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RESEARCH

A Comprehensive Investigation of Risk Association Between the -786 T > C, + 884 G > A, VNTR, rs743506, rs3918226 of *eNOS* and Susceptibility of Migraine: A Updated Meta-Analysis Utilizing Trial Sequential Analysis

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Abstract

With a feature of complex pathogenic mechanisms, migraine is a well-known common neurovascular disorder. Multiple genes are responsible for hindering the susceptibility of pain threshold one of which is the *eNOS* gene and its variants. Multiple independent observational studies with case–control design produced conflicting findings, which can be attributed to a variety of factors including varying sample sizes, demographic stratification, technique application, etc. Therefore, in the present study we aimed to find out the precise risk between the selected variant of *eNOS* and the risk of migraine and its clinical subtypes using a meta-analysis approach. To find the association between the risk variants of the *eNOS* gene and migraine, a PRISMA-based systematic literature review strategy was utilized to search via online resources including PubMed and Google Scholar. Using several genetic models, odds ratios with 95% confidence intervals were computed to pool the data. To access heterogeneity, Cochran's Q Test and I2 statistics were utilized, while Begg's and Egger's tests were used to determine publication bias. A p-value of 0.05 or below was deemed statistically significant for all two-sided tests. The present meta-analysis was able to find out the significant protective association between rs743506 and migraine after using dominant (OR: 0.66, CI [0.49–0.86]), over-dominant (OR: 0.56, CI [0.42–0.75]), codominant model (OR: 0.58, CI[0.43–0.77]). Only significant risk association was found between rs1799983, rs3918226, and risk of migraine with aura after utilizing recessive and codominant models i.e., HR vs HW and HR vs HT. The present meta-analysis showed that rs743506 showed a protective association in comparison to rs1799983, rs3918226 which showed significant risk in the MA group. Also, TSA showed non-significant results and therefore, in conclusion, more studies are required to establish risk.

Keywords Migraine · -786 T > C · + 884 G > A · VNTR · rs743506 · rs3918226 · *eNOS*

Introduction

Featured with high prevalence and the third most debilitating disorder, migraine is considered a complex neurovascular disorder (Steiner et al. 2020; Sudershan et al. 2023a, b). The International Classification of Headache Disorder-III has classified migraine into two sub-clinical types i.e., Migraine with Aura (MA) or Migraine without Aura (MWA) based on the criteria of presence or absence of aura phenotype" (ICHD-3.org/1-Migraine/). Susceptibility to migraine is generally attributed to various risk

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variables which are roughly classified as environmental and genetic factors where the former is accountable for hindering the sensitivity threshold of pain established by the latter, i.e., genetic risk factors (Sudershan et al. 2022). The significance of genes for vascular endothelial function in migraine, including eNOS (Endothelial Nitric Oxide Synthase), has been demonstrated by a large number of independent candidate gene association studies carried out in a variety of populations (Toriello et al. 2008; Gruber et al. 2010; Güler et al. 2015; Eröz et al. 2014; Logan et al. 2005; Gonçalves et al. 2011, 2012; Zakerjafari et al. 2016; García-Martín et al. 2020).

*eNOS*is an endothelial form of NOS (Nitric Oxide Synthase) which is long (26 exons spanning 21 kb DNA) and located on the 17th chromosome (7q36.1) (Fig. 1A) and encodes a long protein that acts as a key enzyme act in homodimer (Fig. 1C) for the synthesis of Nitric Oxide (NO) from L-arginine (Oliveira-Paula et al. 2016).Nitric oxide (NO) is an effective, reactive, endogenous vasodilator that easily oxidizes to form stable byproducts like nitrate and nitrite. There are multiple biological processes that it regulates which include platelet aggregation inhibition, cerebral blood flow regulation, activation of nociceptors in the trigeminovascular system, and release of vasoactive neuropeptides during the neurogenic inflammatory response (Chen et al. 2008) and has been implicated in the pathophysiology of migraine (Olesen 2008; Sudershan et al. 2023a, b). *eNOS* represent multiple variants such as -786 T > C, + 884 G > A, VNTR, rs743506, and rs3918226 which are responsible for altering the enzymatic activity and thus have a direct correlation with the level of NO (Oliveira-Paula et al. 2016). These variations (Table 1) have been investigated by several studies to determine the risk of diseases such as rs2070744 (Logan et al. 2005; Gonçalves et al. 2011, 2012; Eröz et al. 2014; Güler et al. 2015; Zakerjafari et al. 2016; García-Martín et al. 2020), rs3918226 Gonçalves et al. 2011, 2012; Güler et al. 2015), VNTR / Intronic-4 variant (Sipahi et al. 2013; Gonçalves et al. 2011, 2012), rs1799983 / + 894 G > A (Borroni et al. 2006; Toriello et al. 2008; Gruber et al. 2010; Gonçalves et al. 2011; Bahadir et al. 2012; Gonçalves et al. 2012; Tajehmiri et al. 2013; Eröz et al. 2014; Güler et al. 2015), rs743506 / Intronic-20 variant (Gonçalves et al. 2011, 2012; Güler et al. 2015) but have shown conflicting results.

As a result, the current study intended to determine the precise risk of migraine and its two sub-clinical types, MA and MWA,

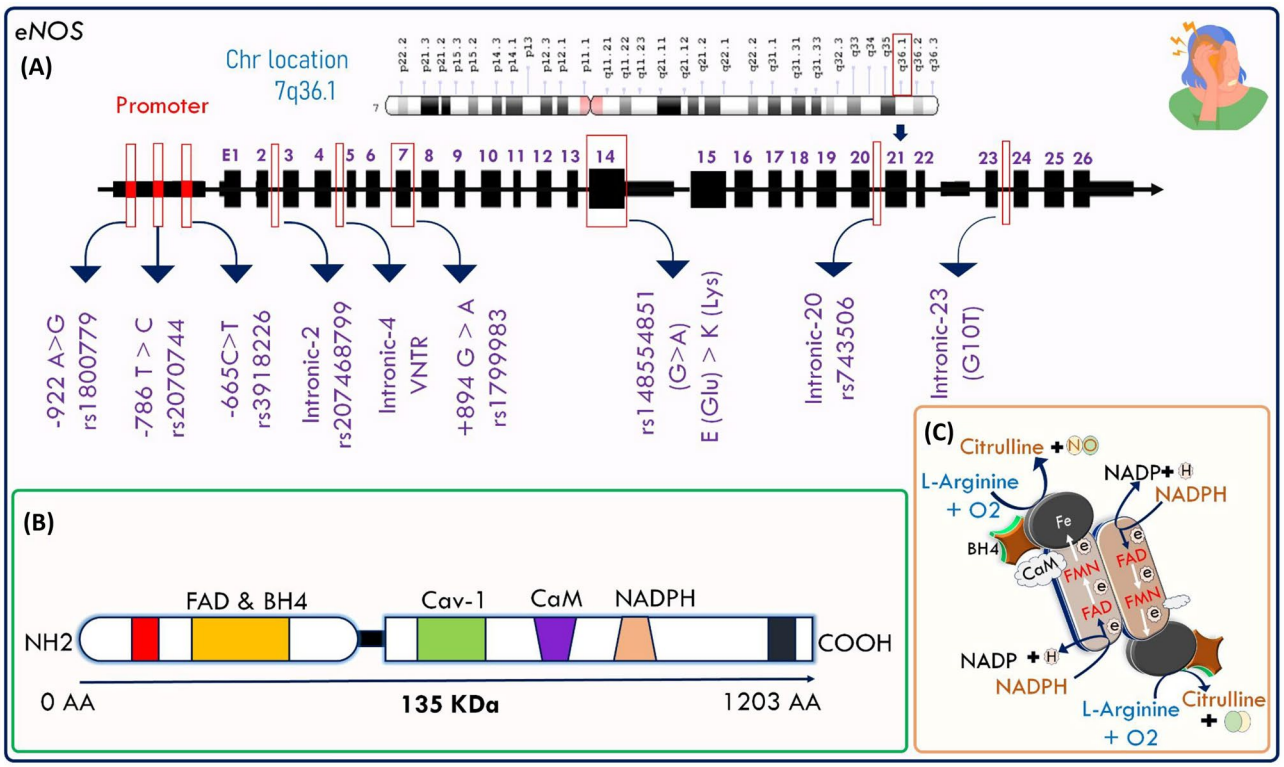

Fig. 1 **A**:eNOS gene with multiple common polymorphisms (MAF > 1%) infographics (Common (1000 Genomes Phase 3 MAF > = 1%) Short Genetic Variants from dbSNP Release 153 (ucsc.edu) **B** Alignment of different regions of eNOS: Cofactors: FAD (flavin adenine dinucleotide) &BH4 tetrahydrobiopterin, Cav-1 (caveolin 1), CaM (calmodulin), NADPH (Nicotinamide adenine dinucleotide phosphate). **C** Alignment of a homodimer of eNOS protein

Table 1 Variant description

S. No.	rs ID	Variant Name	Molecular position	Type	Functional effect	Ref Allele	Risk Allele	Description
1	rs1800779	-922 A > G	Chr-7: 150992855	SNP	Promoter	A	G	An -922 A > G of the eNOS gene is an important functional locus for regulating eNOS gene expression
2	rs2070744	-786 T > C	Chr-7: 150992991	SNP	Promoter	T	G	–786 T > C, leads to a thymine to cytosine change on the eNOS 5' promoter region –786 nt, which causes a reduction of both eNOS gene promoter activity and basal NO production
3	rs3918226	Intronic-1- variant	Chr-7: 150993088	SNP	Intronic	C	T	This SNPis found in close proximity to a possible transcription factor binding site for the Ets family domain, which is essential for the activation of the NOS3 promoter
4	rs207468799	Intronic-2- variant	Chr-7: 150993957	SNP	Intronic	G	A	-
5	rs61722009	Intronic-4- variant	Chr-7: 150997171–7268	In-Del	Intronic	4b	4a	The VNTR in eNOS intron 4 modifies the levels of a 27-nt small interference RNA, which inhibits eNOS expression
6	rs1799983	+ 894 G > A	Chr-7: 150999023	SNP	Missense	G	A	The SNP is located in exon 7 (leads to a glutamine to aspartate substitution in the 298 position of the protein and probably affects the amounts of eNOS located within the caveolae and eNOS activity
7	rs148554851	Exonic	Chr-7: 151002142	SNP	Missense	G	A	The variant is responsible for altering the amino acide sequence E-564 (Glu) > K (Lys)
8	rs743506	Intronic-20-variant	Chr-7: 151010400	SNP	Intronic	A	G	Tag SNP
9	rs7830	Intronic-23-variant	Chr 7: 151012483	SNP	Intronic	G	T	Tag SNP

related to the presence of risk variants such as -786 T > C, + 884 G > A, VNTR, rs743506, and rs3918226 of eNOS.

Method

Literature Survey

A systematic way of literature survey was utilized in the present meta-analysis which was aimed to find out the precise association between the selected variants such as -786 T > C, + 884 G > A, VNTR (Variable number of tandem repeats), rs743506, rs3918226and the risk of migraine and its clinical subtypes (MA and MWA). Online databases and search engines such as PubMed-NCBI (National Center for Biotechnology Information) (Pubmed.NCBI.nlm.nih.gov), and Google Scholar (Scholar.google.com.tw), Semantic Scholars (Research Dashboard | Semantic Scholar) respectively were utilized.

For the literature survey, we used multiple key terms such as "gene polymorphism and risk of migraine", *OR* "*eNOS* variants and risk of migraine", *OR* "rs743506 and risk of migraine", *OR* "intronic-20 variant and risk of migraine", *AND* "rs1799983 and risk of migraine", *OR* "+ 894 G > A and risk of migraine", *AND* "rs2070744, and risk of migraine" *OR* "-786 T > C and risk of migraine", *AND* "rs3918226 and risk of migraine" *OR* "-665 C > T and risk of migraine", *AND* "VNTR and risk of migraine" *OR* "intron-4 variant and risk of migraine".

Both the search strategy and the decision-making process for choosing appropriate information for the review complied with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria (www.prisma-statement.org). We went to considerable measures to eliminate any unfavorable study data that was either unpublished / Research Square (www.researchsquare.com), incomplete, or only partially available, book chapters, conference papers, etc., to reduce the possibility of bias. Three

of our authors (A.S, M.B,& K.S) were responsible for the literature survey.

Inclusion and Exclusion Features

Concerning the study inclusion criteria as mentioned in the previous study (Sudershan et al. 2023a, b), such as (1): variants of interest such as -786 T > C, + 884 G > A, VNTR (Variable number of tandem repeats), rs743506, rs3918226 must be investigated with a case–control or cohort study design must be the prime requirement, (2): the "authors must have diagnosed the patients according to the criteria of the International Headache Society (IHS) or ICHD-2/3" (International Classification of Headache Disorders-II/ III), (3): "the genotype frequencies for the polymorphisms studied among migraineurs and non-migraineurs must be reported in the paper in detail", (4): "all studies must be within the Hardy–Weinberg Equilibrium (HWE)", (5):" studies should provide clear data to calculate the odds ratios (ORs) and the corresponding 95% confidence intervals (CI)". Studies that were based on pedigrees, as well as genome scans, were not accepted for analysis because both of these types of studies investigate linkage. Also, if an article does not contain the necessary data, the necessary data were either extracted from a previously published article or the author of the article in question was contacted.

Data Extraction

Different features from each included study were extracted such as the number of subjects (case and control), source of control such as population-based (PB) or Hospital Based (HB), source of patients such as Clinic/Hospital Based or Population-Based, and what diagnostic criteria were used. Also, location, ethnicity, the genotypic and allelic frequency of both cases and controls, the first authors along with the publication year, and, lastly, the genotyping method that was applied were collected. Additionally, it was verified that each study had received ethical board clearance before conducting its observation study. Prior research and references were checked if any statistical or numerical data were identified to be missing. Our three authors, M.B, A.C.P, and K.S., were in charge of extracting all of the data and characteristics, and our other two authors (A.S. and P.K.) were responsible for analyzing the quality of the data.

Quality Assessment-Newcastle–Ottawa Scale (NOS)

The evaluation of the quality of the included studies is one of the most essential factors to consider when conducting a meta-analysis. Therefore, the current study evaluated the quality of all prior published studies using the criteria present in the Newcastle–Ottawa scale (NOS). (Ottawa Hospital Research Institute (ohri.ca). If any differences in decision-making were noticed concerning article inclusion, data extraction, or quality assessment/ NOS, the third investigator (H.K) investigated and concluded the matter.

Statistical Analysis

First, the genotypic and allelic frequencies were determined for each of the studies that were incorporated into the meta-analysis. Next, the Chi-square test was performed to determine whether or not the population is in HWE ($p > 0.05$ for populations in HWE; $p < 0.05$ for populations not in HWE). The tests of Begg's and Egger's, as well as the $\chi 2$ based on Cochran's Q Test with I-square (I2) tests, were used to evaluate the publication bias, which included the reporting bias, and the heterogeneity of the research papers, respectively.

The current study used logistic regression using the OR (Odds Ratio) model along with a 95% CI (Confidence Interval) and a p-value of ≤ 0.05 to determine the precise association between the variations of interest and migraine. Different genetic models such as allelic (Rare/R vs Wild/W), dominant (HR + HT vs HW), recessive (HR vs HW + HT), and over-dominant (HT vs HW + HR), Codominant model (HR vs HW, HR vs HT, HT vs HW) were used to observe the strength of association (OR) using random: Dersimonian and Laird method or fixed model (Inverse variance method) based on I2 (I2 > 50%: Random model).

Additionally, using the criteria "exclusion of each study," we performed a sensitivity analysis to determine how different studies affected the pooled ORs. Every test was conducted using a two-sided approach, and a result of less than 0.05 was regarded as statistically significant. Every step of the current meta-analysis procedure, from choosing the statistical tests to use to assess the findings, was carried out following the Cochrane principles (Training.cochrane.org/handbook/current). The meta-analysis was conducted by the online tool "MetaGenyo: Meta-Analysis of Genetic Association Studies (GAS), which has been exclusively designed by Marugan and group to carry out the meta-analysis of gene association study (Martorell-Marugan et al. 2017).

Trial Sequential Analysis

Trial Sequential Analysis (TSA), a modern technique, was employed in the current meta-analysis to reduce random errors by determining whether or not the studies included in the meta-analysis had insufficient sample sizes. TSA tool was used (Copenhagen Trial Unit, Denmark) based on a 5% overall risk and a relative risk reduction of 10–20% (with 80% power) (TSA – ctu.dk).

Result

Study Characteristics

By conducting a systematic literature search following the PRISMA guidelines, we found 123 studies after an initial search in the database. After critical analysis as depicted in the PRISMA flow diagram (Fig. 2), we selected 15 final studies that describe the association of different variants of the eNOS gene with migraine. After selection, the features (depicted in Section "Data Extraction") were recorded, where it was found incomplete data of Papasavva and group (Papasavva et al. 2023) was therefore excluded from the study. After the inclusion of the studies, we checked the quality of each independent study using the New-Ottawa Scale (NOS) where a threshold of > 5 points was considered a good study (Table 2). After the quality assessment, two studies were excluded (Ishii et al. 2014; Tutunchi & Akhavan, 2016) due to its poor quality (quality score: <5). After a close look at the published studies by Güler et al. (2015), Gruber et al. (Gruber et al. 2010), and Toriello et al. (2008), we found that they gave the wrong rsID (for example, rs1800779) for the -786 variation. Also, the prior meta-analysis by Ding et al. (2018) made an error by misrepresenting the work/ genotypic data of Eroz et al. (2014).

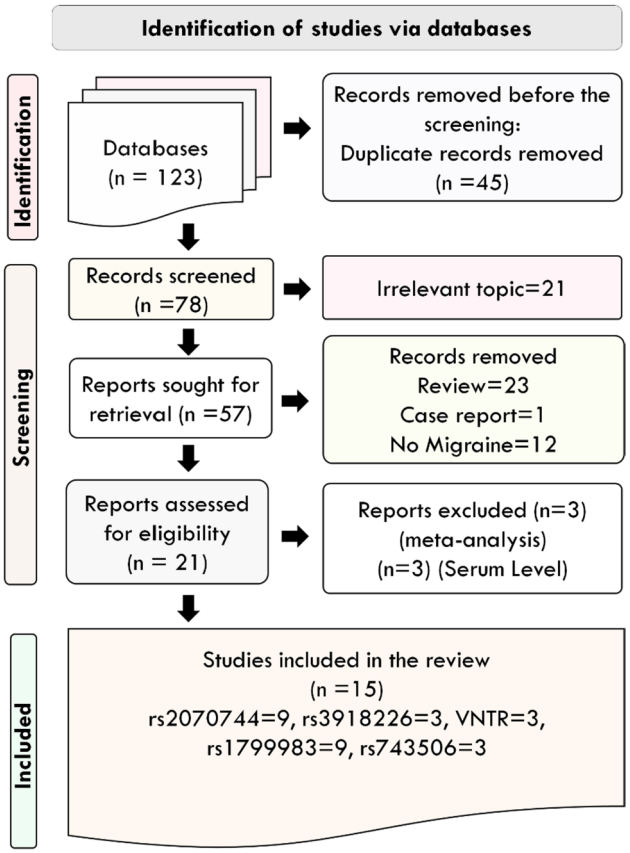

Fig. 2 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram representing the literature survey

Meta-Analysis

rs2070744 / -786 T > C

Another upstream variant ie., -786 T > C located in the promoter region and is found to be studied by 9 independent studies (Logan et al. 2005; Gonçalves et al. 2011, 2012; Gruber et al. 2010; Eröz et al. 2014; Güler et al. 2015; Toriello et al. 2008; Zakerjafari et al. 2016; García-Martín et al. 2020) in different regions (Table 3). The pooled sample size was 1437 for cases and 1265 for control and it was observed that the pooled risk variant genotypic frequency is decreased (HR: 20.66%) than control (HR: 22.21%) in the overall migraine group as well as in MA and MWA types (Table 4). The pooled frequency of risk variants was found slightly higher in overall migraine cases (q = 0.4606) than in controls (q = 0.4573).

After pooling, we observed a non-significant risk association using different genetic models such as allelic (OR: 1.08, CI [0.82–1.42], I2:81.16%-random model) (Fig. 3A), recessive (OR: 0.91, CI [0.62–1.32], I2: 65.66%- random model), etc. (Table 5). After subgrouping into clinical subtypes, a non-significant association was observed for all genetic models utilized in MWA and MA (Table 5). Also, subgroups using the criteria of "ethnicity" non-significant were observed for all genetic models in overall migraine as well in both types (SF: Tables 1–4).

Egger's test performed using a linear regression model revealed no evidence of publication bias (Fig. 3B). By removing each study one at a time, sensitive analysis was carried out for all genetic models (Fig. 4). It was shown that none of the pooled ORs were significantly impacted, demonstrating strong stability of the meta-analysis findings (SF: Tables 1–4).

rs3918226/ Intronic-1 Variant

The third variant i.e., intronic-1 variant (T > C)/ rs3918226, three studies explored the risk (Gonçalves et al. 2011, 2012; Güler et al. 2015) (Table 6). The pooled sample was n = 503 cases and n = 341 in controls which showed that the risk variant genotypic frequency was slightly increased in overall migraine as well as in both of its types (Table 4). However, a slight decrease in risk allele frequency was observed in case (q = 0.062) in contrast to control (q = 0.068).

Using the pooled OR, the present didn't observe any significant association with overall migraine in contrast to recessive, codominant variant (HR vs HT and HT vs HW) which showed significant risk association with an association value of OR: 7.20, CI [1.12–46.1], OR: 6.95, CI

Table 2 Newcastle–Ottawa Scale (NOS) for assessing the quality of non-randomized studies in meta-analyses[a]

Author/ Reference	Selection				Comparability	Exposure			Final Score
	Is the case definition adequate+	Representativeness of the cases	Selection of Controls	Definition of Controls	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate	
Logan et al., 2005	Specialist	Hospital	PB	Yes	Yes (1)	Interview	Yes	NG	7
Borroni et al., 2006	IHS	Hospital	HB	Yes	Yes (1)	Interview	Yes	NG	6
Toriello et al., 2008	IHS	Hospital	HB	Yes	Yes (1)	Interview	Yes	NG	6
Gruber et al., 2010	IHS	Hospital	HB	Yes	Yes (1)	Interview	Yes	NG	6
Goncalves et al., 2011	ICHD	Hospital	PB	Yes	Yes (1)	Interview	Yes	NG	7
Bahadir et al., 2012	ICHD-II	Hospital	HB	Yes	Yes (1)	Interview	Yes	NG	6
Goncalves et al., 2012	ICHD	Hospital	PB	Yes	Yes (1)	Interview	Yes	NG	7
Sipahi et al., 2013	ICHD-II	Hospital	HB	Yes	Yes (1)	Interview	Yes	NG	6
Tajehmiri et al., 2013	NG	Hospital	HB	Yes	Yes (1)	Interview	Yes	NG	5
Eroz et al., 2014	ICHD-II	Hospital	HB	Yes	Yes (1)	Interview	Yes	NG	6
Guler et al., 2014	ICHD-II	Hospital	HB	Yes	Yes (1)	Interview	Yes	NG	6
Ishii et al., 2014	ICHD-II	Hospital	NG	Yes	NG	Interview	NG	NG	4
Garcia-Martin et al., 2020	ICHD-II	Hospital	HB	Yes	Yes (1)	Interview	Yes	NG	6
Papasavva et al., 2022	ICHD-III	Hospital	HB	Yes	Yes (2)	Interview	Yes	NG	7
Tutunchi & Akhavan, 2016	NG	NG	NG	NG	Yes (1)	NG	NG	NG	1

HB: Hospital Based, **PB:** Population Based, **IHS:** International Headache Society, **ICHD-2/3:** International Classification of Headache Disorders-2/3, **NG:** Not Given

Positive Point **No Point/ Study Excluded** **Study Included**

Note: ≥ 5 points / stars out of 9 are consider as a good study and "< 5 points" are considered as a high risk/ biased study

[1.08–44.6] and 8.76, CI [1.27–60.3] respectively in MA group (Table 5). After subgroup analysis in different ethnic groups such as Caucasian and Brazilian, no significant association was observed in the overall migraine group as well as in either group (SF: Tables 3 and 5). All genetic models had a p-value larger than 0.05, indicating that there was no publication bias. A sensitive study performed on all genetic models by methodically deleting individual studies revealed that the pooled ORs were not significantly affected, validating the meta-analysis's high stability (SF: Tables 3 and 5).

VNTR / Intronic-4 Variant/rs61722009

Concerning the VNTR, 3 studies (Sipahi et al. 2013; Gonçalves et al. 2011, 2012) have explored the risk (Table 7). After pooling the result of independent studies which showed a pooled sample size of n = 433 and n = 313 in case and control respectively. The pooled risk genotypic frequency (HR: 20.78%) (q = 0.3475) was lower in overall migraine cases than in control (HR: 23.32%) (q = 0.3945). However, after subgrouping, it was observed that the risk variant genotypic

Table 3 Details of -786 t > c / rs2070744

S. No	Country	Ethnicity	Ethical Permission	Patient Source	Diagnosis	Control Source	Case/ Control	Technique	Type	Case			Control			HWE	Study Name
										TT	TC	CC	TT	TC	CC		
1	UK	Caucasian	✓	HB	NM	PB	24/37	PCR–RFLP	Migraine	10	11	3	14	18	5	0.93	Logan et al. (2005)
2	Brazil	Brazilian	✓	HB	ICHD	PB	178/117	Taq-MAN	Migraine	73	85	20	51	51	15	0.93	Gonçalves et al. (2011)
									MA	17	21	6					
									MWA	56	64	14					
3	Brazil	Brazilian	✓	HB	ICHD	PB	150/99	Taq-MAN	Migraine	65	71	14	45	44	10	0.93	Gonçalves et al. (2012)
									MA	17	21	5					
									MWA	48	50	9					
4	Austria	Caucasian	✓	HB	IHS	HB	54/76	Taq-MAN	Migraine	16	26	12	25	39	12	0.61	Gruber et al. (2010)
									MA	6	11	3					
									MWA	10	15	9					
5	Turkey	Caucasian	✓	HB	ICHD-II	HB	176/123	PCR–RFLP	Migraine	36	100	40	14	51	58	0.93	Eröz et al. (2014)
									MA	18	59	15					
									MWA	18	41	25					
6	Turkey	Caucasian	✓	HB	ICHD-II	HB	175/125	Pyro-sequencing	Migraine	18	86	71	12	67	46	0.54	Güler et al. (2015)
									MA	7	26	25					
									MWA	11	60	46					
7	Spain	Caucasian	✓	HB	IHS	HB	337/341	RT-PCR	Migraine	78	175	84	88	176	77	0.6177	Toriello et al. (2008)
									MA	41	100	47					
									MWA	37	75	37					
8	Iran	Caucasian	✓	HB	NM	HB	60/60	PCR–RFLP	Migraine	22	32	6	51	8	1	0.93	Zakerjafari et al. (2016)
9	Spain	Caucasian	✓	HB	ICHD-II	HB	283/287	Taq-MAN	Migraine	92	144	47	89	141	57	0.931	García-Martín et al. (2020)

Table 4 Pooled genotypic frequency of all eNOS gene variants

Variant	Genotype	Migraine		MA		MWA	
		Case	Control	Case	Control	Case	Control
rs2070744	HW	28.53%	30.75%	23.82%	26.67%	29.78%	26.67%
	HT	50.80%	47.03%	53.48%	48.58%	49.40%	48.58%
	HR	20.66%	22.21%	22.69%	24.74%	20.81%	24.74%
rs3918226	HW	88.46%	86.21%	87.58%	86.21%	88.82%	86.21%
	HT	10.53%	13.78%	10.34%	13.78%	10.61%	13.78%
	HR	0.99%	0%	2.06%	0%	0.55%	0%
VNTR	HW	51.27%	44.40%	66.66%	63.88%	65.56%	63.88%
	HT	27.94%	32.26%	26.43%	32.40%	30.29%	32.40%
	HR	20.78%	23.32%	6.89%	3.70%	4.14%	3.70%
rs1799983	HW	45.27%	46.84%	37.31%	42.47%	47.46%	42.47%
	HT	43.19%	43.30%	47.53%	46.83%	42.40%	46.83%
	HR	11.52%	9.85%	15.15%	10.69%	10.13%	10.69%
rs743506	HW	58.44%	48.09%	55.03%	48.09%	59.88%	48.09%
	HT	32.00%	45.45%	32.21%	45.45%	31.92%	45.45%
	HR	9.54%	6.45%	12.75%	6.45%	8.19%	6.45%

frequency was quite higher in both MA as well in MWA than the control (Table 4). The present meta-analysis observed a non-significant association in both MA and MWA as well with overall migraine (Table 5). After subgrouping into different ethnic groups, no significant association was observed (SF: Tables 7–8).

Using Egger's test, it was observed that all genetic models had p-values larger than 0.05, indicating that there was no publication bias. The pooled ORs were not significantly changed, according to the results of a sensitive analysis that was conducted on all genetic models by methodically deleting individual studies, demonstrating the high stability of the meta-analysis(SF: Tables 7–8).

rs1799983 / + 894 G > A

Concerning rs1799983, a total of 9 studies (Table 9) with a sample size of n = 1345 in cases and n = 1157 in controls, were found to be eligible for pooling the result (Borroni et al. 2006; Toriello et al. 2008; Gruber et al. 2010; Gonçalves et al. 2011, 2012; Bahadir et al. 2012; Tajehmiri et al. 2013; Eröz et al. 2014; Güler et al. 2015). Concerning the genotypic and allelic (q) frequency, the risk genotype and allele (q)were found higher in the overall migraine (HR: 11.52%) (q = 0.3312 vs q = 0.3150) group as well as in MA (HR: 15.15%) than in control (HR: 9.85% & 10.69%) respectively in contrast to MWA cases (Table 4).

After pooling, we observed a non-significant association between the risk variant and overall migraine (Table 5). But after subgrouping into clinical sub-type, a significant risk association was observed after utilizing recessive (OR: 1.57, CI [1.14–2.16]) codominant (HR vs HW and HR vs HT) with an association value of OR: 1.69, CI [1.18–2.41] and OR: 1.58, CI [1.12–2.22] respectively in MA group in contrast to MWA where the non-significant association was observed (Table 5).

Subgrouping into different ethnic groups, we observed a significantly increased risk in Iranian (dominant, over-dominant, and codominant model) and Brazilian populations (SF: Table 5). In the case of MA and MWA sub-types, an overall decreased risk was observed in the Brazilian population using different models in both groups (SF: Tables 10 and 11).

All genetic models exhibited p-values greater than 0.05, according to Egger's test, proving that there was no publication bias. A sensitive analysis was performed on all genetic models by eliminating individual trials, and the findings showed that the pooled ORs were not significantly changed, demonstrating the great stability of the meta-analysis(SF: Tables 5–11).

rs743506 / Intronic-20 Variant

Concerning the intronic-20 variant which was studied by 3 independent groups (Gonçalves et al. 2011, 2012; Güler et al. 2015) (Table 8) and after pooling, we observed a significant increase in risk genotypic frequency in overall migraine as well as in both subtype (Table 4). However, a decreased frequency of risk variant in case (q = 0.2554) in contrast to control (q = 0.2917) was observed.

Concerning the risk attribution, the present meta-analysis was able to observe a decreased risk of overall migraine after utilizing different genetic models such as dominant (OR: 066, CI [0.49–0.86]) (Fig. 5A), over-dominant (OR: 0.56,

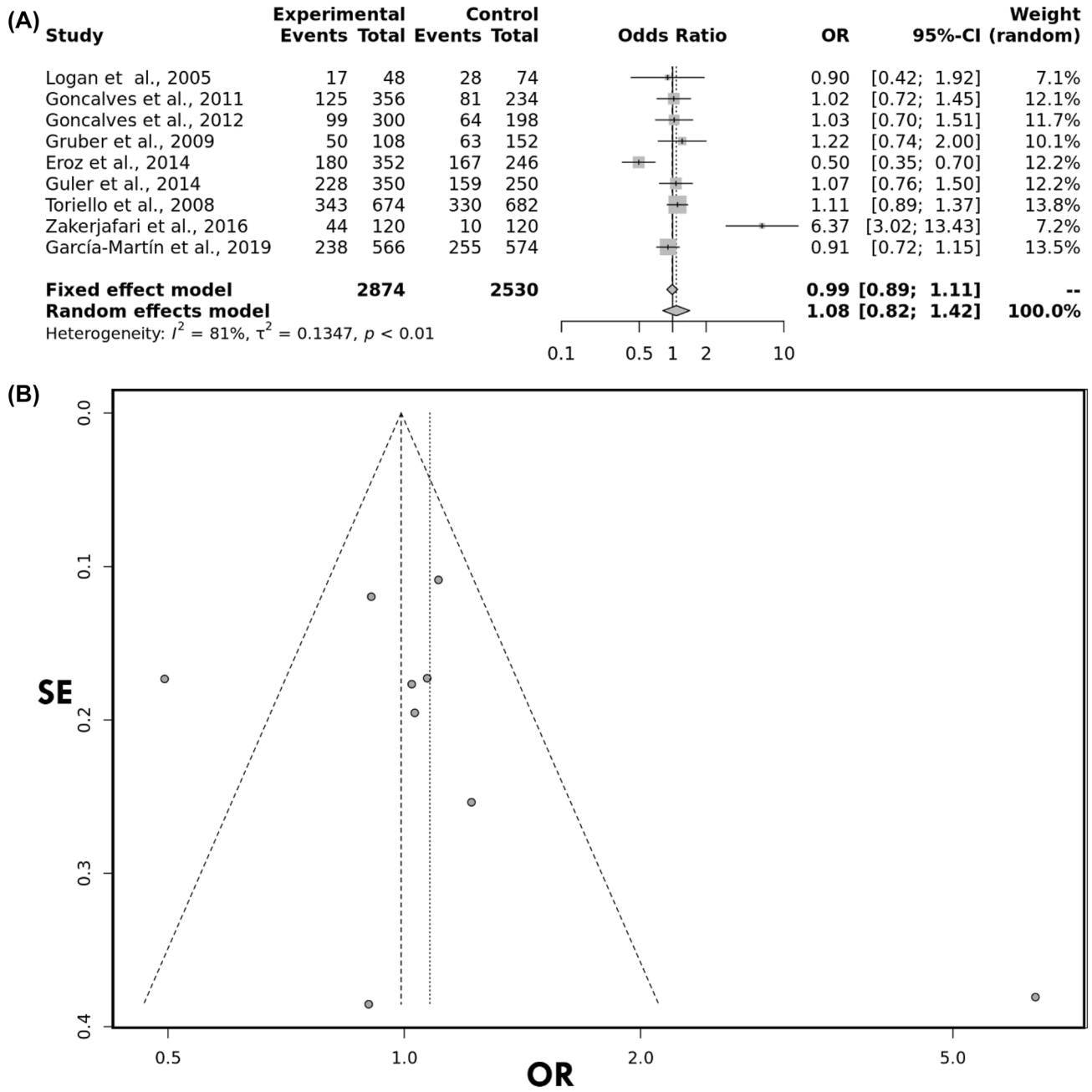

Fig. 3 **A** Forest plot representing the non-significant risk association using allele genetic model of rs2070744. **B** Funnel plot representing no publication bias rs2070744

CI [0.42–0.75], and codominant model (HT vs HW) with an association value of OR: 0.58, CI [0.43–0.77] (Table 5). After subgrouping into MA and MWA, the decreased risk was observed for both sub-types (Table 5) in contrast to significantly increased risk in MA using recessive and codominant model (HR vs HT) with an association value of OR: 2.10, CI [1.08–4.06] and OR: 2.79, CI [1.37–5.65] respectively.

Egger's test, which is based on the connection between standard error and strength of association (log of OR), was used to examine publication bias across all studies included in the meta-analysis. By placing the most accurate research on top and the least precise studies at the bottom of a scatter plot, we were able to create a "funnel plot" that displays the distribution of accuracy across all investigations. All genetic models resulted in symmetrical funnel plots, indicating that no publication bias existed (Fig. 5B). The findings of a sensitive analysis performed on all genetic models by systematically removing individual studies showed that the pooled

Table 5 Pooled Odds Ratio with 95% Confidence Interval of all variants of eNOS gene

Type	Genetic Model	rs743506			rs1799983			rs2070744			rs3918226			VNTR		
		OR	CI	I^2 (M)	OR	CI	I^2 (M)	OR	CI	I^2 (M)	OR	CI	I^2 (M)	OR	CI	I^2 (M)
Migraine	Allele	0.83	0.66-1.03	0	1.14	0.88-1.47	0.729	1.08	0.81-1.42	0.8116	0.9	0.61-1.33	0	0.97	0.74-1.26	0
	Recessive	1.55	0.53-4.54	0.724	1.25	0.95-1.63	0	0.91	0.62-1.32	0.6566	2.8	0.46-17.0	0	1.22	0.74-1.99	0
	Dominant	0.66	0.49-0.86	0	1.24	0.79-1.93	0.83	1.16	0.80-1.68	0.7363	0.82	0.54-1.23	0	0.88	0.61-1.24	0.15
	Over-Dom	0.56	0.42-0.75	0.504	1.14	0.74-1.73	0.825	1.24	0.92-1.65	0.6673	0.74	0.48-1.12	0	0.81	0.58-1.10	0
	HR vs HW	1.22	0.47-3.11	0.620	1.41	0.87-2.25	0.484	0.94	0.61-1.43	0.5986	2.7	0.44-16.4	0	0.97	0.43-2.18	0.2578
	HR vs HT	2.15	0.62-7.39	0.773	1.29	0.97-1.70	0	0.87	0.62-1.19	0.5081	3.72	0.59-23.4	0	1.43	0.85-2.38	0
	HT vs HW	0.58	0.43-0.77	0.004	1.22	0.75-1.97	0.841	1.19	0.85-1.67	0.6442	0.75	0.49-1.13	0	0.83	0.57-1.20	0.2812
MA	Allele	0.99	0.73-1.33	0	1.18	0.88-1.56	0.642	0.94	0.97-1.32	0.7197	1.06	0.62-1.81	0	1.01	0.65-1.57	0
	Recessive	2.1	1.08-4.06	0.148	1.57	1.14-2.16	0	0.83	0.43-1.57	0.7579	7.2	1.12-46.1	0	1.92	0.64-5.70	0
	Dominant	0.77	0.51-1.12	0	1.19	0.70-2.00	0.779	1.07	0.80-1.40	0	0.89	0.49-1.60	0	0.88	0.52-1.49	0
	Over-Dom	0.58	0.38-0.86	0	0.99	0.59-1.66	0.789	1.22	0.85-1.73	0.5041	0.73	0.39-1.35	0	0.75	0.43-1.30	0
	HR vs HW	1.73	0.87-3.41	0.036	1.69	1.18-2.41	0.328	0.84	0.43-1.62	0.6253	6.95	1.08-44.6	0	1.77	0.59-5.35	0
	HR vs HT	2.79	1.37-5.65	0	1.58	1.12-2.22	0.163	0.80	0.42-1.50	0.7257	8.76	1.27-60.3	0	2.28	0.71-7.25	0
	HT vs HW	0.63	0.41-0.96	0	1.1	0.61-1.96	0.800	1.12	0.75-1.51	0	0.75	0.40-1.38	0	0.78	0.44-1.37	0
MWA	Allele	0.77	0.60-0.97	0	1.04	0.80-1.34	0.641	0.97	0.83-1.13	0.4406	0.85	0.55-1.31	0	0.96	0.69-1.33	0
	Recessive	1.2	0.29-4.85	0.774	1.13	0.82-1.56	0	0.91	0.69-1.18	0.3118	4.43	0.21-93.3	0	1.12	0.43-2.91	0
	Dominant	0.62	0.45-0.83	0	0.97	0.66-1.40	0.682	1.01	0.78-1.29	0.0381	0.8	0.51-1.25	0	0.93	0.63-1.36	0
	Over-Dom	0.56	0.32-0.96	0.668	0.96	0.66-1.39	0.688	1.08	0.87-1.33	0	0.75	0.48-1.19	0	0.91	0.60-1.34	0
	HR vs HW	0.94	0.27-3.27	0.702	1.1	0.77-1.56	0.379	0.89	0.64-1.24	0.322	4.14	0.2-87.14	0	1.09	0.41-2.85	0
	HR vs HT	1.67	0.32-8.50	0.820	1.2	0.85-1.68	0	0.91	0.69-1.20	0.0462	7.61	0.33-173	0	1.2	0.44-3.22	0
	HT vs HW	0.57	0.41-0.77	0.39	0.94	0.63-1.39	0.684	1.05	0.80-1.35	0	0.76	0.48-1.20	0	0.91	0.61-1.35	0

*Legends

Non-significant Association
Decreased risk-association/ Protection
Increased risk-association
High Heterogeneity using Random effect Model
Low Heterogeneity using Fixed effect Model

ORs were not significantly altered, confirming the excellent stability of the meta-analysis (Fig. 5C).

After subgrouping into different ethnic group, we observed decreased risk in Brazilian population in overall migraine using dominant (OR: 0.63, CI [0.44–0.88], over-dominant (OR: 0.46, CI [0.31–0.64], and codominant model (OR: 0.50, CI [0.34–0.71]) in contrast to recessive model (OR: 2.70, CI [1.31–5.54]) (SF: Table 12). Also, decreased risk was observed in both MA and MWA in the Brazilian population (SF: Tables 12–14).

Using Egger's test, it was observed that all genetic models had p-values larger than 0.05, indicating that there was no publication bias. The pooled ORs were not significantly changed, according to the results of a sensitive analysis that was conducted on all genetic models by methodically deleting individual studies, demonstrating the high stability of the meta-analysis(SF: Tables 12–14).

Enclosing the section, the pooled analysis has shown that only rs743506 was found to be associated with migraine and its clinical subtype where it decreases the risk of decrease. Another important variant i.e., rs1799983 showed increased risk only in MA patients.

Trial Sequential Analysis

After combining the independent studies to find whether the pooled sample is adequate for confirming the risk of diseases or not? TSA graphs have shown that the last point of

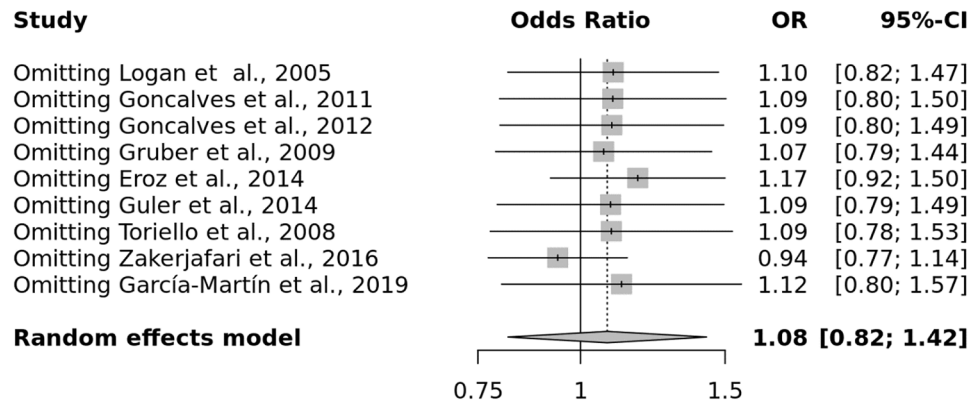

Fig. 4 Sensitivity analysis of rs2070744

Table 6 Details of rs3918226

S. No.	Country	Ethnicity	Ethical Permission	Patient Source	Diagnosis	Control Source	Case/ Control	Technique	Type	Case			Control			HWE	Study Name
										HW	HT	HR	HW	HT	HR		
9	Brazil	Brazilian	✓	HB	ICHD	PB	178/117	Taq-MAN	Migraine	159	16	3	100	17	0	0.48	Gonçalves et al. (2011)
									MA	38	5	1					
									MWA	121	11	2					
14	Brazil	Brazilian	✓	HB	ICHD	PB	150/99	Taq-MAN	Migraine	135	14	1	86	13	0	0.48	Gonçalves et al. (2012)
									MA	37	5	1					
									MWA	98	9	0					
20	Turkey	Caucasian	✓	HB	ICHD-II	HB	175/125	Pyro-sequencing	Migraine	151	23	1	108	17	0	0.48	Güler et al. (2015)
									MA	52	5	1					
									MWA	99	18	0					

Table 7 Details of Intronic 4-VNTR

S. No.	Country	Ethnicity	Ethical Permission	Patient Source	Diagnosis	Control Source	Case/ Control	Technique	Type	Case			Control			HWE	Study Name
										HW	HT	HR	HW	HT	HR		
1	Turkey	Caucasian	✓	HB	ICHD-II	HB	105/97	PCR–RFLP	Migraine	6	25	74	1	31	65	0.58	Sipahi et al. (2013)
2	Brazil	Brazilian	✓	HB	ICHD	PB	178/117	Taq-MAN	Migraine	116	53	9	75	38	4	0.97	Gonçalves et al. (2011)
									MA	29	12	3					
									MWA	87	41	6					
3	Brazil	Brazilian	✓	HB	ICHD	PB	150/99	Taq-MAN	Migraine	100	43	7	63	32	4	0.97	Gonçalves et al. (2012)
									MA	29	11	3					
									MWA	71	32	4					

Table 8 Details of rs743506

S. No	Country	Ethnicity	Ethical	Patient Source	Diagnosis	Control Source	Case/ Control	Technique	Type	Case			Control			HWE	Study Name
										HW	HT	HR	HW	HT	HR		
1	Brazil	Brazilian	✓	HB	ICHD	PB	178/117	Taq-MAN	Migraine	102	53	23	53	58	6	0.07	Gonçalves et al. (2011)
									MA	23	15	6					
									MWA	79	38	17					
2	Turkey	Caucasian	✓	HB	ICHD-II	HB	175/125	Pyro-sequencing	Migraine	104	61	10	64	49	12	0.56	Güler et al. (2015)
									MA	37	18	7					
									MWA	67	43	3					
3	Brazil	Brazilian	✓	HB	ICHD	PB	150/99	Taq-MAN	Migraine	88	47	15	47	48	4	0.07	Gonçalves et al. (2012)
									MA	22	15	6					
									MWA	66	32	9					

the Z-curve reached or is positioned within the conventional boundary which is considered a statistically non-significant zone therefore, we cannot conclude that there is any risk association between the variant under study (rs1799983) and diseases (Fig. 6). Therefore, to achieve power further studies are required (SF: Figs. 1–4).

Discussion

Polygenic inheritance is believed to be responsible for determining the pain threshold in migraine, which contributes to the disorder's complexity (Sudershan et al. 2022). The eNOS gene is recognized as one of the candidate genes that is crucial for the normal functioning of endothelial cells / vascular systems (Tran et al. 2022). The gene is long (26 exons spanning 21 kb DNA) (Fig. 1A) and encodes a long protein (1203 aa) (Fig. 1B) (NOS3 Gene—GeneCards | NOS3 Protein | NOS3 Antibody) which consists of multiple domains (Fig. 1B) such as FAD (flavin adenine dinucleotide) & BH4 tetrahydrobiopterin, Cav-1 (caveolin 1), CaM (calmodulin), NADPH (Nicotinamide adenine dinucleotide phosphate) important for the binding of the respective cofactors (*See review*) (Alderton et al. 2001). The active form of protein occurs in homodimer form (Fig. 1C) and is responsible for the synthesis of Nitric Oxide (NO) (Nathan and Xie 1994) which is an effective endogenous vasodilator that regulates cerebral blood flow regulation, nociceptors in the trigeminovascular system, and regulate the release of vasoactive neuropeptides during the neurogenic inflammatory response (Chen et al. 2008; Olesen 2008; Sudershan et al. 2023a, b). Also, NO enhances the CGRP release which in turn results in enhanced vasodilation and mast cell degranulation (Messlinger et al. 2012).

For enzymes to be activated, a high concentration of calcium must be present in the cytoplasm, which is secreted from the endoplasmic reticulum after being activated by numerous signaling molecules such as estrogen, membrane depolarization, neurotransmitters autocoid (serotonin), etc. (Fig. 7) (KEGG: Kyoto Encyclopedia of Genes and Genomes). Upon production of NO in endothelial cells, the NO is transported into smooth muscle cells where it acts as a ligand for soluble Guanine Cyclase which is responsible for the production of cGMP (Cyclic Guanosine Monophosphate) (Fig. 8). NO is immediately converted into nitrate and nitrite where migraine patients it's both types (MA and MWA) have shown a significantly increased nitrate (Gruber et al. 2010).

The eNOS protein is necessary for nitric oxide production, however, any change in the regulation of the enzyme's activity will affect nitric oxide production (Forstermann and Sessa 2012). Numerous variations of eNOS, including -786 T > C, + 884 G > A, VNTR, rs743506, and rs3918226

Table 9 Details of894 g > t rs1799983 Glu298Asp

S. No.	Country	Ethnicity	Ethical	Patients Source	Diagnosis	Control Source	Case/ Control	Technique	Type	Case			Control			HWE	Study Name
										HW	HT	HR	HW	HT	HR		
1	Italy	Caucasian	✓	HB	IHS	HB	156/125	NM	Migraine	80	53	23	59	50	16	0.34	Borroni et al. (2006)
									MA	24	16	13					
									MWA	56	37	10					
2	Spain	Caucasian	✓	HB	IHS	HB	337/341	RT-PCR	Migraine	103	165	69	104	177	60	0.34	Toriello et al. (2008)
									MA	53	94	42					
									MWA	50	71	28					
3	Austria	Caucasian	✓	HB	IHS	HB	54/76	Taq-MAN	Migraine	21	28	5	31	34	11	0.735	Gruber et al. (2009)
									MA	8	12	0					
									MWA	13	16	5					
4	Brazil	Brazilian	✓	HB	ICHD	PB	178/117	Taq-MAN	Migraine	109	54	15	61	51	5	0.341	Gonçalves et al. (2011)
									MA	29	11	4					
									MWA	80	43	11					
5	Turkish	Caucasian	✓	HB	ICHD-II	HB	51/51	PCR–RFLP	Migraine	2	43	6	22	27	2	0.32	Bahadir et al. (2012)
									MA	2	23	4					
									MWA	0	20	2					
6	Brazil	Brazilian	✓	HB	ICHD	PB	150/99	Taq-MAN	Migraine	95	44	11	51	44	4	0.34	Gonçalves et al. (2012)
									MA	28	11	4					
									MWA	67	33	7					
7	Iran	Iranian	✓			HB	67/100	ARMS-PCR	Migraine	56	12	0	93	6	1	0.258	Tajehmiri et al. (2013)
8	Turkey	Caucasian	✓	HB	ICHD-II	HB	176/123	PCR–RFLP	Migraine	44	116	16	62	54	7	0.34	Eröz et al. (2014)
									MA	20	64	8					
									MWA	24	52	8					
9	Japan	Asian	✓	HB	ICHD-II	HB	47/NC	PCR–RFLP	Migraine	39	8	0	NA			NA	Ishii et al. (2014)
10	Turkey	Caucasian	✓	HB	ICHD-II	HB	175/125	Pyro-sequencing	Migraine	99	66	10	59	58	8	0.341	Güler et al. (2015)
									MA	33	20	5					
									MWA	66	46	5					
11	Greece	Caucasian	✓	HB	ICHD-3	HB	221/265	NM	Migraine	-			-				Papasavva et al. (2023)

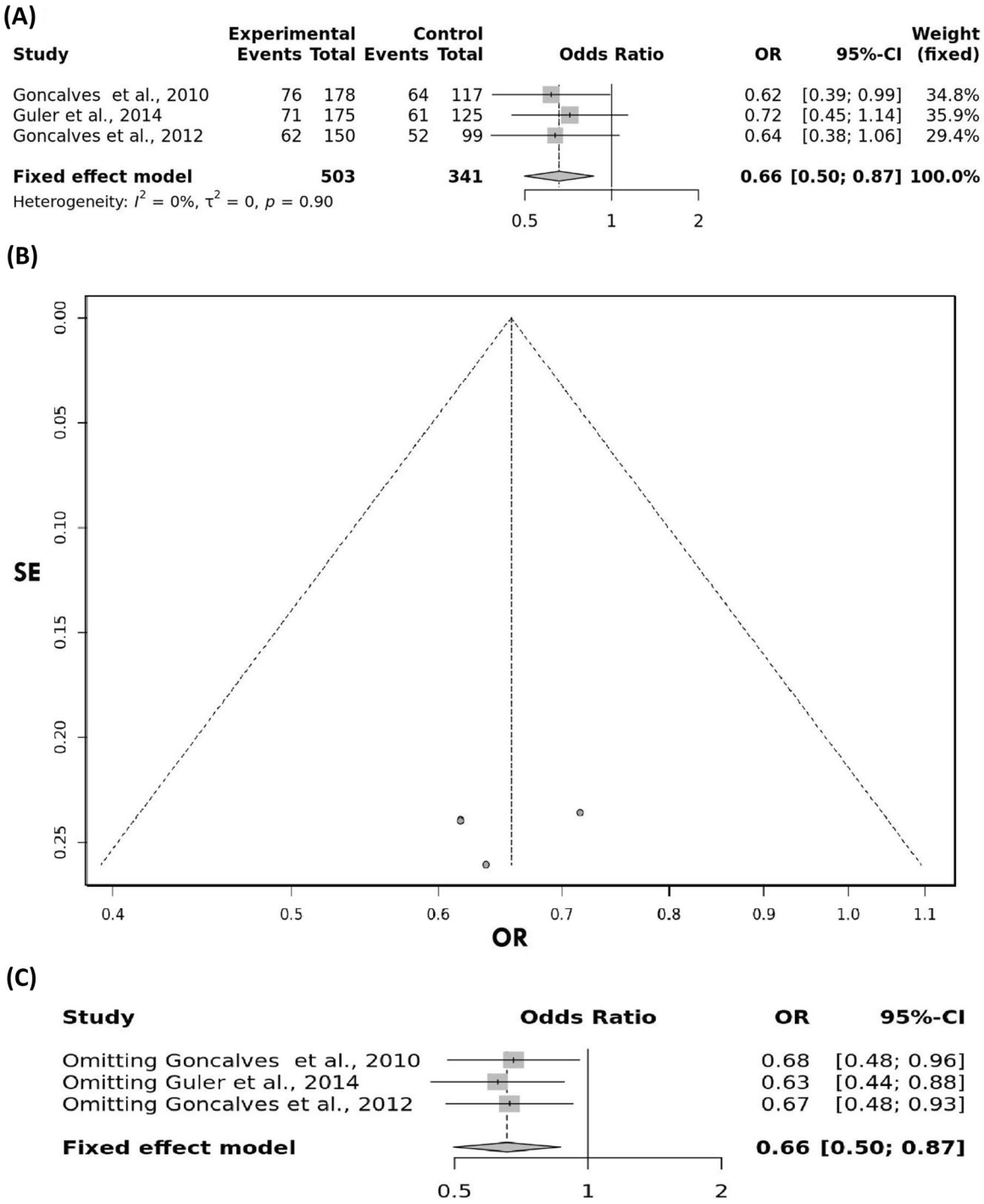

Fig. 5 **A** Forest plot representing a significant protective effect using the dominant model of rs743506 with the risk of migraine. **B** Funnel plot representing no publication bias. **C** Sensitivity analysis

alter the regulation of enzyme's function and thus have a direct impact on nitric oxide levels (Oliveira-Paula et al. 2016). Many independent candidate gene association studies have been carried out in various populations (Toriello et al. 2008; Gruber et al. 2010; Güler et al. 2015; Eröz et al. 2014; Logan et al. 2005; Gonçalves et al. 2011, 2012; Zakerjafari et al. 2016; García-Martín et al. 2020) but have shown conflicting results. Determining the precise risk of migraine and

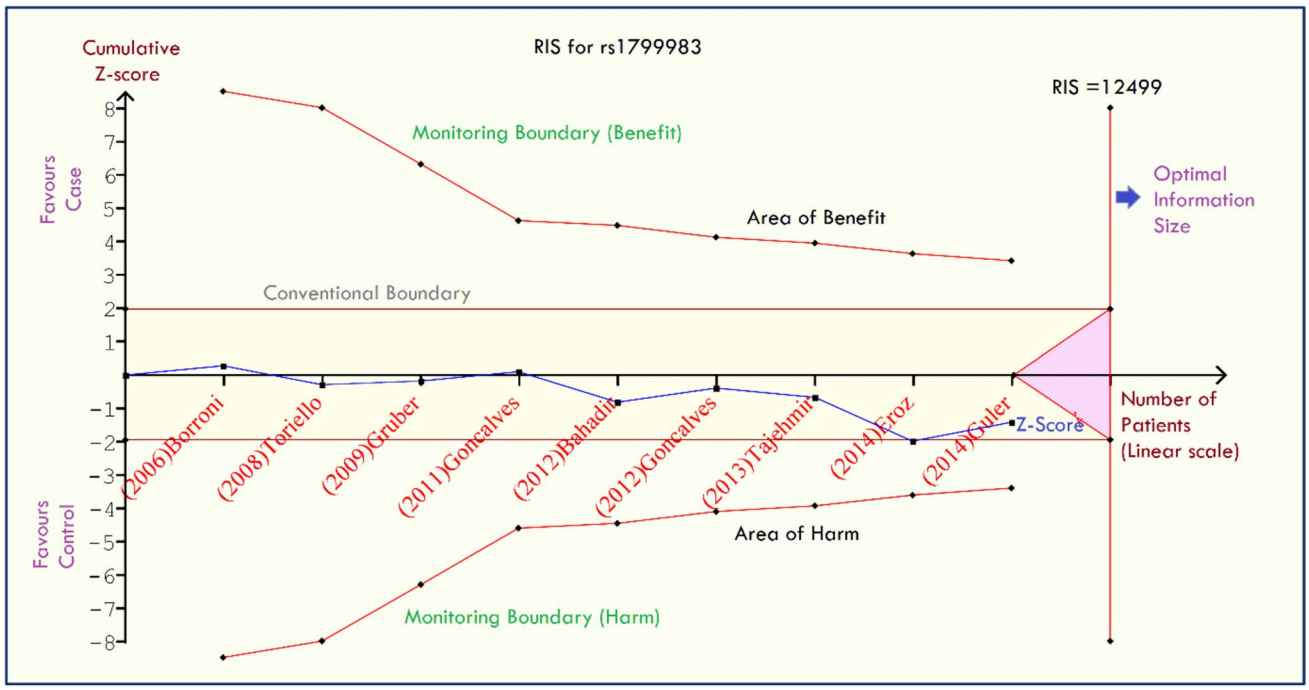

Fig. 6 TSA graph for rs1799983 (allele model) showed a non-significant result with less sample size/ power, therefore, more studies to find out the association

its two subclinical kinds, MA and MWA, with the presence of risk variants such -786 T > C, + 884 G > A, VNTR, rs743506, and rs3918226 of eNOS was the goal of the current study.

In the present study, we have observed a non-significant association between different risk variants such as rs2070744/ -786 T > C and VNTR with the risk of migraine

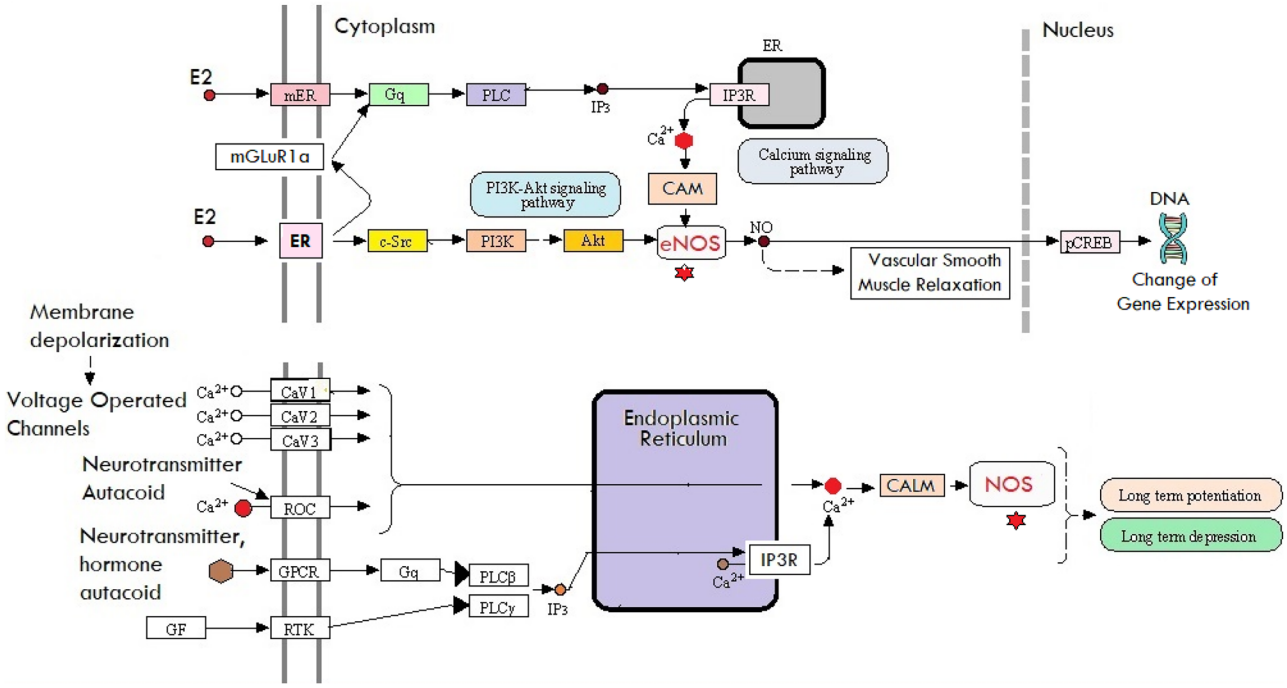

Fig. 7 KEGG pathways showed that multiple primary signaling molecules such as Estrogen (E2), membrane depolarization, and Neurotransmitters autacoids such as serotonin activates multiple pathway which increase the calcium/ calmodulin complex required for the activation of eNOS. (KEGG: Kyoto Encyclopedia of Genes and Genomes)

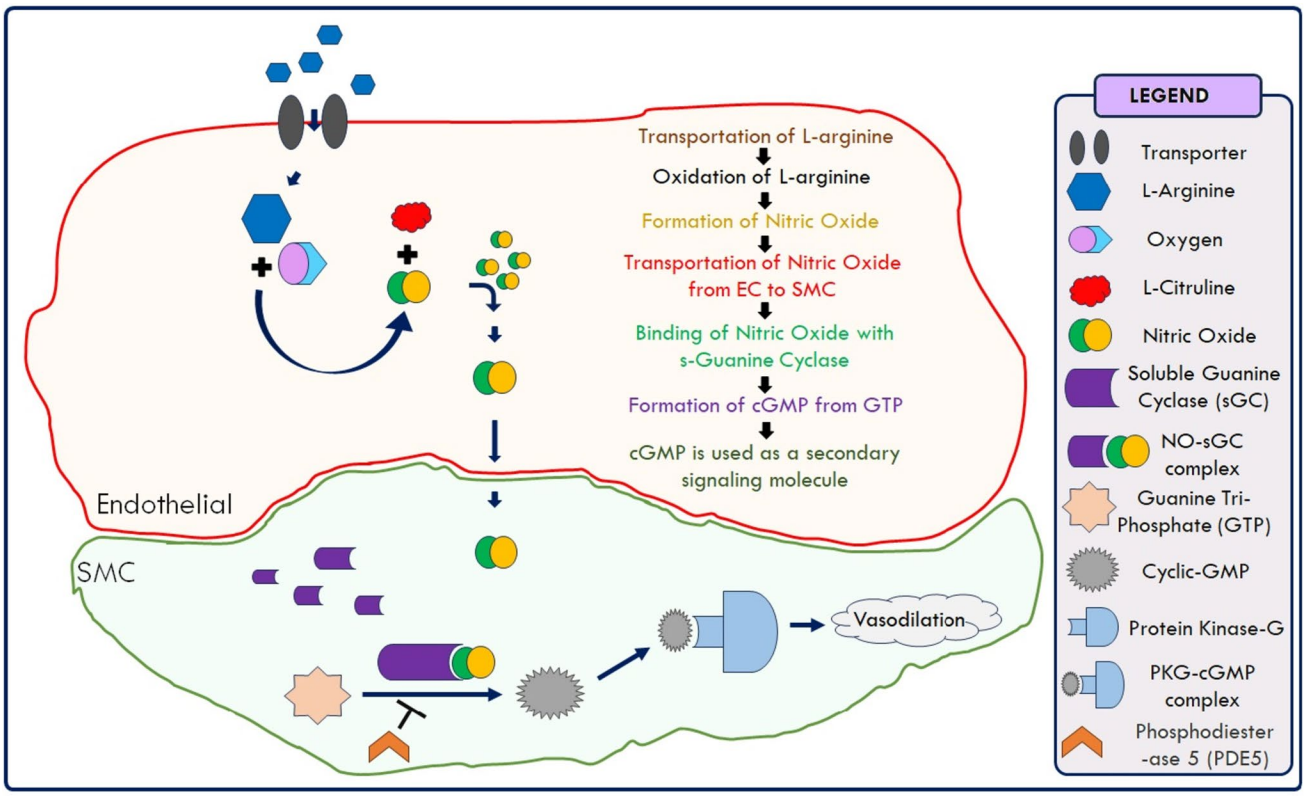

Fig. 8 Synthesis of Nitric Oxide in the presence of eNOS protein in the endothelial cells which is then transported in the smooth muscle cells where it regulates the vascularity

or both types i.e., MA and MWA. However, a considerable risk has been identified between other variations such as rs3918226 which significantly increases the risk of MA in the general population (SF: Table 8), and after using dominant, over-dominant, and co-dominant models, rs179983 was found to be a critical risk factor which considerably increases the susceptibility of total migraine in the Iranian population. (SF: Table 12). However, a decreased risk of MA and MWA was found in the Brazilian population after using the dominant, over-dominant, and codominant models (SF: Tables 13 and 14).Another variant i.e., rs743506 significantly decreases the risk of migraine and MWA in the Brazilian population (SF: Tables 15 and 17) in contrast to what was observed in the MA group (SF: Table 16).

Also, we utilize LDmatrix Tool (Genome Assembly: GRCh37 for All Population setting) to create an interactive heatmap matrix of pairwise linkage disequilibrium statistics of multiple eNOS gene variants which have been studied in association with migraine such as rs1800779, rs2070744, rs3918226, rs61722009, rs1799983, rs148554851, rs743506, rs7830. Multiple query variant such as rs207468799 rs61722009 rs148554851 is missing from 1000G (GRCh37) data but rs2070744 and rs1800779 are found in high disequilibrium ($r^2 = 0.977$) (Fig. 9) (SF: Table 15) (LDlink | An Interactive Web Tool for Exploring Linkage Disequilibrium in Population Groups (nih.gov).

Concerning the previously published meta-analysis studied + 894 G > T/ rs1799983 (Chen et al. 2015), rs179983 and rs1800779 (Qin et al. 2016), and -786 T > C (Dong et al. 2018). + 894 G > T analyzed by Chen et al. (2015) and Qin et al. (2016) where Qin and group showed rs1799983 was associated with susceptibility to MA in the genetic recessive (OR = 1.41 [1.02- 1.96]) and co-dominant (TT vs GG) (OR = 1.50 [1.04–2.15]) models. But no sub-grouping based on ethnicity was done by Qin and group. Also, a non-significant association was observed between rs1800779 and the risk of migraine of MA and MWA by Qin et al. (2016). Whereas Chen and group observed an increased risk of migraine among the non-caucasian population and with MA in both the overall and non-caucasian populations. Dong and group have observed that risk variant (-786 T > C) increased the risk of overall migraine in the Caucasian population after pooling the results of six studies (Logan et al. 2005; Gonçalves et al. 2011, 2012; Eröz et al. 2014; Güler et al. 2015; Zakerjafari et al. 2016) in contrast to the present study. Such contradiction might be due to the inclusion of more studies (García-Martín et al. 2020; Toriello et al. 2008).

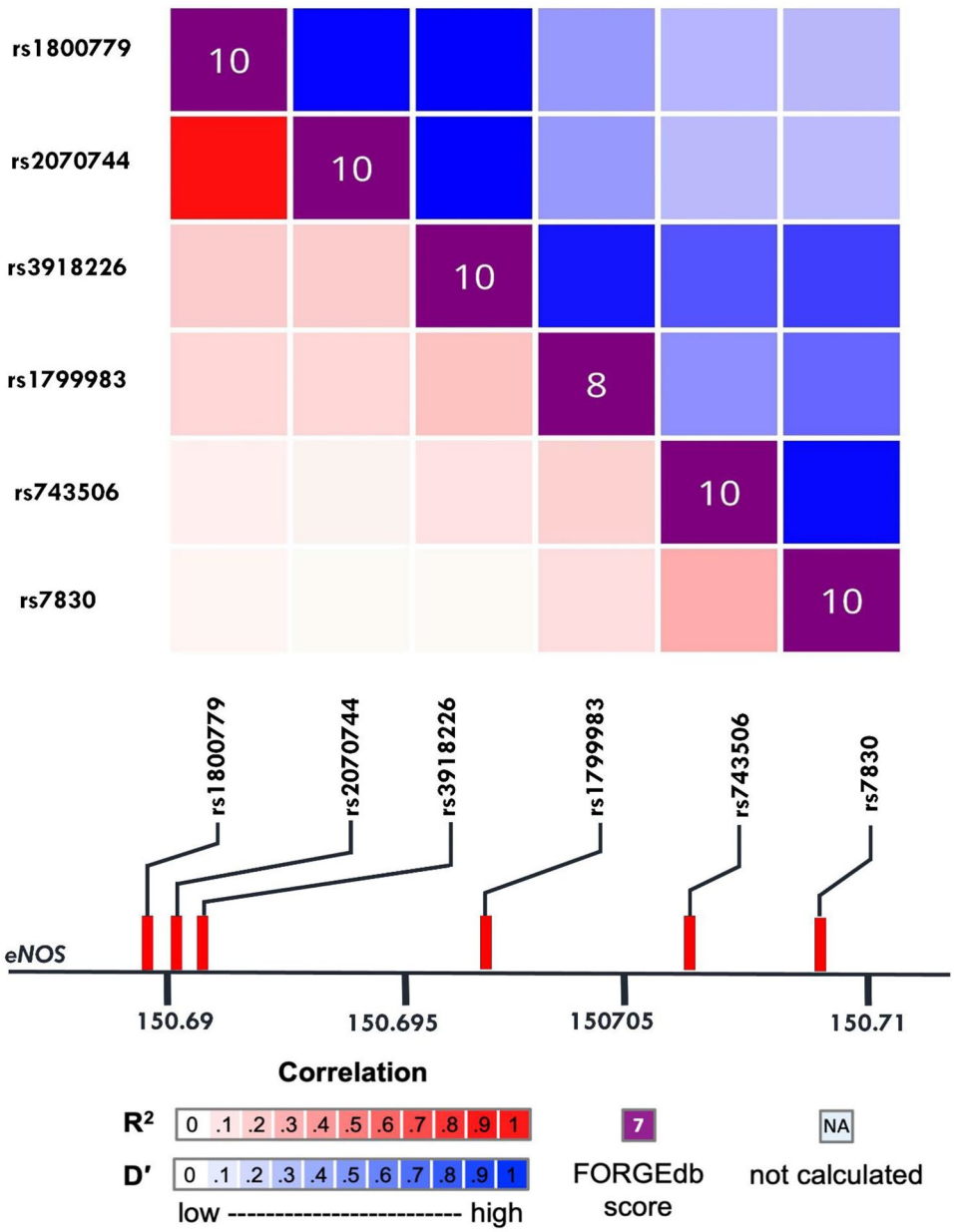

Fig. 9 An interactive heatmap matrix of pairwise linkage disequilibrium statisticsof multiple variants of the eNOS gene (LDlink | An Interactive Web Tool for Exploring Linkage Disequilibrium in Population Groups (nih.gov)

Enclosing the section, the present meta-study might not support the fact that eNOS is a good candidate gene for increasing the risk of migraine or its two clinical subtypes. This is possible due to the lack of power in the present meta-study as supported by trial sequential analysis (SF: Figs. 1–4). However, this is not the actual scenario because studies conducted on a model organism have shown that there is an enhanced expression of eNOS in the dura mater and pia mater, an expression that was not suppressed by sumatriptan or other similar drugs (Zinck et al. 2006). Other evidence has shown that the formation of NO from organic nitrates like nitroglycerin (NTG), which is extremely permeable, lipophilic, and causes headaches comparable to migraines, is controlled by the enzyme eNOS (*See review*) (Sureda-Gibert et al. 2022). Further evidence for the importance of the NO-sGC pathway in migraine has been provided by studies showing that a PDE5 inhibitor (sildenafil) can also cause headaches in healthy controls and delayed migraine headaches in patients (Kruuse et al. 2002, 2003). Also, the present study hasn't looked into the combined risk potential of variants on risk of disease which was supported by independent studies (described in Section "Strengths and Limitations with Future Perspective") (Gonçalves et al. 2012). Cumulative all the pieces of evidence mentioned above, have shown a possible relation of the eNOS gene with the pathophysiology of migraine. Therefore, we cannot rule out the possibility that the NOS3 gene may have a minor impact/

no association with migraine given that it is already known that it is a complicated and heterogeneous condition.

Strengths and Limitations with Future Perspective

The primary strength of the present meta-analysis is that it is the first of the kind in which multiple variants of the gene of interest have been analyzed. The second strength is the method used to conduct the literature review, followed by a selection of studies that met the inclusion criteria outlined in subsection "Inclusion and Exclusion Features". Third, precise statistical analysis is used to determine the risk association between the various risk variations and the diseases under review. Fourth, we found out the risk attribution that exists between the selected polymorphisms and the two subtypes of migraines, which are MA and MWA. Fifth, a precise risk attribution has been established for the migraine and its clinical subtypes risk among various ethnicities. In addition, we determine the necessary sample size for the meta-analysis using a novel approach known as TSA.

The first limitation is the small sample size of the present meta-analysis which was evaluated by trial sequential analysis. Also, we tried to contact the author (Papasavva et al. 2023) but no reply was received but if we had the data we could be able to find the significant result. Secondly, despite all studies having used the ICHD-3 / HIS criteria to diagnose the suspected individual, there is still a chance of misclassification because migraine is such a heterogeneous disorder. Third, the present study was only able to do clinical sub-grouping, and there was no attempt to subgroup the data according to gender. Fourth, the risk posed by variations that are not statistically related can be altered by a variety of modifier genes that were not investigated in this particular study. Furthermore, the interaction between markers of the same gene can be linked to the risk of disease. Unfortunately, this potential connection was not investigated in the current study.

Concerning future perspective as shown by TSA graphs, there are more studies needed to confirm the risk due to the presence of a variant of interest of the eNOS gene. Also, it is very important to find out the combined impact of variants of the same gene and other genes (gene–gene interaction) on the risk of diseases of interest.

Conclusion

Migraine has been known as a complex neuro-vascular disorder with a polygenic inheritance and eNOS is one of the genes which is responsible for the disease's susceptibility. In the present study, we have observed that the rs743506 showed a protective association in comparison to rs1799983, rs3918226 which showed significant risk in the MA group.

Abbreviations **ICHD-3**: International Classification of Headache Disorder 3rd edition; **MA**: Migraine Aura; **MWA**: Migraine without Aura; ***eNOS***: Endothelial Nitric Oxide Synthase; **ICHD-3**: International Classification of Headache Disorders; **NCBI**: National Centre for Biotechnology Information; **MEDLINE**: Medical Literature Analysis and Retrieval System Online; **PRISMA**: Preferred Reporting Items for Systematics Reviews and Meta-Analysis; **IHS**: International Headache Society; **HWE**: Hardy-Weinberg Equilibrium; **OR**: Odds Ratio; **CI**: Confidence Interval; $\mathbf{I^2}$: I Square; **GAS**: Gene Association Study

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Data Availability All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare that they have no conflict of interest.

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