TRPM8* have been successfully replicated by GWAS in Dutch and German populations (Freilinger et al., 2012). Keeping in view the potential role of these three genes and their strong association ($p < 5 \times 10–6$) with migraine in genome-wide analysis, we genotyped rs11172113 (*LRP1*), rs2651899 (*PRDM16*) and, rs10166942 (*TRPM8*) in our Pakistani population to ascertain its link with migraine susceptibility.

2. Materials and methods

2.1. Subjects

This case-control study involved 127 migraine patients (21 with MA and 106 with MO) recruited from two different hospitals in Lahore, Punjab, Pakistan. Diagnosis for migraine was made by neurologists strictly following the guidelines of IHS (Society HCSotIH, 2004). All the patients suffering from some other neurological conditions like epilepsy were excluded from the study. Migraineurs with secondary causes linked to head trauma event considered ineligible for the current research. For comparison, 120 control subjects were also included from the same ethnic region to avoid potential biases. The individuals included in the control group were healthy volunteers without a history of any disorder.

Advance studies and research board (AS&RB) of the University of the Punjab, Lahore, Pakistan approved to conduct this study. Furthermore, written informed consent was taken from each subject of the study before blood sampling. Demographic attributes and other disease-related information of the patients were also recorded through a structured questionnaire while healthy individuals were only asked for the demographics.

2.2. Blood sampling and DNA isolation

3 mL of peripheral venous blood was collected from each subject in EDTA-coated vials and stored at −20 °C until further experimentation. A modified organic method was used for DNA extraction from lymphocytes (Sambrook, 1989). The Quantity and quality of each DNA sample were assessed by NanoDrop (Optizen NanoQ) before subjecting it to amplification.

2.3. Genotyping

Previously reported primers were used for the amplification of rs11172113 (*LRP1*), rs2651899 (*PRDM16*), and rs10166942 (*TRPM8*) (21]. Primers were ordered from the Macrogen. To make the final 25uL volume of PCR reaction mixture, 9 uL of PCR reaction mixture (amaR OnePCR; Cat. No. SM213-0250), 1.5uL of each 10 pmol forward and reverse oligonucleotide primers, 1.5uL DNA template and, 11.5uL of nuclease-free water (ROTH Art.-Nr. T143.3) were added in a microcentrifuge tube. The following cycling conditions were used for the amplification of selected SNPs: Initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 45 s, annealing step at 60 °C (rs11172113 (*LRP1*)), 61 °C (rs2651899 (*PRDM16*)), and 59 °C (rs10166942 (*TRPM8*)) for 30 s followed by extension for 30 s at 72 °C and final extension at 72 °C for 10 min. Amplicons of 142 bp (rs11172113), 270 bp (rs2651899), and 180 bp (rs10166942) were resolved on 2% agarose gel. The purification of amplicons was done by using the purification Kit of GeneJET PCR (Thermo Scientific™ #K0702) and sequencing was done from a commercial source (1st BASE Singapore).

2.4. Statistical analysis

Sequence alignment editor BioEdit 7.2 software was used to detect the changes in the sequenced DNA samples of cases and controls. NCBI-BLAST was also performed for each sample. Categorical variables were presented in number and percentage, while a continuous variable in mean ± standard deviation (SD) and *t*-test was applied for comparison among subgroups. All the SNPs were checked for Hardy-Weinberg equilibrium and no violation was noted for any SNP. Allelic and genotypic frequencies were calculated by the chi-square test. A significance level of $p < 0.05$ was set for the current study. The association of SNPs with migraine was checked by the dominant models using unconditional logistic regression. For both rs11172113 and rs10166942 with minor alleles as C, the dominant model can be TT versus CT + CC. For rs2651899 with a minor allele as G, a dominant model can be AA versus AG + GG. The risk of migraine was calculated as odds ratio (OR) and 95% confidence intervals (CI) between groups for those holding the mutant alleles. All the analyses were performed using Statistical Package for the Social Sciences (SPSS) IBM 20.0, and GraphPad Prism.

3. Results

3.1. Characteristics of subjects

The current study included total of 247 participants, of which 120 were in the control group and 127 were migraineurs. The mean age of the control group having 82 females and 38 males was 26.26 ± 5.57 while that of the migraine group including 96 females and 31 males was 25.79 ± 5.19 years. Migraine patients were further classified on the basis of a particular migraine type as MA (20.47%) and MO (79.53%). No statistically significant difference (p = 0.49) was observed by comparing the case and control group in terms of age. However, a statistically significant difference (p = 0.0012) was noted between both groups when compared by gender due to the predominant presence of the female gender. Information regarding demographics and clinical features is presented in Table 1.

3.2. Genetic analysis of PRDM16 rs2651899 polymorphism

Significant differences between migraineurs and control group at allelic (p=<0.001, OR 3.088), genotypic level (p=<0.001) as well as for dominant model (p=<0.001, OR 5.437, 95% Cl 3.112–9.498) were observed in the current study as presented in Table 2 and 3. Minor allele G was found overrepresented in patients as compared to the control group (46.06 vs. 21.67%) and presented a significant risk for migraine. Logistic regression analysis (adjusted for age and sex) showed a significant association of *PRDM16* rs2651899 with migraine when compared at the subgroup level. Comparison of MO with control showed a significant difference in allelic (p=<0.001; OR 3.407) and genotypic distributions (p=<0.001) between the two groups presented in Table 4. The frequency of AG and GG genotype was higher in patients (55.45, 20.79%) compared to the control group (26.67, 8.33%). The same trend of significant association of rs2651899 with migraine was observed in MA vs. control given in table 5. Moreover, female and male subgroups of migraine were individually compared with the whole control cohort and observed significant differences at allelic, genotypic level given in tables 6 and 7.

Table 1
Demographic and clinical attributes of migraine patients.

Characters	Cases (N)	Percentage
Sex		
Male	31	24.40
Female	96	75.60
Age (Years)	25.79 ± 5.19	
Migraine subgroups		
MA	26	20.47
MO	101	79.53
Frequency of attack		
< 5 times per month	26	20.47
6-10 times per month	42	33.07
greater than 10 times per month	59	46.46
Associated symptoms		
Nausea	101	79.53
Vomiting	77	60.63
Photophobia	68	53.54
Phonophobia	39	30.71
Vertigo	15	11.81
Lacrimation	19	14.96
Trigger factors		
Menstruation	80	62.99
Emotional stress	56	44.09
Weather changes	39	30.71
Noise	78	61.41
Family history		
First degree relatives	22	17.32
Second degree relatives	37	29.13

*Only yes responses are included for associated symptoms, Trigger factors and Family history

3.3. Genetic analysis of LRP1 rs11172113 polymorphism

Chi-square test showed significant differences in the frequency between case and control group at allelic (p = 0.0055; OR 1.686, 95% Cl 1.165–2.441) and genotypic (p = 0.01) level shown in table 2. Logistic regression analysis indicated significant difference for genotype CC (p = 0.005; OR 3.977, 95% Cl 1.524–10.375) between case and control group while non-significant difference was noted for genotype CT (p = 0.052; OR 1.749 95% Cl 0.996–3.073). Comparison between MO and control showed significant difference at all levels; allelic, genotypic, and dominant model.

The frequency of genotype CC was marked higher in the MO group compared to the control and showed the significant risk for classical migraine patients (p = 0.002, OR 4.880, 95% Cl 1.794–13.270). The same trend of association of rs11172113 with migraine was found in female migraine patients and the control group. No significant association was detected in MA vs. control and migraine male vs. control group at any level.

3.4. Genetic analysis of TRPM8 rs10166942 polymorphism

The difference in genotypic distribution of rs10166942 between the case and control group was not found statistically significant (p = 0.0512) while it was significant at the allelic level (p = 0.0291; OR 1.621, 95% Cl 1.048–2.508) when checked by chi-square test as presented in table 2. Comparison of CC vs. TT genotype in regression model showed non-significant differences (p = 0.486, OR 1.484 95 %Cl 0.489–4.511) while in CT vs.TT significant (p = 0.013 OR 2.044, 95% Cl 1.63–3.594) association was noted in case vs. control group presented in Table 3. The same trend was observed in MO vs. control and migraine female vs. control group where CT genotype presented a significant risk for migraine susceptibility. Moreover, it was noted that individual comparison of MA and Male migraine patients with control showed no significant association at any level as shown in tables 5 and 7.

3.5. Novel intronic variant near LRP1 (rs11172113) polymorphic site

A novel intronic variant was also found in 36 migraine patients in the present study. No control subject carried this variant. The c.67 + 4436_67 + 4438delA (NM_002332.2) lied near *LRP1* (rs11172113) and the prediction of the functional effects was made by Mutation Taster (http://www.mutationtaster.org/). According to the results of Mutation Taster, this deletion of the A allele was found deleterious. phyloP and phastCons score in Mutation Taster showed that the mutation point conserved in nature. It could change the splice site leading to altered protein structure.

4. Discussion

To unravel the genetic cause of complex neurological disorders like migraine, a series of replication studies is required in different populations and ethnicities. Variants like rs11172113 (*LRP1*), rs2651899 (*PRDM16*), and rs10166942 (*TRPM8*) found associated with migraine in GWAS have been successfully replicated long ago in different ethnic groups including the Chinese and Indian population but no study from Pakistan added data for these variants (Kaur et al., 2019; An et al., 2013). To the best of our knowledge, this is the first study that investigated the association of these well-known SNPs with migraine susceptibility in the Pakistani population.

Table 2
Allelic and genotypic frequencies of case and control.

variant	Allele/Genotypes	Control N = 120 (%)	Case N = 127 (%)	P value	OR(95 %CI)
rs2651899 (*PRDM16*)	A	188(78.33)	137(53.94)	<0.001***	3.088(2.082–4.579)
	G	52(21.67)	117(46.06)		
	AA	78(65)	34(26.77)	<0.001***	
	AG	32(26.67)	69(54.33)		
	GG	10(8.33)	24(18.90)		
rs11172113 (*LRP1*)	T	166(69.16)	145(57.09)	0.0055**	1.686(1.165–2.441)
	C	74(30.83)	109(42.91)		
	TT	53(44.16)	38(29.92)	0.010*	
	CT	60(50)	69(54.33)		
	CC	07(5.83)	20(15.75)		
rs10166942 (*TRPM8*)	T	198(82.5)	189(74.41)	0.0291*	1.621(1.048–2.508)
	C	42(17.5)	65(25.59)		
	TT	84(70)	70(55.12)	0.0514	
	CT	30(25)	49(38.58)		
	CC	06(5)	08(6.30)		

OR; Odds ratio,Cl; confidence interval

Table 3
comparison between migraine and control group through genetic models.

SNP	Model	Allele/ Genotype	Migraine N = 127 %	Control N = 120 %	P value	Crude Analysis COR(95% Cl)	▫Adjusted Analysis AOR(95% Cl)	P value
	Allele	A	137(53.94)	188(78.33)	<0.001***	3.088(2.082–4.579)		
		G	117(46.06)	52(21.67)				
		AA	34(26.77)	78(65)				
	Genotype	AG vs. AA	69(54.33)	32(26.67)	<0.001***	4.947(2.766–8.847)	5.452(2.983–9.965)	<0.001***
rs2651899 (*PRDM16*)		GG vs. AA	24(18.90)	10(8.33)	<0.001***	5.506(2.376–12.760)	5.394(2.319–12.550)	<0.001***
	Dominant	AA	34(26.77)	78(65)				
		AG + GG	93(73.23)	42(35)	<0.001***	5.080(2.951–8.744)	5.437(3.112–9.498)	<0.001***
	Allele	T	145(57.09)	166(69.16)	0.0055***	1.686(1.165–2.441)		
		C	109(42.91)	74(30.83)				
rs11172113 (*LRP1*)		TT	38(29.92)	53(44.16)				
	Genotype	CT vs. TT	69(54.33)	60(50)	0.087	1.604(0.93–2.757)	1.749(0.996–3.073)	0.52
		CC vs. TT	20(15.75)	07(5.84)	0.005**	3.985(1.582–10.369)	3.977(1.524–10.375)	0.005**
	Dominant	TT	38(29.92)	53(44.16)				
		CT + CC	89(70.08)	67(55.84)	0.021*	1.853(1.098–3.127)	2.028(1.180–3.487)	0.011*
rs10166942 (*TRPM8*)	Allele	T	189(74.41)	198(82.5)	0.0291*	1.621(1.048–2.508)		
		C	65(25.59)	42(17.5)				
		TT	70(55.12)	84(70)				
	Genotype	CT vs. TT	49(38.58)	30(25)	0.017*	1.960(1.126–3.411)	2.044(1.63–3.594)	0.013*
		CC vs. TT	08(6.30)	06(5)	0.404	1.600(0.530–4.831)	1.484(0.489–4.511)	0.486
	Dominant	TT	70(55.12)	84(70)				
		CT + CC	57(44.88)	36(30)	0.016*	1.900(1.125–3.209)	1.943(1.143–3.305)	0.014*

Data presented as number (N) and percentage (%)
COR; Crude odds ratio, AOR; Adjusted odds ratio Cl; Confidence Interval
*p < 0.05, **p < 0.01, ***p < 0.001 statistically significant
Adjusted for age and sex

In the present study, we found that rs2651899 (*PRDM16*) could be a potential risk factor for migraineurs. This variant showed a significant association when compared between migraine types and both genders. The same results were reported in the Han-Chinese population from Southern China where they found this variant as a probable genetic risk factor for migraine further strengthening our findings (An et al., 2013). However, in contrast to our results, they did not find this association in the subgroup analysis in terms of gender and migraine type. In the present study, *PRDM16* rs2651899 showed highly significant differences at the allelic and genotypic level when compared across gender and the particular migraine type with the overall control group which is consistent with the findings of Kaur et al. reporting significant association of this SNP with migraine in the Indian population except for minor allele as they reported T allele as a risk factor compared to G allele in our study (Kaur et al., 2019).

Moreover, our results are consistent with the findings reported by Chasman et al. concerning allelic and genotypic distribution among cases and control subjects. Unlike the current findings, they observed no association in the subgroup analysis (Chasman et al., 2011). In contrast to these results in a GWAS study, no association was found between rs2651899 and migraine (Freilinger et al., 2012). However, this lack of association can be attributed to ethnic differences.

In case of rs11172113 (*LRP1*), an association was detected at the genotypic and allelic level in an overall comparison of case and control but this association reversed in MA vs. control and Male vs. control group. This discrepancy could be due to the small sample size in MA (Ghosh et al., 2013) and the migraine male group [31] compared to control subjects [120]. The findings of the current study are inconsistent with Gosh et al. where this variant presented a protective effect to migraine susceptibility. The exact

Table 4
Comparison between MO and control group.

SNP	Model	Allele/ Genotype	MO N = 101 %	Control N = 120 %	P value	Crude Analysis COR(95% CI)	Adjusted Analysis AOR(95% CI)	P value
	Allele	A	104(51.49)	188(78.33)	<0.001	3.407(2.254–5.149)		
		G	98(48.51)	52(21.67)				
rs2651899 (*PRDM16*)		AA	24(23.76)	78(65)				
	Genotype	AG vs. AA	56(55.45)	32(26.67)	<0.001***	5.687(3.027–10.688)	6.058(3.168–11.585)0.000	<0.001***
		GG vs. AA	21(20.79)	10(8.33)	<0.001***	6.825(2.828–16.473)	6.598(2.724–15.978)	<0.001***
	Dominant	AA	24(23.76)	78(65)				
		AG + GG	77(76.24)	42(35)	<0.001***	5.958(3.296–10.772)	6.200(3.391–11.335)	<0.001***
	Allele	T	110(54.45)	166(69.16)	0.0015**	1.876(1.271–2.770)		
		C	92(45.55)	74(30.83)				
rs11172113 (*LRP1*)		TT	26(25.74)	53(44.16)				
	Genotype	CT vs. TT	58(57.43)	60(50)	0.025*	1.971(1.090–3.562)	2.083(1.129–3.841)	0.019*
		CC vs. TT	17(16.83)	07(5.84)	0.002**	4.951(1.826–13.423)	4.880(1.794–13.270)	0.002**
	Dominant	TT	26(25.74)	53(44.16)				
		CT + CC	75(74.26)	67(55.84)	0.005**	2.282(1.286–4.049)	2.430(1.347–4.383)	0.003**
	Allele	T	145(71.78)	198(82.5)	0.0071**	1.853(1.179–2.914)		
		C	57(28.22)	42(17.5)				
rs10166942 (*TRPM8*)		TT	49(48.51)	84(70)				
	Genotype	CT vs. TT	47(46.54)	30(25)	0.001**	2.686(1.507–4.786)	2.735(1.523–4.909)	0.001**
		CC vs. TT	5(4.95)	06(5)	0.572	1.429(0.414–4.927)	1.292(0.371–4.501)	0.687
	Dominant	TT	49(48.51)	84(70)				
		CT + CC	52(51.49)	36(30)	0.001**	2.476(1.426–4.300)	2.476(1.419–4.321)	0.001**

Data presented as number (N) and percentage (%)
COR; Crude odds ratio, AOR; Adjusted odds ratio Cl; Confidence Interval
*p < 0.05, **p < 0.01, ***p < 0.001 statistically significant
Adjusted for age and sex

Table 5
Comparison between MA and control group.

SNP	Model	Allele/ Genotype	MA N = 26 %	Control N = 120 %	P value	Crude Analysis COR(95% CI)	Adjusted Analysis AOR(95% CI)	P value
	Allele	A	33(63.46)	188(78.33)	0.0234*	2.082(1.095–3.958)		
		G	19(36.54)	52(21.67)				
rs2651899 (*PRDM16*)		AA	10(38.46)	78(65)				
	Genotype	AG vs. AA	13(50)	32(26.67)	0.014*	3.3169(1.261–7.962)	4.165(1.552–11.175)	0.005**
		GG vs. AA	3(11.54)	10(8.33)	0.250	2.340(0.550–9.960)	2.389(0.552–10.346)	0.244
	Dominant	AA	10(38.46)	78(65)				
		AG + GG	16(61.54)	42(35)	0.015*	2.971(1.239–7.7125)	3.620(1.445–9.066)	0.006**
	Allele	T	35(67.31)	166(69.16)	0.7930	1.090(0.5739–2.069)		
		C	17(32.69)	74(30.83)				
rs11172113 (*LRP1*)		TT vs. TT	12(46.15)	53(44.16)				
	Genotype	CT	11(42.31)	60(50)	0.645	0.810(0.330–1.987)	1.012(0.386–2.652)	0.981
		CC vs. TT	3(11.54)	07(5.84)	0.401	1.893(0.426–8.404)	1.857(0.416–8.276)	0.417
	Dominant	TT	12(46.15)	53(44.16)				
		CT + CC	14(53.85)	67(55.84)	0.853	0.923(0.394–2.162)	1.144(0.464–2.819)	0.770
	Allele	T	44(84.62)	198(82.5)	0.713	0.8571(0.376–1.954)		
		C	8(15.38)	42(17.5)				
rs10166942 (*TRPM8*)		TT	21(80.77)	84(70)				
	Genotype	CT vs. TT	2(7.69)	30(25)	0.086	0.267(0.059–1.206)	0.306(0.066–1.429)	0.312
		CC vs. TT	3(11.54)	06(5)	0.354	2.000(0.462–8.664)	1.873(0.426–8.233)	0.406
	Dominant	TT	21(80.77)	84(70)				
		CT + CC	5(19.23)	36(30)	0.273	0.556(0.194–1.588)	0.631(0.216–1.842)	0.399

Data presented as number (N) and percentage (%)
COR; Crude odds ratio, AOR; Adjusted odds ratio Cl; Confidence Interval
*p < 0.05, **p < 0.01, ***p < 0.001 statistically significant
Adjusted for age and sex

functional role of this SNP could not be found. However, they reported that the SNP is in strong linkage disequilibrium with rs11172113 affects the transcription factor binding site (Ghosh et al., 2013). The same results have been reported from She ethnic and Han-Chinese populations where they were unable to find any association of this SNP with migraine susceptibility (An et al., 2013; Fu et al., 2019).

For rs10166942 *TRPM8*, we found significant differences in allelic frequencies between control and cases while we were unable to find an association of this SNP at genotypic level. However, it showed significant association when compared across gender and migraine type. In two other replication studies, non-significant association was observed at the genotypic level as presented in the current study (Kaur et al., 2019; An et al., 2013). Incongruent to our results, Esserlind et al. and Sintas et al. reported this SNP as a strong predictor of migraine susceptibility (Esserlind et al., 2013; Sintas et al., 2015). Consistent with our results, Gosh et al. reported that rs10166942 may not be a risk factor for migraine (Ghosh et al., 2013). The association of any of these variants to migraine in the current study does not imply causation. To

Table 6
comparison between female migraine patients and control.

SNP	Model	Allele/ Genotype	Migraine Female N = 96 %	Control N = 120 %	P value	Crude Analysis COR(95% CI)	Adjusted Analysis AOR(95% CI)	P value
	Allele	A	102(53.12)	188(78.33)	<0.001***	3.190(2.100–4.846)		
		G	90(46.88)	52(21.67)				
rs2651899 (*PRDM16*)		AA	25(26.04)	78(65)				
	Genotype	AG vs. AA	52(54.17)	32(26.67)	<0.001***	5.070(2.700–9.579)	6.813(3.247–14.293)	<0.001***
		GG vs. AA	19(19.79)	10(8.33)	<0.001***	5.928(2.438–14.412)	5.409(2.077–14.086)	0.001**
	Dominant	AA	25(26.04)	78(65)				
		AG + GG	71(73.96)	42(35)	<0.001***	5.274(2.923–9.518)	6.354(3.246–12.437)	<0.001***
	Allele	T	109(56.77)	166(69.16)	0.0078**	1.708(1.150–2.538)		
		C	83(43923)	74(30.83)				
rs11172113 (*LRP1*)		TT	28(29.17)	53(44.16)				
	Genotype	CT vs. TT	53(55.21)	60(50)	0.087*	1.672(0.928–3.011)	2.305(1.177–4.513)	0.015*
		CC vs. TT	15(15.62)	07(5.84)	0.006**	4.056(1.481–11.106)	3.652(1.259–10.595)	0.017*
	Dominant	TT	28(29.17)	53(44.16)				
		CT + CC	68(70.83)	67(55.84)	0.024*	1.921(1.088–3.393)	2.529(1.331–4.806)	0.005**
	Allele	T	141(73.44)	198(82.5)	0.0228*	1.705(1.074–2.707)		
		C	51(26.56)	42(17.5)				
rs10166942 (*TRPM8*)		TT	51(53.13)	84(70)				
	Genotype	CT vs. TT	39(40.62)	30(25)	0.011*	2.141(1.187–3.861)	2.653(1.326–5.307)	0.006**
		CC vs. TT	06(6.25)	06(5)	0.409	1.647(0.504–5.381)	1.112(0.337–3.67)	0.862
	Dominant	TT	51(53.13)	84(70)				
		CT + CC	45(46.87)	36(30)	0.011*	2.059(1.177–3.603)	2.231(1.184–4.203)	0.013*

Data presented as number (N) and percentage (%)
COR; Crude odds ratio, AOR; Adjusted odds ratio Cl; Confidence Interval
*p < 0.05, **p < 0.01, ***p < 0.001 statistically significant
Adjusted for age and sex

Table 7
Comparison between male migraine patients and control.

SNP	Model	Allele/ Genotype	Migraine Male N = 31 %	Control N = 120 %	P value	Crude Analysis COR(95% CI)	Adjusted Analysis AOR(95% CI)	P value
	Allele	A	35(56.45)	188(78.33)	<0.001***	2.789(1.548–5.025)		
		G	27(43.55)	52(21.67)				
rs2651899 (*PRDM16*)		AA	09(29.03)	78(65)				
	Genotype	AG vs. AA	17(54.84)	32(26.67)	0.003**	4.604(1.859–11.40)	5.033(1.983–12.772)	0.001**
		GG vs. AA	05(16.13)	10(8.33)	0.024*	4.333(1.209–15.525)	4.185(1.164–15.043)	0.028*
	Dominant	AA	09(29.03)	78(65)				
		AG + GG	23(70.97)	42(35)	0.001**	4.540(1.98–10.744)	3.765(1.364–10.394)	0.011*
	Allele	T	36(58.06)	166(69.16)	0.0977	1.620(0.912–2.877)		
		C	26(41.93)	74(30.83)				
rs11172113 (*LRP1*)		TT	10(32.26)	53(44.16)				
	Genotype	CT vs. TT	16(51.61)	60(50)	0.437	1.413(0.591–3.381)	1.490(0.601–3.694)	0.390
		CC vs. TT	05(16.13)	07(5.84)	0.050	3.786(0.999–14.340)	3.696(0.971–14.065)	0.055
	Dominant	TT	10(32.26)	53(44.16)				
		CT + CC	21(67.74)	67(55.84)	0.233	1.661(0.721–3.828)	1.130(0.409–3.125)	0.814
	Allele	T	48(77.42)	198(82.5)	0.358	1.375(0.695–2.720)		
		C	14(22.58)	42(17.5)				
rs10166942 (*TRPM8*)		TT	19(61.29)	84(70)				
	Genotype	CT vs. TT	10(32.26)	30(25)	0.383	1.474(0.616–3.524)	1.545(0.636–3.749)	0.337
		CC vs. TT	02(6.45)	06(5)	0.650	1.474(0.276–7.875)	1.402(0.260–7.547)	0.694
	Dominant	TT	19(61.29)	84(70)				
		CT + CC	12(38.71)	36(30)	0.355	1.474(0.648–3.351)	1.363(0.503–3.696)	0.543

Data presented as number (N) and percentage (%)
COR; Crude odds ratio, AOR; Adjusted odds ratio Cl; Confidence Interval
*p < 0.05, **p < 0.01, ***p < 0.001 statistically significant
Adjusted for age and sex

imply causation with certitude, multiple studies with larger samples size from the different regions of the country are requisite.

Limited success to replicate the GWAS variant in different studies may be due to smaller sample sizes and ethnic differences. Furthermore, subgroup analysis needs a larger sample size to be surer about the association of a particular variant with the gender or migraine subtype.

5. Conclusion

In conclusion, of this first-ever report of GWAS variants from Pakistan, we have found a strong association of rs2651899 (*PRDM16*) with the migraine. While *LRP1* (rs11172113) was found associated at the allelic and genotypic level in case and control group irrespective of subgroups and *TRPM8* (rs10166942) was

not observed as a potential genetic marker of migraine susceptibility.

6. Limitations

This study holds a relatively smaller sample size to replicate GWAS variants. Subgroups had an uneven distribution of patients in a way that female and MO patients were predominant. Furthermore, no biological assays were performed to check the functional changes caused by these variants.

7. Future prospects

Pakistan is a country where consanguineous marriages are strongly preferred in all ethnic and religious groups that ultimately make the narrower genetic pool leading to a superabundance of genetic disorders. No or very few studies exist on the genetic basis of many common neurological disorders for the Pakistani population. For better management and pre-diagnosis of this disorder, genetic studies, especially the replication studies for different variants at a larger scale are requisite in this understudied population so that population-specific genetic biomarkers can be developed. To strengthen these results, further replication studies at a larger scale are required from different ethnicities in Pakistan.

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