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ARTICLE

Association between endothelial nitric oxide synthase (NOS3) rs2070744 and the risk for migraine

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Abstract

Because nitric oxide could play an important role in the pathogenesis of migraine (suggested by experimental, neuropathological, biochemical, and pharmacological data), and a recent meta-analysis showed an association between the single-nucleotide polymorphism (SNP) rs2070744 in the endothelial nitric oxide synthase (*eNOS* or *NOS3)* gene (chromosome 7q36.1) and the risk for migraine in Caucasians, we attempted to replicate the possible association between this SNP and the and the risk for migraine in the Caucasian Spanish population. The frequencies for the *NOS3* rs2070744 genotypes and allelic variants were assessed in 283 migraine patients and 287 healthy controls with a *TaqMan*-based qPCR Assay. The putative influence on genotype frequency of age at onset of migraine attacks, gender, family history of migraine, absence or presence of aura, and triggering of migraine attacks by ethanol, were also analyzed. The frequencies of *NOS3* rs2070744 genotypes and allelic variants were not associated with the risk for migraine (OR [95%] CI for the minor allele = 0.91 [0.72–1.15]) and were not influenced by age at onset of migraine, gender, presence of aura, or triggering of migraine attacks by ethanol. *NOS3* rs2070744CC genotypes were significantly more frequent in patients with a family history of migraine. *NOS3* rs2070744 SNP is not associated with the risk for migraine in Caucasian Spanish people although it might be related to family history.

Introduction

Migraine is one of the most frequent neurological disorders, affecting between 10 and 18% of the population with a higher incidence in women than in men (2–3/1). Both, migraines without aura (MWoA) and with aura (MWA) show a high frequency of positive family history, ranging from 50 to 70%, and it is well known that the risk for both MWoA and MWA is increased in subjects with first-degree parents affected by this disease. However, migraine genetics is not well established to date. In patients with familial hemiplegic migraine, several genes such as *CACNA1A*, *ATP1A2*, and *SCN1A* have been identified as causative [1]. A recent meta-analysis of 22 hypothesis-free genome-wide association studies involving migraine patients ($n =$ 59,764) and healthy controls ($n = 316{,}078$) identified 38 susceptibility loci for migraine [2]. During the last 20 years, there have been reported a substantial number of hypothesis-driven case-control association studies trying to establish a possible relationship between genetic variants in many candidate genes and the risk for migraine, leading to variable and inconsistent results, but a detailed description of these studies is out of the scope of the present report.

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Table 1 Demographic and clinical data of the series studied

Group	Migraine patients ($n = 283$)	Healthy controls ($n = 287$)
Age (years): mean (SD); range	39.0 (12.8); 13–73	38.9 (13.2); 19–77
Age at onset (years): mean (SD); range	17.8 (10.7); 2–67	NA
Age at onset <15 years: *N* (%); range	157 (55.5); 2–15	NA
Female: *N* (%)	207 (73.1)	210 (73.2)
Positive family history: *N* (%)	226 (79.9)	NA
Aura: *N* (%)	147 (51.9)	NA

Nitric oxide (NO) has many important biological functions, which include the inhibition of platelet aggregation, the maintaining of the arterial vasodilatory tone, actions on neurotransmission processes, antitumor and antimicrobial activities, and mediation of macrophage cytotoxicity [3]. In addition, depending on its redox form, NO can act as a reactive free radical or have neuroprotective effects through N-methyl-D-aspartate glutamatergic receptor blockade [3]. The synthesis of NO from L-arginine is mediated by the three isoforms of NO synthase (NOS): neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3).

It is well known that NO has important implications in the pathophysiology of migraine, which are supported by many experimental, biochemical, neuropathological, and pharmacological data, involving mainly, but not exclusively, NOS1 (revised in [4, 5]). Several studies have addressed the possible association of certain allelic variants in *NOS* genes with the risk for migraine [4, 6–19] with variable results (revised in [4]).

NOS3 or *eNOS* gene (chromosome 7q36.1; gene identity 4846, MIM 163729) encodes the protein eNOS (http://www.ncbi.nlm.nih.gov/gene/4846). Two studies have shown an association between the *NOS3* rs2070744 single-nucleotide polymorphism (SNP) and the risk for migraine [17, 19], while others failed to find this association [8, 13, 15, 18]. A recent meta-analysis showed an increased risk for migraine in carriers of the rs2070744CC genotype in Caucasian populations, and a lack of association in non-Caucasian populations and in the whole series [20].

In this replication study, we investigated the possible association between the rs2070744 and the risk for migraine in Caucasian Spanish people. We also studied, as a secondary analysis, the possible influence of this SNP in age at onset of migraine, gender, positive family history of migraine, the presence or absence of aura in migraineurs, and in the triggering of migraine attacks by alcohol.

Patients and methods

Patients and controls

The demographic data of the 283 patients fulfilling standardized diagnostic criteria for migraine (none of them suffered from other headache types) [21] and the 287 age- and gender-matched controls (who did not have either personal or familial positive history of migraine and did not suffer from other headache types) who participated in this study are summarized in Table 1. Migraine patients were recruited in the general neurologic clinics of three university hospitals during the periods between September 2006–September 2007 and June 2017–February 2019. Many of these patients were involved in other case-control genetic association studies published by our group [4, 22–29]. Details of the recruitment of both migraine patients and healthy controls were described elsewhere [28].

Ethical aspects

The principles of the Declaration of Helsinki were applied. All the participants were included in the study after written informed consent. The study protocol was approved by the Ethics Committees of the University Hospital "Príncipe de Asturias" (Alcalá de Henares, Madrid, Spain), University Hospital "Infanta Cristina" (Badajoz, Spain), and Hospital La Mancha-Centro (Alcázar de San Juan, Ciudad Real, Spain).

Genotyping of rs2070744 variants

Genotyping was performed in genomic DNA obtained from venous blood samples of participants using specific TaqMan probes for the rs2070744 SNP (C__15903863_10, Life Technologies, Alcobendas, Madrid, Spain). Full details of the procedure were described elsewhere [4].

Statistical analysis

Statistical analyses were done by using the SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The Hardy–Weinberg equilibrium was confirmed, both in patients and in controls, by using the online program https://ihg.gsf.de/cgi-bin/hw/hwa1.pl. The chi-square test (or the Fisher's exact test where appropriate) was used for assessing intergroup comparison values (both between the whole series of migraine patients and controls and between migraine patients and controls considering each gender

Table 2 *NOS3* genotypes and allelic variants of patients with migraine and healthy volunteers

	Migraine patients ($N = 283$, 566 alleles)	Controls ($N = 287$, 574 alleles)	Intergroup comparison OR (95% CI), P; NPV (95% CI)
Genotypes			
NOS3 rs2070744 (T/T)	92 (32.5; 27.1–38.0)	89 (31.0; 25.7–36.4)	1.07 (0.75–1.53); 0.701; 0.51 (0.48–0.54)
NOS3 rs2070744 (T/C)	144 (50.9; 45.1–56.7)	141 (49.1; 43.3–54.9)	1.07 (0.77–1.49); 0.676; 0.51 (0.47–0.56)
NOS3 rs2070744 (C/C)	47 (16.6; 12.3–20.9)	57 (19.9; 15.2–24.5)	0.80 (0.52–1.23); 0.315; 0.49 (0.47–0.51)
Alleles			
NOS3 rs2070744 (T)	328 (58.0; 53.9–62.0)	319 (55.6; 51.5–59.6)	1.10 (0.87–1.39); 0.418; 0.52 (0.48–0.55)
NOS3 rs2070744 (C)	238 (42.0; 38.0–46.1)	255 (44.4; 40.4–48.5)	0.91 (0.72–1.15); 0.418; 0.49 (0.47–0.52)

The values in each cell represent: number (percentage; 95% confidence intervals). *NPV* negative predictive value

separately). The 95% confidence intervals and the negative predictive values [30] were also calculated.

The sample size was determined from the allele frequencies observed for healthy individuals by using a genetic model that analyzed the minor allele frequency with an odds ratio (OR) value = 1.5 ($\alpha = 0.05$). The statistical power (two-tailed association) for rs2070744 variant alleles, according to the sample size of this study, was 92.6%.

The genotype and allelic frequencies between subgroups of migraine patients according to the age at onset of migraine attacks (≤16 years vs. ≥16 years), negativity vs. positivity of family history of migraine, absence or presence of aura, and triggering or non triggering migraine attacks with alcohol were well calculated by using the chi-square test or the Fisher's exact test when appropriate as well. The comparison of mean age at onset of migraine attacks between the three genotypes of rs2070744 SNP was done with a *T*-test for independent samples.

Results

The frequencies of rs2070744 genotypes and allelic variants were in Hardy–Weinberg's equilibrium both in migraine patients and in healthy controls groups, did not differ significantly between the two groups (Table 2), and were not influenced by gender (Table 3) and age at onset of migraine (Table 4), and were similar in patients diagnosed with MWA than in those with MWoA (Table 4), and in migraine patients in which ethanol triggered migraine attacks than in those in which ethanol did not trigger migraine attacks (Table 5). The OR (95% CI; *P*) values obtained using the dominant and recessive models for the risk of developing migraine were 1.07 (0.75–1.52; 0.700) and 0.80 (0.49–1.29; 0.359), respectively. Armitage test for trend with the number of variant alleles indicated an OR equal to 0.9, with a *P* value = 0.414.

The frequencies of rs2070744CC genotype and rs2070744C allele were significantly higher in patients with a positive family history of migraine compared with those without a family history of this disease (Table 4). Mean ± SD age at onset of migraine was similar in migraine patients with rs2070744CC, CT, and TT genotypes (Table 6).

The OR (95% CI; *P*) values obtained using the dominant and recessive models for the comparison regarding family history positivity were 0.65 (0.37–1.16; 0.147) and 3.36 (1.20–9.44; 0.017), respectively. Armitage test for trend with the number of variant alleles indicated an OR value equal to 1.73, with a *P* value = 0.026.

Discussion

NOS3 rs2070744 SNP could affect NOS3 expression, which is related with endothelial dysfunction [31, 32]. *NOS3* rs2070744 SNP has been associated with increased risk of coronary artery disease [33–35], essential hypertension [36], diabetes mellitus [34], diabetic nephropathy [34], diabetic retinopathy [37], cancer [38], periodontal disease [39], preeclampsia [40], normal tension glaucoma [41], psoriasis [42], multiple sclerosis [43], hypoxic-ischemic encephalopathy [44], delayed cerebral ischemia following aneurismal subarachnoid hemorrhage [45], and the power athletic status [46–48]. A population-based study in USA showed association between *NOS3* rs2070744 and increased risk for stroke [49], while a case-control association study in Turkish did not find that association [50]. Finally, Gao et al. [51] describe decreased risk for immunoglobulin A nephropathy (in interaction with *NOS3* rs1799983) in Chinese.

Despite NO could play an important role in the pathogenesis of migraine, as it was suggested by many previous data [4], to date it is not well established the possible contribution of polymorphisms in the NOS genes with the risk of developing this disease. Together with the description of increased risk for migraine described in Caucasian individuals carrying the *NOS3* rs2070744CC genotype in a recent meta-analysis [20], Gonçalves et al. [15] described interaction between *NOS3* rs743506 and *NOS2* rs2297518 SNPs in the risk for migraine, and Güler et al. [18] a higher

Table 3 *NOS3* genotypes and allelic variants of patients with migraine and healthy volunteers distributed by gender

	Migraine women ($N = 207$, 414 alleles)	Control women ($N = 210$, 420 alleles)	Intergroup comparison values OR (95%CI); P	NPV	Migraine men ($N = 76$, OR 152 alleles)	Control men $N = 77$, 154 alleles)	Intergroup comparison values OR (95%CI); P	NPV
Genotypes								
NOS3 rs2070744 (T/T)	68 (32.9; 26.5–39.2)	65 (31.0; 24.7–37.2)	1.09 (0.72–1.65); 0.678	0.51 (0.48–0.55)	24 (31.6; 21.1–42.0)	24 (31.2; 20.8–41.5)	1.02 (0.52–2.02); 0.957	0.51 (0.45–0.56)
NOS3 rs2070744 (T/C)	104 (50.2; 43.4–57.1)	103 (49.0; 42.3–55.8)	1.05 (0.71–1.54); 0.808	0.51 (0.46–0.56)	40 (52.6; 41.4–63.9)	38 (49.4; 38.2–60.5)	1.14 (0.61–2.15); 0.686	0.52 (0.43–0.61)
NOS3 rs2070744 (C/C)	35 (16.9; 11.8–22.0)	42 (20.0; 14.6–25.4)	0.81 (0.50–1.34); 0.416	0.50 (0.47–0.52)	12 (15.8; 7.6–24.0)	15 (19.5; 10.6–28.3)	0.78 (0.34–1.79); 0.551	0.49 (0.46–0.53)
Alleles								
NOS3 rs2070744 (T)	240 (58.0; 53.2–62.7)	233 (55.5; 50.7–60.2)	1.11 (0.84–1.46); 0.467	0.52 (0.48–0.56)	88 (57.9; 50.0–65.7)	86 (55.8; 48.0–63.7)	1.09 (0.69–1.71); 0.718	0.52 (0.45–0.58)
NOS3 rs2070744 (C)	174 (42.0; 37.3–46.8)	187 (44.5; 39.8–49.3)	0.90 (0.69–1.19); 0.467	0.49 (0.46–0.52)	64 (42.1; 34.3–50.0)	68 (44.2; 36.3–52.0)	0.92 (0.59–1.45); 0.718	0.49 (0.44–0.55)

The values in each cell represent: number (percentage; 95% confidence intervals). *NPV* negative predictive value

Table 4 *NOS3* genotypes and allelic variants of patients with migraine and healthy volunteers distributed by age of onset, positivity of family history of migraine, and presence or absence of aura

	Age at onset ≤ 15 years ($N = 150$, 300 alleles)	Age at onset ≥ 16 years ($N = 133$, 266 alleles)	Intergroup comparison values OR (95% CI); P	Positive family history of migraine ($N = 214$, 428 alleles)	Negative family history of migraine ($N = 65$, 130 alleles)	Intergroup comparison values OR (95% CI); P	Migraine with aura ($N = 139$, 278 alleles)	Migraine without aura ($N = 141$, 282 alleles)	Intergroup comparison values OR (95% CI); P
Genotypes									
NOS3 rs2070744 (T/T)	47 (31.3; 23.9–38.8)	45 (33.8; 25.8–41.9)	0.89 (0.54–1.47); 0.654	65 (30.4; 24.2–36.5)	26 (40.0; 28.1–51.9)	0.65 (0.37–1.16); 0.148	46 (33.1; 25.3–40.9)	46 (32.6; 24.9–40.4)	1.02 (0.62–1.68); 0.933
NOS3 rs2070744 (T/C)	75 (50.0; 42.0–58.0)	69 (51.9; 43.4–60.4)	0.92 (0.58–1.48); 0.753	107 (50.0; 43.3–56.7)	34 (52.3; 40.2–64.5)	0.91 (0.52–1.59); 0.745	73 (52.5; 44.2–60.8)	69 (48.9; 40.7–57.2)	1.15 (0.72–1.85); 0.550
NOS3 rs2070744 (C/C)	28 (18.7; 12.4–24.9)	19 (14.3; 8.3–20.2)	1.37 (0.73–2.60); 0.324	42 (19.6; 14.3–24.9)	5 (7.7; 1.2–14.2)	2.93 (1.11–7.75); 0.025	20 (14.4; 8.6–20.2)	26 (18.4; 12.0–24.8)	0.74 (0.39–1.41); 0.361
Alleles									
NOS3 rs2070744 (T)	169 (56.3; 50.7–61.9)	159 (59.8; 53.9–65.7)	0.86 (0.62–1.21); 0.408	237 (55.4; 50.7–60.1)	86 (66.2; 58.0–74.3)	0.64 (0.42–0.96); 0.029	165 (59.4; 53.6–65.1)	161 (57.1; 51.3–62.9)	1.10 (0.78–1.54); 0.588
NOS3 rs2070744 (C)	131 (43.7; 38.1–49.3)	107 (40.2; 34.3–46.1)	1.15 (0.82–1.61); 0.408	191 (44.6; 39.9–49.3)	44 (33.8; 25.7–42.0)	1.58 (1.05–2.37); 0.029	113 (40.6; 34.9–46.4)	121 (42.9; 37.1–48.7)	0.91 (0.65–1.28); 0.588

The values in each cell represent: number (percentage; 95% confidence intervals)

Table 5 NOS3 genotypes and allelic variant frequencies in patients with migraine and their relationship with response to ethanol as a triggering factor

	Triggering effect of ethanol (90 individuals, 180 alleles)	Lack of effect of ethanol (193 individuals, 386 alleles)	Intergroup comparison values OR (95% CI); *P*
Genotypes			
NOS3 rs2070744 (T/T)	33 (36.7; 26.7–46.6)	59 (30.6; 24.1–37.1)	1.32 (0.78–2.23); 0.309
NOS3 rs2070744 (T/C)	46 (51.1; 40.8–61.4)	98 (50.8; 43.7–57.8)	1.01 (0.61–1.67); 0.958
NOS3 rs2070744 (C/C)	11 (12.2; 5.5–19.0)	36 (18.7; 13.2–24.1)	0.61 (0.29–1.26); 0.177
Alleles			
NOS3 rs2070744 (T)	112 (62.2; 55.1–69.3)	216 (56.0; 51.0–60.9)	1.30 (0.90–1.86); 0.160
NOS3 rs2070744 (C)	68 (37.8; 30.7–44.9)	170 (44.0; 39.1–49.0)	0.77 (0.54–1.11); 0.160

The values in each cell represent: number (percentage; 95% confidence intervals)

Table 6 Mean (SD) age at onset of migraine for the different genotypes

	Age at onset (SD); range	*T*-test *P* value	*T*-test *P* value
Genotypes		NOS3 rs2070744 (T/C)	NOS3 rs2070744 (C/C)
NOS3 rs2070744 (T/T)	18.6 (11.8); 2–67	0.4975	0.1789
NOS3 rs2070744 (T/C)	17.8 (11.2); 3–50		0.2655
NOS3 rs2070744 (C/C)	16.0 (6.7); 4–36		

NOS3 rs1799983TT genotype frequency in migraine patients who had a headache duration of longer than 24 h compared with patients who had migraine of shorter duration. These data should suggest a possible association between *NOS3* genes and migraine.

In contrast with the results of the previously mentioned meta-analysis describing a modest increase in the risk for migraine related with the *NOS3* rs2070744CC genotype in Caucasians (435 migraine patients vs. 345 controls; OR [95% CI] = 1.62 [1.03–2.56] compared with TT + TC genotypes) [20], in the present study, involving Caucasian Spanish people, we failed to find any association between *NOS3* rs2070744 variants with the risk for migraine, both analyzing the frequency of the different genotypes of this SNP under dominant, recessive of allelic models. Moreover, the pooled data of the present study with those of Caucasian series in the previously reported meta-analysis showed a lack of association of rs2070744 SNP as well (718 migraine patients vs. 632 controls; OR [95% CI] = 1.16 [0.86–1.57], $P = 0.334$). In addition, a secondary analysis failed to show any relationship between *NOS3* rs2070744 variants and the age at onset of migraine, gender, presence or absence of aura, or provocation of migraine attacks by ethanol. However, we found an association between rs2070744CC genotype and rs2070744C alleles with the positivity of family history of migraine, but the statistical significance of this finding was not enough strong.

The relatively low sample size of the migraine patient and control cohorts involved in the current study (although it should be adequate to detect ORs of 1.5, it could be insufficient to detect more modest associations) should be considered as its main limitation. Taking into account the limitations of the present study, and in contrast with a previous meta-analysis [20], *NOS3* rs2070744 variants are apparently unrelated with the risk of migraine in the Caucasian Spanish people. The fact that this particular SNP showed a lack of association with the risk of developing migraine in the population analyzed in the present study does not preclude the possibility of an association between other SNPs in the *NOS3* gene and a modification in the risk for this disease.

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Compliance with ethical standards

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

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