Original Research

Genetic predisposition of *TNFα* gene polymorphism in South-Indian Migraineurs and meta-analysis

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1. Abstract

Migraine (Mg) is a multifaceted neurovascular disorder caused by genetic and several environmental etiologies. We have implemented a case-control study of *TNFα* gene polymorphism in 212 Mg patients and 218 healthy controls utilizing the ARMS-PCR technique, followed by Sanger sequencing. Besides, we have conducted a meta-analysis of different genetic models (five genetic models) to combine and summarize the available data from 11 studies (including this present research). The strength of genetic associations in the meta-analysis used to assess by the pooled odds ratio (OR) and 95% confidence intervals (CI). The results of this case-control study discovered a significant relationship with Mg in recessive and homozygous genotype with OR = 2.35 (95% CI [0.96–5.74]), *p*-value = 0.045. Also, the outcomes of meta-analysis suggested an irrelevant relationship between *TNFα* gene (*rs1800629*) polymorphism and Mg susceptibility in the five genetic models. However, subgrouping based on ethnic background showed a significant association in the allelic genetic model with OR = 1.53 (95% CI [1.02–2.31]), $p = 0.040$ respectively. The meta-analysis results of *TNFα* gene polymorphism may represent a risk factor for Mg among Asians. In the future, large scale, multicentric case-control study by classification of patients with Mg with or without aura can be performed worldwide to identify the potential genetic risk factors leading to Mg pathogenesis.

Submitted: 28 April 2021 Revised: 2 August 2021 Accepted: 10 August 2021 Published: 20 December 2021

2. Introduction

Migraine (Mg) is one among the neurovascular disorder with characteristic heterogeneous clinical symptoms, which includes unilateral pulsatile headache that might last 4–72 hours, hypersensitivity such as phonophobia, photophobia, nausea and vomiting [1]. This disorder is of two distinct types based on their symptoms: (MgA), Mg with aura also called classical Mg and MO, Mg without aura common Mg also called Mg [2]. Previously, a meta-analysis was showed to evaluate the global occurrence of Mg relating 6,216,995 participants, which explained the pooled Mg occurrence of 10.4% in Africa, 11.4% in Europe, 16.4% in South and Central America, 9.7% in North America and 10.15% in Asia [3]. The essential features to be considered in Mgs can be explained as follows, the physiological basis for the aura, head pain anatomy, and genetics of Mg [4]. A genome-wide association study (GWAS) with broadly-defined headache, have documented 28 susceptible loci in the genes such as LRP1, STAT6, SDR9C7, FHL5, UFL1, TRPM8, HJURP, LINC02210-CRHR1, MAPT, MYO1H, IFT81, PTBP2, and MACF1 respectively [5].

The Tumor Necrosis Factor alpha (*TNF*α) gene positioning in the major histocompatibility complex (MHC)-class III on the 6th chromosome on p-arm at 21.33, which encodes for *TNF*α protein consisting 233 amino acids [6]. It encodes for multipurpose pro-inflammatory cytokines that belong to TNF superfamily and those are typically secreted by using macrophages. This cytokine can bind with receptors such as the TNFR1-TNFRSF1A and TNFBR-TNFRSF1B respectively [7]. Cytokines are considered as essential mediators in the inflammatory pathways and found to be associated with Mg pathogenesis. *TNF*α is concerned with the regulatory mechanism of biological processes, including coagulation, apoptosis, cell differentiation, cell proliferation, and lipid metabolism [8]. Studies focusing on central and peripheral levels of cytokines such as IL1a, IL1b, IL4, IL5, and *TNF*α revealed conflicting results, and demonstrated the role of inflammation in Mg and also in headache [9].

Previous studies documented the function of cytokines in regulating biological processes, such as, *TNF*α in the regulation of pain threshold and Mg pathogenesis. Further, inflammatory activation by cytokines was observed in the pathological process of Mg, which is attributed to tissue damage and Mg infarction [10]. Recent studies have identified a wide range of single nucleotide polymorphisms (SNPs) in the promoter site of *TNF*α, thus leading to changes at the transcriptional and posttranscriptional levels. Among several SNPs identified in the *TNF*α gene (*rs1800629*; g.-308 G>A) has been found to be associated with MA and MO. This SNP has been observed with the increase of *TNF*α gene expression thereby leading to higher levels of cytokine, further contributing to Mg pathogenesis [11]. In this study, the *rs1800629* polymorphism was selected, since no case-control studies were published from Indian population of Asian ethnicity. To define the relationship between selected SNP (*rs1800629*) predispositions with Mg risk, this case-control study was performed with participants from the south Indian population. To acquire more strong and conclusive results, we additionally executed a meta-analysis from Asian and Caucasian ethnic background by considering the available studies on the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [12].

3. Methodology

3.1 Stage 1: case-control study

3.1.1 Subjects

The case-control study comprised 212 Mg patients and 218 healthy controls were enrolled from Neurology Department, Chettinad Super Speciality Hospital, Tamil Nadu, India, among January–July 2018. Migraineurs were diagnosed by an experienced neurologist (KS) based on International Classification of Headache Disorders (ICHD) (3rd Edition) strategy by International Headache Society (IHS) for Mg [13]. The controls were also from the same ethnic background (Asian) and free from disease. The clinical and demographic characteristics were recorded for each of the study participants using a questionnaire framed by the investigators. The entire study design was accepted by the Chettinad Academy of Research and Education, Institutional Human Ethics Committee (390/IHEC/10-17). Prior to the sampling permission was attained from the participants of this study. The subjects with cardiac ailments, HIV-seropositive, and other neurodegenerative disorders were excluded from this study.

3.1.2 DNA extraction and *rs1800629* genotyping

Peripheral blood (4 mL) was collected from the Migraineurs and controls, the salting-out method with some minor modifications was used to isolate the genomic DNA [14]. Genotyping of *rs1800629* polymorphism was carried out using (Table 1) by Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) [15]. The PCR was performed in Applied Biosystems Veriti® Thermal Cycler (USA) in 10 μL total volume, including 2.5 μL of 10$\times$ PCR buffer, 10 pmol/μL each primer, 1.5 μL of genomic DNA, 0.5 μL of dNTPs, and 0.3 μL of Taq DNA polymerase. The reaction of PCR condition was: 5 min at 94 °C, followed by 32 cycles of 45 s at 94 °C, for 45 s at 66 °C, for 45 s at 72 °C and with a final extension for 5 min at 72 °C. The PCR yields were checked on 1.8% agarose gel together with 100 base pair DNA Ladder (Dye Plus, Takara). At last, the selected samples of Mg = 12 and Controls = 8, the results of ARMS-PCR were confirmed using DNA sequencing (ABI 3130, Foster City,

Table 1. Designed primers for *rs1800629* genotyping.

Primer-ID	Primer sequence (5′–3′)	Allele	No of base pairs	Tm (°C)	Total length (Bp)
TNFα-OF	CAACACAGCTTTTCCCTCCAACCCCGTT		28	69	
					400
TNFα-OR	TGGTGGAGAAACCCATGAGCTCATCTGG		28	69	
TNFα-IF	GGAGGCAATAGGTTTTGAGGGGCAGGG	G	28	69	251
TNFα-IR	GTAGGACCCTGGAGGCTGAACCCCGTACT	A	29	69	202

IF, inner forward; IR, inner reverse; OF, outer forward; OR, outer reverse.

USA). Further to define, the interaction of chromosomes with the *rs1800629* SNP, the 3DSNP tool [16] was used to create Circos plots for visualizing the complete genomic data based on r2 values.

3.1.3 Statistical analyses

Pearson χ^2 assessment was used to calculate the allelic and genotypic frequencies of *rs1800629* SNP between Migraineurs and controls. The distribution of genotype in the controls was determined by Hardy-Weinberg Equilibrium (HWE value >0.05). The relationship of *rs1800629* SNP with Mg was analyzed by calculating the OR and with 95% CIs. The statistical analysis was performed by SPSS V-21 (IBM Analytics, Chicago, IL, USA) and $p < 0.05$ would be considered as statistically significant. Further, the effects of polymorphism was also examined by calculating the OR, and 95% CIs in dominant (GA + AA vs. GG) and recessive (AA vs. GG + GA) genetic models (G-major, A-minor allele). Further, the association of parameters such gender, pain severity, and location of headaches with genotypes of Mgrs were examined.

3.2 Meta-analysis

3.2.1 Literature search for meta-analysis

Comprehensive literature was implemented in the available records such as Cochrane Library, NCBI-PubMed, MEDLINE, EMBASE, and Google Scholar upto December 2018 that addressed the association of *TNFα* gene polymorphisms with Mg. The keywords such as "Mg", "MA, MO", "Tumor Necrosis Factor alpha gene", "and *TNFα* gene", "SNP", "*rs1800629*" were helped to identify the literature available in English Language only. Further relevant publications were identified by screening the references of the original research/full-text articles.

3.2.2 Selection criteria

Studies incorporated in this meta-analysis were necessary to meet the subsequent criteria. (i) Studies should have two groups (Case-Controls). (ii) Second, studies should have assessed the relationship of *TNFα* gene polymorphism (*rs1800629*) with Mg. (iii) Articles must provide adequate data on genotype frequencies. The studies conducted on human cell lines, editorials, case reports, letters and studies which lack genotype frequencies were excluded from this analysis.

Table 2. Demographic characteristics of Migraineurs and controls.

Factors	Migraineurs (N = 212)	Controls (N = 218)
Men:Women	70:142	78:140
Mean Age	37.75 ± 7.68	35.33 ± 5.56
Mg with:without aura	45:167	Nil
Age of disease onset	26.9 ± 8.90	Nil
Family history of Mg	36	Nil
Pain severity	Low: 47	Nil
	Moderate: 63	Nil
	Severe: 102	
Location of headaches	One side: 79	Nil
	Both Sides: 71	
	Entire head: 62	

Data are presented as mean ± SD.

3.2.3 Data mining and quality evaluation

The bibliographic search and records were mined by two investigators [PK and AN] and any contradictions were solved by a mutual conversation (RK, SSJ and AH). The information such as publication year, first author name, region of study, origin, Mg diagnostic criteria, DNA source, the sample size of Migraineurs and controls, genotype frequency and methods of genotyping has been extracted. The quality assessment was independently performed for each of the incorporated studies by HWE [17] and the Newcastle Ottawa Scale (NOS) [18].

3.2.4 Meta-analysis for 1800629 polymorphism

The relationship between *TNFα rs1800629* polymorphism and Mg risk in overall and subgroup (Caucasian and Asian) analysis was assessed by OR and 95% CI under allelic (A vs. G), homozygote (AA vs. GG), heterozygote (GA vs. GG), recessive (AA vs. GG + GA), and dominant (GA + AA vs. GG) (G-major, A-minor allele) genetic models respectively. The heterogeneity (I^2) between the included studies in this meta-analysis was assessed by Q-statistics and I^2 test [19]. If $I^2 > 50\%$, the random-effects model was used, otherwise the fixed-effects model [20] was implemented. Further, we employed funnel plot analysis and Egger's test [21] was executed by RevMan V.5 (Cochrane Community, London, UK) or STATA V.12 (Stata-corp, LLC, US)

Table 3. Allele frequencies and genotype distribution of *TNFα* gene polymorphism in Migraineurs and controls.

Polymorphism	Frequencies	Migraineurs (%) n = 212	Controls (%) n = 218	HWE	OR	95% CI	χ^2	*p*-value
rs1800629	Allele							
	G	354 (83.49)	360 (78.28)	-		Reference		
	A	70 (16.50)	76 (17.43)	-	0.93	[0.65–1.33]	0.71	0.394
	Genotype							
	GG	158 (74.52)	152 (69.72)			Reference		
	GA	38 (17.92)	56 (25.68)	0.11	0.65	[0.40–1.04]	3.21	0.073
	AA	16 (07.54)	10 (04.58)		2.35	[0.96–5.74]	3.67	0.045*
Genetic models								
Dominant	GA + AA vs GG	-	-	-	0.58	[0.26–1.32]	1.66	0.138
Recessive	AA vs GG + GA	-	-	-	1.27	[0.83–1.93]	1.23	0.157

HWE, Hardy Weinberg equilibrium; OR, Odd's ratio; χ^2, Chi-square; *p*-value, one tailed test; *, Significant *p*-value.

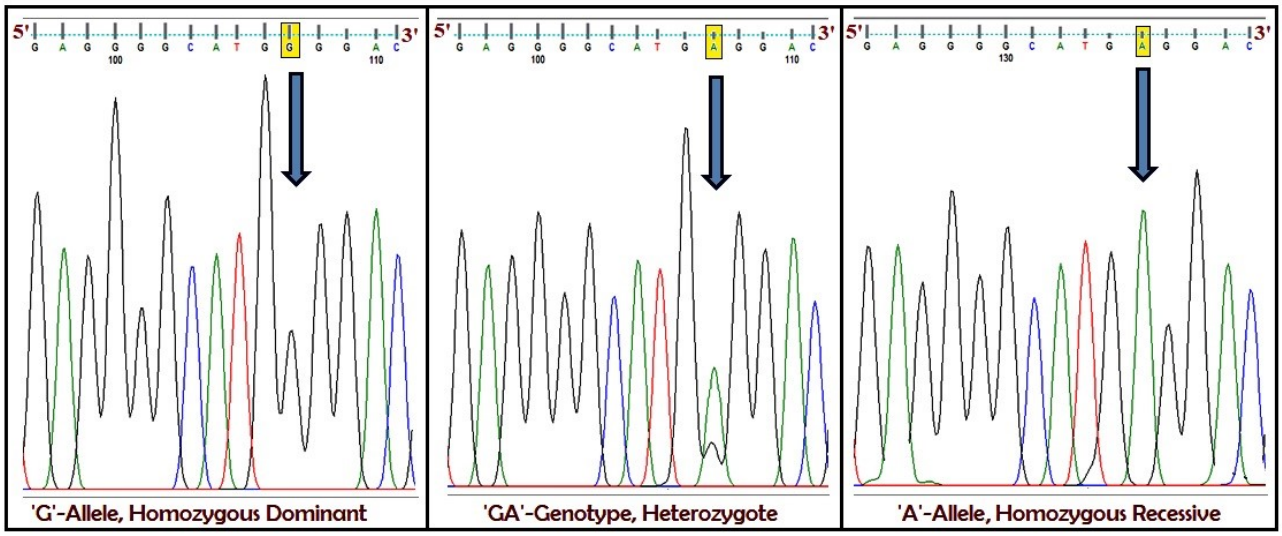

Fig. 1. DNA sequence electropherogram of *rs1800629* polymorphism in *TNFα* gene.

4. Results

4.1 Geographical attribute

The geographical attribution of migraineurs and controls are summarized in Table 2. Amidst the 430 participants joined in this study, the age range in migraineurs and controls were 37.75 ± 7.68 and 35.33 ± 5.56. Further, the age at the disease (migraineurs) onset was 26.9 ± 8.90.

4.2 Genotypic frequencies of *rs1800629* SNP among South-Indian Migraineurs

The genotype distribution of *TNFα rs1800629* polymorphism in the experimental group and healthy volunteers was reliable with HWE ($p > 0.05$), signifying a good representation of the study population. Allele and genotype frequencies of *rs1800629* polymorphism in the Migraineurs and controls along with HWE, Chi-square, ORs and 95% CIs, and *p*-value were explained in Table 3. The 'A' allele frequency for *rs1800629* polymorphism in the Migraineurs was lower than the controls. The examination of *rs1800629* polymorphism discovered a positive relationship with Migraineurs when linked to the controls (*p*-value < 0.05). The homozygous recessive (AA) genotype showed a substantial relationship with Mg, OR = 2.35 (95% CI [0.96–5.74]) *p*-value = 0.045, respectively.

Additionally, dominant and recessive genetic models were also analyzed, which showed insignificant associations between the Migraineurs and controls for *rs1800629* polymorphism. The DNA sequence electropherograms representing the homozygous dominant, heterozygous and homozygous recessive genotypes of *rs1800629* polymorphism are illustrated in Fig. 1. The sequences of *TNFα* genotypes were submitted in NCBI Genbank with Accession numbers: MH102359, MH105039, and MH105072. The chromosomal interactions with the *rs1800629* SNP are represented by a Circos plot in Fig. 2. Simultaneously, the genotype association with gender, pain severity, and location of headaches were assessed in Mgrs,

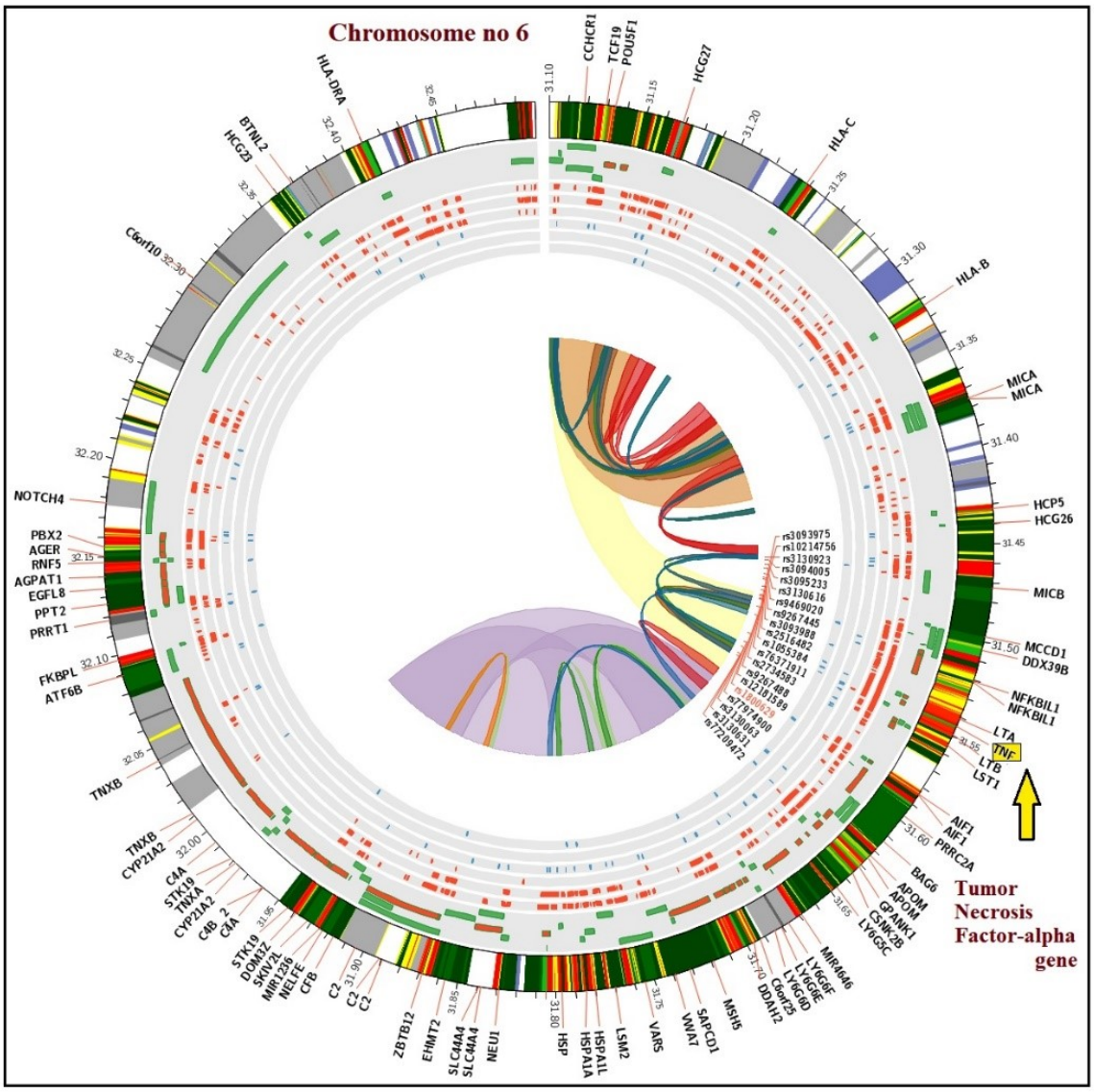

Fig. 2. Circos plot illustrating the chromosomal interactions among the current SNP (*rs1800629*) and its associated SNPs.

Table 4. The characteristics of included studies in this meta-analysis.

Study name	Year	Country	Ethnicity	Source of DNA	Diagnostic criteria	No of cases	No of controls	NOS score	Genotyping method
Asuni *et al.* [22]	2009	Italy	Caucasian	Blood	ICHD-II	301	278	7	PCR
Ates *et al.* [23]	2011	Turkey	Asian	Blood	HIS	203	202	6	ARMS-PCR
Fawzi *et al.* [11]	2015	Egypt	Egyptian	Blood	HIS	200	200	8	PCR-RFLP
Ghosh *et al.* [24]	2010	North India	Asian	Blood	HIS	216	216	7	PCR-RFLP
Herken *et al.* [25]	2005	Turkey	Asian	Blood	HIS	60	62	6	PCR-RFLP
Lee *et al.* [26]	2007	Korea	Asian	Blood	HIS	439	382	6	PCR
Pappa *et al.* [27]	2010	Greece	Caucasians	Blood	ICHD-II	103	178	7	PCR-RFLP
Rainero *et al.* [28]	2004	Italy	Caucasian	Blood	HIS	299	306	7	PCR-RFLP
Stuart *et al.* [29]	2013	Australia	Caucasian	Blood	HIS	335	345	7	HRM, RFLP
This study	2018	India	Asian	Blood	HIS	212	218	7	ARMS-PCR
Yilmaz *et al.* [30]	2010	Turkey	Asian	Blood	ICHD-II	67	96	7	PCR-RFLP

PCR, Polymerase Chain Reaction; RFLP, Restriction Fragment Length Polymorphism; ARMS-PCR, Amplification-Refractory Mutation System Polymerase Chain Reaction; HRM, High Resolution Melting analysis; HIS, International Headache Society; ICHD-II, International Classification of Headache Disorders; NOS, Newcastle-Ottawa Scale.

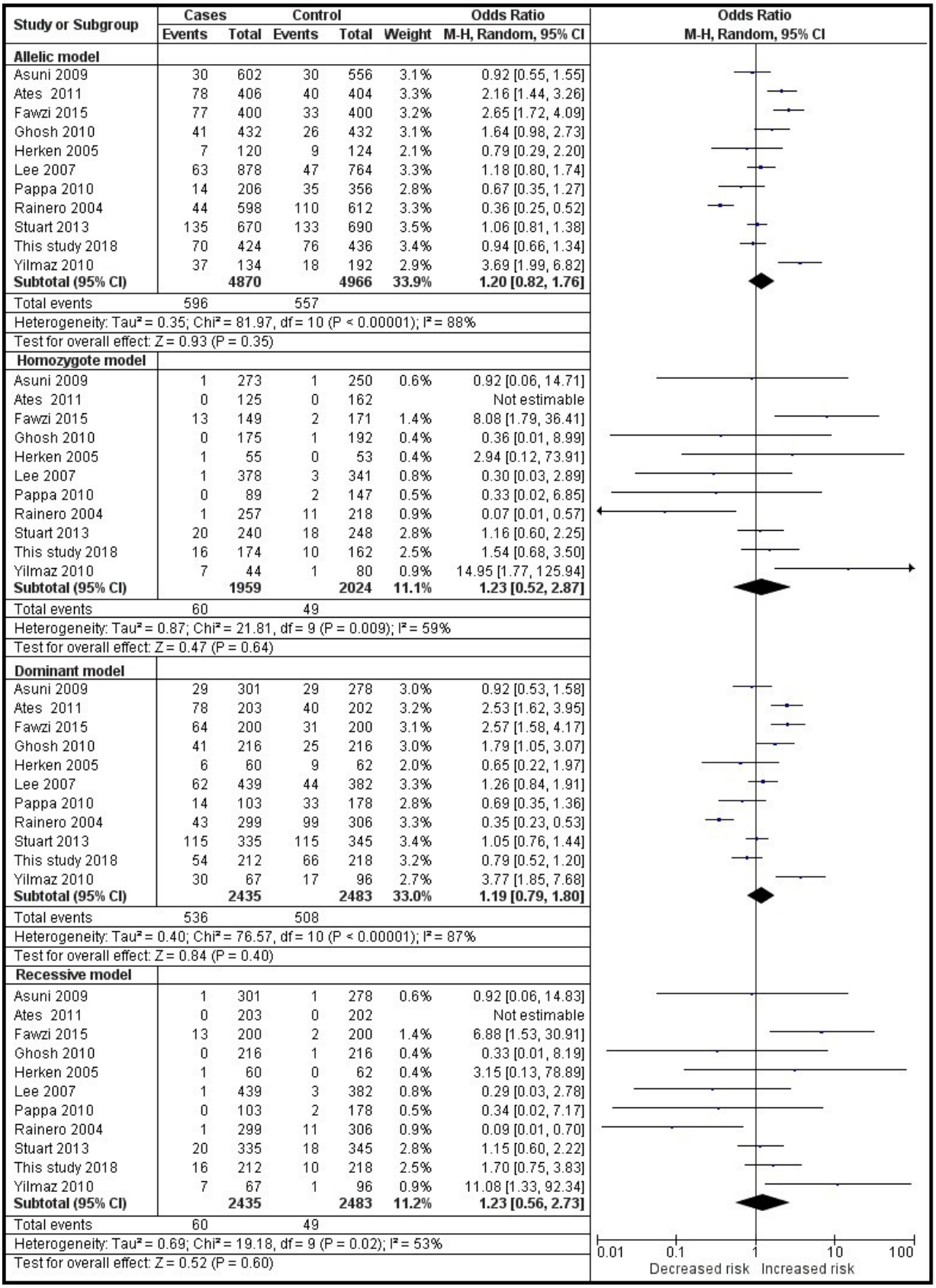

Fig. 3. Forest plot representing the overall risk for SNP *rs1800629*.

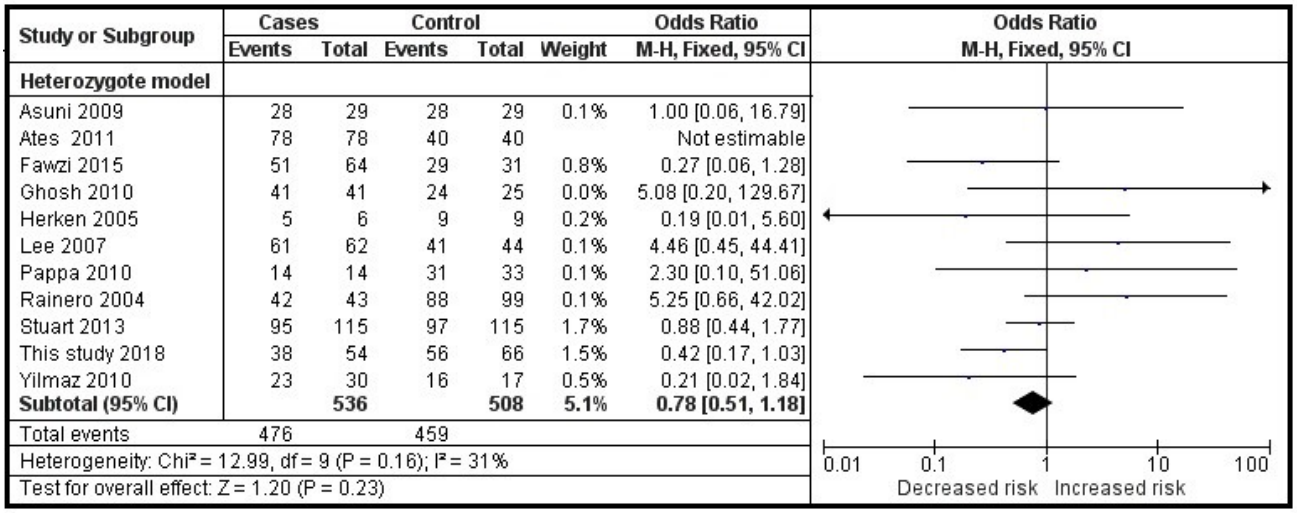

Fig. 4. Forest plot representing the overall risk for SNP *rs1800629* under heterozygote model.

Table 5. Genotypic and allele frequencies of *TNFα* gene (*rs1800629*) polymorphism.

Study name	Cases (GG/GA/AA)	Controls (GG/GA/AA)	Cases (G/A-Allele)	Controls (G/A-Allele)	HWE	Chi-square
Asuni *et al.* [22]	272/28/1	249/28/1	572/30	526/30	0.822	0.05
Ates *et al.* [23]	125/78/0	162/40/0	328/78	364/40	0.118	2.43
Fawzi *et al.* [11]	136/51/13	169/29/2	323/77	367/33	0.55	0.35
Ghosh *et al.* [24]	175/41/0	191/24/1	391/41	406/26	0.793	0.06
Herken *et al.* [25]	54/5/1	53/9/0	113/7	115/9	0.537	0.37
Lee *et al.* [26]	377/61/1	338/41/3	815/63	717/47	0.168	1.89
Pappa *et al.* [27]	89/14/0	145/31/2	192/14	321/35	0.813	0.05
Rainero *et al.* [28]	256/42/1	207/88/11	554/44	502/110	0.665	0.18
Stuart *et al.* [29]	220/95/20	230/97/18	535/135	557/133	0.072	3.21
This study	158/38/16	152/56/10	354/70	360/76	0.11	2.52
Yilmaz *et al.* [30]	37/23/7	79/16/1	97/37	174/18	0.851	0.03

HWE, Hardy-Weinberg equilibrium.

which showed significant influence of polymorphism with pain severity, and location of headaches (**Supplementary file 1**).

4.3 Literature search and study characteristics

The comprehensive literature search in the available databases using PRISMA guidelines retrieved a total of 176 publications of which 10 studies were selected for meta-analysis. All the selected studies [11, 22–30] were assessed for quality using on HWE and Newcastle Ottawa Scale. The main characteristics such as study name with year, ethnic origin, country, DNA isolation source, Mg diagnostic criteria, total no of study participants (Migraineurs and healthy controls), NOS Score, genotyping methods, allele and genotypic frequencies, HWE, and chi-square are summarized in Tables 4 (Ref. [11, 22–30]), 5 (Ref. [11, 22–30]). Between the selected articles, seven studies have used PCR-RFLP, whereas two studies (including our study) have adopted ARMS-PCR and the outstanding two researches used further techniques for genotyping.

4.4 Meta-analysis of *rs1800629* polymorphism with Mg risk

4.4.1 The meta-analysis results (*rs1800629*) are demonstrated via forest plots in Figs. 3 and 4

The heterogeneity analysis of *TNFα rs1800629* polymorphism, showed high heterogeneity in allelic (I^2 = 88%), dominant (I^2 = 87%), homozygote (I^2 = 59%), recessive (I^2 = 53%), and mild heterogeneity in heterozygote (I^2 = 31%), genetic models. The relationship between *rs1800629* polymorphism and Mg risk, revealed no obvious links under A vs. G OR = 1.20 (95% CI [0.82–1.76]), p = 0.35, AA vs. GG OR = 1.23 (95% CI [0.52–2.87]), p = 0.64, GA vs. GG OR = 0.78 (95% CI [0.51–1.18]), p = 0.23 GA + AA vs. GG OR = 1.19 (95% CI [0.79–1.80]), p = 0.402, and AA vs. GG + GA OR = 1.23 (95% CI [0.56–2.73]), p = 0.60 genetic models respectively. Further, Begg's and Egger's test were implemented which shown no noticeable publication preference in the present study. The sub-set stratification was performed based on ethnic background (Asian =

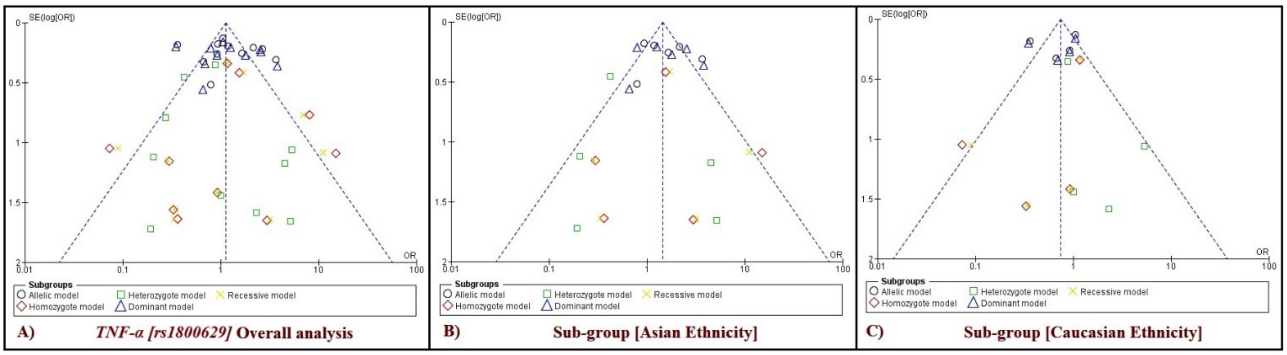

Fig. 5. Funnel plot of publication biases on the relationships between *TNFα* (*rs1800629*) polymorphism with Mg risk in overall and sub-group analysis.

Table 6. Meta-analyses of *rs1800629* polymorphism and Mg risk among subgroups.

Genetic models	Ethnicity	Q	Q-*p*	I^2	Model	OR 95% CI	Z-test	*p*-value
Allelic model	Caucasian	21.92	<0.0001	86	Random	0.70 [0.40–1.23]	1.25	0.210
A vs G	Asian	20.93	0.0008	76	Random	1.53 [1.02–2.31]	2.03	0.040*
Homozygote model	Caucasian	7.23	0.06	58	Random	0.46 [0.10–2.09]	1.00	0.310
AA vs GG	Asian	7.34	0.12	45	Fixed	1.75 [0.93–3.27]	1.74	0.080
Heterozygote model	Caucasian	2.89	0.41	0	Fixed	1.21 [0.66–2.20]	0.62	0.540
GA vs GG	Asian	6.49	0.17	38	Fixed	0.57 [0.29–1.13]	1.61	0.110
Dominant model	Caucasian	18.31	0.0004	84	Random	0.69 [0.39–1.22]	1.26	0.210
GA + AA vs GG	Asian	23.85	0.0002	79	Random	1.54 [0.95–2.50]	1.75	0.080
Recessive model	Caucasian	6.20	0.10	52	Random	0.51 [0.13–2.02]	0.96	0.341
AA vs GA + GG	Asian	6.52	0.16	39	Fixed	1.77 [0.94–3.31]	1.78	0.070

*Significant (p value < 0.05).

06, Caucasian = 04 and others = 01). The sub-grouping of *rs1800629* polymorphism in the background of Asian population discovered a substantial relationship in the allelic OR = 1.53 (95% CI [1.02–2.31]), $p = 0.040$ genetic model. Moreover, subgroup analysis of *rs1800629* polymorphism in Caucasian ethnicity revealed an irrelevant relationship under five genetic models. The OR interprets a 95% CIs for the subgrouping of Asian and Caucasian ethnicity is signified in Table 6 and the funnel plot (Fig. 5).

5. Discussion

Mg is a multi-faceted neurovascular disorder with recurring attacks of a severe headache which are often accompanied by neurological disturbances and nausea. They have a solid genetic foundation such as disorders of Mg caused by variations in monogenic forms, as well as familial clustering in Mg which is related to polymorphisms in several genes (polygenic forms) [31]. The Global Burden of Diseases (GBD), Risk Factors and Injuries studies performed a large worldwide ethnicity-based cross-sectional survey on Mg and with the pressure-type headache from the year 1990 and 2016 using Bayesian meta-regression model. The results revealed that 3000 million individuals were approximated to have pressure-type headache/Mg in the year 2016, in which 1040 million (95% uncertainty interval [UI] 1.00–1.09) with Mg and 1089 million (95% UI 1.71–2.10) with pressure-type headache respectively. These estimates provide us the need for more focus on headache and its related disorders [32].

TNFα is a pro-inflammatory cytokine and a polypeptide effector that plays a major role in inflammatory reactions. They have the ability to activate the transcription of Calcitonin Gene-Related Peptide (CGRP) and thus contribute to their role in the pathology and physiology of Mg [33]. The *TNFα* promoter region harbors various SNPs which might have the ability to influence the serum *TNFα* levels. These SNPs are mainly considered as enhancers of transcriptional activation which are found to be associated with higher *TNFα* levels and they have also been documented with increased susceptibility in several neoplastic, autoimmune and communicable diseases [34, 35]. In this research, the relationship between *TNFα rs1800629* polymorphism with Mg risk was carried out. This is the first case-control study to the best of our knowledge, which examines the relationship between *TNFα rs1800629* genetic alteration and Mg vulnerability in the South Indian populace. The analysis of genotypic frequencies revealed significant relationship *p*-value (<0.05) between Migraineurs and controls recommending that *TNFα rs1800629* genetic predisposition might be a positive vulnerable factor for Mg

in the South Indian populace. Additionally, our analysis showed the influence of polymorphism on the pain severity, and location of headaches of Migraineurs. Overall, our results were in agreement with previous reports from an Egyptian population comprising 200 Migraineurs and 200 healthy controls [11].

The meta-analysis of *TNFα rs1800629* polymorphism indicated irrelevant relationship in A vs G OR = 1.20 (95% CI [0.82–1.76]), AA vs GG OR = 1.23 (95% CI [0.52–2.87]), GA vs GG OR = 0.78 (95% CI [0.51–1.18]), GA + AA vs GG OR = 1.19 (95% CI [0.79–1.80]) and AA vs GA + GG OR = 1.23 (95% CI [0.56–2.73]), genetic models with p value > 0.05 respectively. The lack of association between *rs1800629* variants might be due to different geographical distribution of study subjects, inherent heterogeneity of the disease. Parallel to our results of meta-analysis, *rs1800629* polymorphism showed irrelevant relationships in Italian [22], and Greek subjects [27], whereas a positive relationship was observed in this study and Egyptian subjects [11]. The sub-grouping results based on Asian and Caucasian ethnicity revealed a significant association of *TNFα rs1800629* polymorphism with Mg vulnerability only in an allelic genetic model of Asian sub-groups. Conversely, these relationships differed by clinical phenotypes and with ethnic background. Further, *TNFα* contributes towards the central sensitization through enhancing the excitatory and also by reducing inhibitory currents and by activation of COX2, these entire events play a crucial role in the development of inflammatory hyperalgesia [36].

This meta-analysis study has some limitations that must be considered such as; first, literatures available only in the English language were included. Second, stratification examination based on Mg with and without aura was not performed due to lack of equal availability of samples in both the conditions. Third, the genotypic frequencies were not correlated with the serum *TNFα* levels. Fourth, we may not enlighten the underlying interactions of gene-gene and gene-environment mechanisms.

6. Conclusions

To conclude, the association study of *TNFα rs1800629* polymorphism revealed a positive association in South Indian Migraineurs. Nonetheless, the confounding variables related with Mg are necessary to approve our present findings. The meta-analysis results of *TNFα* -308G>A genetic predisposition may represent a vulnerable factor for Mg among Asians but not in Caucasians. Hence, well planned, enormous scale multicentric case-control studies have to be performed in Caucasian, Asian and other populations to identify the potential genetic risk factors and differences leading to Mg pathogenesis.

7. Author contributions

Conceived and designed the experiments: PK, APS, RSRAH, KS, SSSJA, and RV. Performed the experiments: APS, UV, RSRAH and PK. Analyzed the data: RSRAH, APS and PK. Writing, Reviewing and Editing: RSRAH, SSSJA and RV. All authors read and approved the final manuscript.

8. Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee (Ethic code: 390/IHEC/10-17) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

9. Acknowledgment

All the authors were thankful to the patients and controls for participating in this current study. The author (AH) wishes to acknowledge Chettinad Academy of Research and Education (CARE) for providing Chettinad Research fellowship.

10. Funding

This research received no external funding.

11. Conflict of interest

The authors declare no conflict of interest.

12. References

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Supplementary material: Supplementary material associated with this article can be found, in the online version, at https://www.fbscience.com/Elite/articles/10.52586/E880.

Abbreviations: PCR, Polymerase Chain Reaction; ARMS-PCR, Amplification-refractory mutation system-PCR; OR, Odds ratio, CI, Confidence Interval; MA, Mg; MO, Mg without aura; GWAS, Genome-wide association study; LRP1, LDL receptor related protein 1; STAT6, Signal transducer and activator of transcription 6; SDR9C7, Short Chain Dehydrogenase/Reductase Family 9C Member 7; FHL5, Four And A Half LIM Domains 5; UFL1, UFM1-protein ligase 1; TRPM8, Transient receptor potential cation channel subfamily M; HJURP, Holliday junction recognition protein; LINC02210-CRHR1, Long Intergenic Non-Protein Coding RNA 2210-Corticotropin Releasing Hormone Receptor 1; MAPT, Microtubule Associated Protein Tau; MYO1H, Myosin 1H; IFT81, Intraflagellar Transport 81; PTBP2, Polypyrimidine Tract Binding Protein 2; MACF1, Microtubule Actin Crosslinking Factor 1; MHC, Major histocompatibility complex; TNF, Tumor necrosis factor; TNFR1-TNFRSF1A, Tumor necrosis factor receptor 1-Tumor necrosis factor receptor superfamily member 1A; TNFBR-TNFRSF1B, Tumor necrosis factor receptor B- TNF receptor superfamily member 1B; IL1a, Interleukin 1a; IL1b, Interleukin 1b; IL4, Interleukin4; IL5, Interleukin5; *TNF*α, Tumor necrosis factor α; SNP, Single nucleotide polymorphisms; PRISMA, Preferred reporting items for systematic reviews and meta-analyses; IHS, International Headache Society;

HIV, Human immunodeficiency virus; DNA, Deoxyribonucleic acid, USA, Unites States of America; HWE, Hardy–Weinberg Equilibrium; SPSS, Statistical Package for the Social Sciences; PCR-RFLP, PCR-restriction fragment length polymorphism; NOS, Newcastle Ottawa Scale; GBD, Global Burden of Diseases; CGRP, Calcitonin Gene-Related Peptide; NCBI, National center for biotechnology information.

Keywords: Mg; *TNFα*; Association; *rs1800629*; Meta-analysis

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