NEWS & VIEWS

Is *SOD2* Ala16Val Polymorphism Associated with Migraine with Aura Phenotype?

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Abstract

Several studies suggest a role of oxidative stress in the physiopathology of migraine, particularly in the form with aura. In a case-control study, we investigated the association between migraine and superoxide dismutase 1 (*SOD1*) and superoxide dismutase 2 (*SOD2*) genes in a cohort of 490 consecutive unrelated Caucasian migraineurs (migraine with aura [MwA], $n=107$; migraine without aura [MwoA], $n=246$; chronic migraine [CM], $n=137$) and 246 healthy controls recruited at our Headache and Pain Unit and stored in the Inter-institutional Multidisciplinary BioBank (BioBIM). Migraine phenotype was carefully detailed using face-to-face interviews. We examined polymorphisms of *SOD1* gene (A/C substitution—rs2234694) and *SOD2* gene (C/T transition—rs4880—Ala16Val). The rs4880 TT (Val/Val) genotype was associated ($p=0.042$) with the presence of unilateral cranial autonomic symptoms (UAs) in MwA patients. We also found a mild correlation between *SOD2* rs4880 genotype and the type of acute migraine treatment ($p=0.048$) in MwA patients. Our findings suggest that *SOD2* is a disease-modifier gene influencing oxidative mechanisms in MwA. These observations lead to the hypothesis that *SOD2* polymorphism may cause a defective control of the oxidative phenomena linked to cortical spreading depression, the neurophysiological hallmark of migraine aura, causing an overstimulation of trigeminal neurons and UAs triggering. *Antioxid. Redox Signal.* 22, 275–279.

Introduction

MIGRAINE IS A PRIMARY BRAIN dysfunction characterized by a dysregulation of cortical excitation–inhibition balance, leading to activation and sensitization of the trigeminovascular system with consequent meningeal inflammation (9). Increased oxidative stress is a key event in migraine physiopathology (2, 7, 8), and generation of diffusible reactive oxygen species (ROS) in the cerebral cortex activates nociceptive transmission during the attack in migraine with aura (MwA) (6). Therefore, antioxidants and ROS scavengers have been suggested as promising therapeutic strategy for migraine (6).

Oxidative stress is characterized by an impairment in balance between free-radical production that leads to damage of DNA, lipids, and proteins and levels of biological antioxidant systems (6). One of the most efficient antioxidant defense system of cells exposed to ROS is the superoxide dismutase (SOD) system. SODs are enzymes that catalyze the dismutation of superoxide (O_2^-) into oxygen (O_2) and hydrogen peroxide (H_2O_2). Among the different SOD isoforms identified, SOD1 is localized in the intracellular cytoplasmic compartments, and SOD2 plays an essential role as a primary mitochondrial antioxidant enzyme. The *SOD1* maps to chromosome 21q22 (#147450; OMIM) and presents in intron 3, 34 base pairs downstream the GT splice donor site

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Innovation

Experimental models reveal that oxidative stress has a significant role in cortical spreading depression, the neurophysiological event underlying migraine aura, and in trigeminal nociceptive activation. This is the first study investigating the association between superoxide dismutase 1 (*SOD1*) and superoxide dismutase 2 (*SOD2*) genetic polymorphisms and migraine. The strength of our study includes the detailed demographic and clinical characterization of migraineurs recruited by headache specialists, and the use of a specific migraine biorepository. We found that a common polymorphism of *SOD2* gene contributes to the clinical phenotype of migraine with aura.

of exon 3, a polymorphism resulting in an A-to-C substitution (rs2234694). This polymorphism is related to *SOD1* activity, resulting in higher activities in subjects carrying AA genotype. Several studies have suggested an association of this SNP with different diseases such as diabetes, autism, and amyotrophic lateral sclerosis (#147450; OMIM). *SOD2* gene, mapping to chromosome 6q25.3 (#147460; OMIM), is characterized by a common polymorphism at codon 16 in exon 2, a C-to-T transition (rs4880) resulting in an Alanine (GCT) to Valine (GTT) substitution (Ala16Val). The presence of Valine (T allele) leads to the production of instable mRNA and reduces transport of the *SOD2* enzyme into the mitochondrial matrix and then its antioxidant function. Several studies have previously demonstrated the association between the *SOD2* Ala16Val polymorphism and breast, prostate, and lung cancers, and cardiovascular, liver, and neurological diseases (#147460; OMIM).

Despite oxidative stress being implicated in the pathophysiology of migraine, to date, the association between migraine and *SOD* genetic polymorphisms has not been investigated. Since we established a biorepository specifically dedicated to migraine patients (Interinstitutional Multidisciplinary BioBank [BioBIM]) in this study, we aimed at exploring the association between *SOD1* and *SOD2* specific genetic variants and migraine in a cohort of carefully clinical characterized Caucasian patients.

Results and Discussion

We consecutively enrolled 490 migraineurs affected by MwA ($n = 107$), migraine without aura (MwoA, $n = 246$), and

chronic migraine (CM, $n = 137$) (5) and 246 healthy control subjects. Sociodemographic and clinical characteristics of the study population, gathered with face-to-face interviews, are presented in Table 1.

Genotypes and the allele frequencies of the *SOD1* and *SOD2* polymorphisms between migraineurs and controls did not significantly differ from those predicted by the Hardy-Weinberg equilibrium (Table 2). We found no association between *SOD1* and *SOD2* polymorphisms and migraine susceptibility, migraine type (MwoA, MwA, and CM), sociodemographic characteristics, or concomitant diseases. However, when focusing in detail on migraine clinical phenotype, in patients with MwA we observed a correlation between *SOD2* TT (Val/Val) genotype and the presence of unilateral cranial autonomic symptoms (UAs) (1) ($p = 0.04$, effect size [ES] = 0.243) (Fig. 1A). We also found a mild association between *SOD2* genotype and acute migraine treatment type in the same patients' group ($p = 0.04$, ES = 0.249): CC (Ala/Ala) genotype carriers used more triptans (45.83%), TT (Val/Val) carriers used more nonsteroidal anti-inflammatory drugs (NSAIDs, 35.13%), while heterozygous CT (Ala/Val) carriers used both triptans and NSAIDs (45.65%) (Fig. 1B).

This is the first study investigating the association between specific *SOD1* and *SOD2* gene polymorphisms and migraine. We found no direct relationship between the *SOD1* and *SOD2* analyzed polymorphisms and migraine susceptibility. However, we report an interesting correlation between *SOD2* Ala16Val variant and the clinical phenotype of MwA, namely the presence of UAs (1).

To date, studies on *SOD* activity in migraine have provided conflicting results. A low concentration of platelet *SOD* levels and a decreased *SOD* activity in MwA have been reported, suggesting a higher vulnerability to oxidative stress in these patients (7). Higher *SOD* activity in red cells in patients with MwA compared with those with MwoA, probably due to a relative increase in oxidative stress due to vasoconstriction induced by ROS and associated with occurrence of aura, has also been described (8). Conversely, no changes in *SOD* concentration in neutrophils and platelets of MwA patients have been described (2), and a not considerable decrease in erythrocytes from 34 young patients suffering from MwoA or MwA was found (2). These discrepancies are probably due to the different parameters and assays used in determining *SOD* activity and level, especially across different ages (adults vs. children), and to the evidence that the enzyme activities may

TABLE 1. BASELINE CHARACTERISTICS OF THE MIGRAINE PATIENTS AND CONTROLS

Variable	Controls (246)	All patients with migraine (490)	Chronic migraine (137)	Migraine with aura (107)	Migraine without aura (246)
Sex, F/M, (F%)	175/71 (71.1)	369/74 (83.1)	122/15 (89.0)	82/25 (76.6)	201/45 (81.7)
Age at observation, mean (SD)	42.1 (12.56)	41.4 (12.72)	41.8 (12.50)	41.1 (12.38)	41.4 (12.36)
Age at onset, mean (SD)		20.3 (11.67)	20.3 (11.26)	19.8 (10.60)	20.1 (11.17)
Family history of migraine, (%)		348 (71.0)	93 (67.8)	78 (72.8)	175 (71.1)
UAs		191 (38.9)	63 (45.9)	35 (32.7)	93 (37.8)
Migraine acute treatment					
NSAIDs		126 (25.4)	28 (20.4)	30 (28.0)	68 (27.6)
Triptans		146 (29.7)	35 (25.5)	26 (24.2)	85 (34.5)
NSAIDs + Triptans		136 (27.7)	60 (43.7)	17 (15.8)	59 (23.9)
Others		82 (16.7)	14 (10.2)	34 (31.7)	34 (13.8)

NSAIDs, nonsteroidal anti-inflammatory drugs; SD, standard deviation; UAs (unilateral cranial autonomic symptoms), conjunctival injection, lacrimation, nasal congestion/rhinorrhea, ptosis, eyelid swelling or forehead/ facial sweating, singly or combined.

TABLE 2. DISTRIBUTIONS OF GENOTYPE AND ALLELE FREQUENCIES OF *SOD1* rs2234694 AND *SOD2* rs4880 POLYMORPHISMS OBSERVED IN PATIENTS AND CONTROLS

	n	<i>rs2234694</i> genotypes (%)			<i>rs2234694</i> alleles (%)	
		AA	AC	CC	A	C
Controls	246	222 (40.25)	19 (45.20)	1 (14.55)	406 (62.85)	240 (37.15)
All patients	490	200 (39.84)	223 (44.42)	79 (15.74)	623 (62.05)	381 (37.95)
MwA	107	98 (39.81)	9 (42.59)	0 (17.59)	132 (61.11)	84 (38.89)
MwoA	246	217 (40.63)	28 (46.09)	0 (13.28)	326 (63.67)	186 (36.33)
CM	137	127 (38.41)	9 (42.75)	1 (18.84)	165 (59.78)	111 (40.22)

	n	<i>rs4880</i> genotypes (%)			<i>rs4880</i> alleles (%)	
		CC	CT	TT	C	T
Controls	246	45 (40.25)	110 (45.20)	77 (14.55)	406 (62.85)	240 (37.15)
All patients	490	200 (39.84)	223 (44.42)	79 (15.74)	623 (62.05)	381 (37.95)
MwA	107	24 (39.81)	46 (42.59)	37 (17.59)	132 (61.11)	84 (38.89)
MwoA	246	54 (40.63)	114 (46.09)	76 (13.28)	326 (63.67)	186 (36.33)
CM	137	33 (38.41)	67 (42.75)	37 (18.84)	165 (59.78)	111 (40.22)

Values are given as No. (%).

CM, chronic migraine; MwA, migraine with aura; MwoA, migraine without aura; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2.

change in different cellular compartments (e.g., platelet vs. erythrocytes) (2).

For this reason, we decided to perform our study using molecular methods and examining SNPs of genes encoding *SOD1* and *SOD2*.

The association between *SOD2* Ala16Val polymorphism and the occurrence of UAs during the attack in MwA is the main result of our study. This is in agreement with current knowledge in the pathophysiology of MwA and UAs (1, 4, 6). Experimental data reveal that oxidative stress has a significant role in cortical spreading depression (CSD), the neurophysiological event underlying migraine aura, and in trigeminal nociceptive activation (6). During CSD, production of ROS in the brain cortex, meninges, and trigeminal ganglia may induce nociceptive activation and release of calcitonin gene-related peptide from peptidergic nerves, inducing the firing of peripheral trigeminal neurons and meningeal inflammation (6). Experimental examination of more than 1176 genes demonstrated that CSD involves the expression of genes implied in responses to oxidative stress such as major prion protein, glutathion-S-transferase-5, and apolipoprotein E (4). UAs, the hallmark of trigeminal autonomic cephalgias that also appear during the attack in approximately 45.8% of migraine patients (1), are known to occur in those migraineurs whose attacks are characterized by an overstimulation of trigeminal afferents that triggers the efferent parasympathetic arm of the trigemino-autonomic reflex (1). Based on the findings mentioned earlier, we hypothesize that patients with MwA and UAs carrying the *SOD2* TT (Val) genotype have a defective control of the CSD oxidative phenomena. This could magnify CSD-induced activation of trigeminal nociceptive firing, leading to an overstimulation of trigeminal neurons, which, in turn, trigger the trigemino-autonomic reflex, giving rise to UAs.

Our study also suggests that in MwA patients, *SOD2* CC genotype is more frequent among triptans users whereas the TT genotype is more common among NSAIDs users, CT genotype carriers behaving midway. These data need to be considered with caution, as in our study patients were asked to

FIG. 1. Genotypic frequencies of *SOD2* rs4880 displayed according to the presence of unilateral cranial autonomic symptoms (A) and acute migraine treatment (B) in patients with MwA. Percentage values of relative frequencies are reported. MwA, migraine with aura; NSAIDs, nonsteroidal anti-inflammatory drugs; SOD2, superoxide dismutase 2.

report their current acute migraine treatment but not specifically the reason of their choice. One possibility is that SOD2 activity in CC carriers counteracts CSD-induced oxidative pro-nociceptive effects, contributing toward preventing or delaying the development of central sensitization, therefore resulting in a better efficacy of triptans, drugs known to work before central sensitization has occurred (3). Conversely, TT carriers could have more pronounced CSD-induced pain consequences, including rapid central sensitization, which reduces triptans response (3), thus preferring NSAIDs, which are also effective when taken during the late stages of the attack, when the central sensitization has been developed.

Data based on a specifically dedicated migraine biobank, coupled to extremely detailed clinical and demographic features gathered with face-to-face interviews by headache specialists, are the main strength of the study. Limitations are that we recruited patients in a tertiary-referral center and that we cannot exclude possible artefacts due to recall bias. Moreover, we acknowledge the need to enlarge the patients' sample in future studies, hoping in replication studies by future headache-dedicated biorepositories.

In conclusion, our findings suggest that *SOD2* is a disease-modifier gene influencing oxidative mechanisms in MwA; *SOD2* polymorphisms may impact MwA phenotype through a defective control of CSD-related oxidative phenomenon that amplifies trigeminal nociceptive activation. Further genetic association studies are imperative to understand the SODs gene involvement in migraine physiopathology that may be useful as a biomarker for detecting asymptomatic individuals at increased risk for migraine and for developing effective treatment strategies (1).

Notes

Population study

From 2009 to 2013, 490 Caucasian unrelated patients affected by MwA, MwA, and CM were consecutively recruited at our Headache and Pain Unit. Migraine was diagnosed according to the International Headache Society (IHS) criteria (5). Detailed information on the patients' demographics and clinical characteristics of migraine, including age of onset, family history of migraine, presence of aura, frequency and duration of attacks, location, side, quality and intensity of pain, presence of UAs (conjunctival injection, lacrimation, nasal congestion/rhinorrhea, ptosis, eyelid swelling, or forehead/facial sweating, singly or combined) (1), duration of CM, preventive and acute treatments, presence and duration of medication overuse, sleep problems, obesity, head or neck injuries, other concomitant diseases, or any other factors currently known to be associated with migraine, was gathered by specifically trained neurologists during the first visit by means of face-to-face interviews using a semi-structured questionnaire. Patients with significant cardiovascular, metabolic, pulmonary, renal, hepatic, endocrine, or other systemic disease were excluded. Informative and consent forms were provided at the time of diagnosis to patients, along with the permission to obtain blood samples for research purposes. As a control, we enrolled 246 healthy subjects that matched with the cases for age, gender, and race-ethnicity. None of the controls had clinical evidence or family history of migraine or other neurological diseases.

Genotyping

All biological samples were stored at the BioBIM of IRCCS San Raffaele Pisana following the biobanking standard operative procedures. DNA was isolated from EDTA anticoagulated whole blood using MagNA Pure LC instrument and the MagNA Pure LC total DNA isolation kit I (Roche Diagnostics, GmbH, Mannheim, Germany) according to the manufacturer's instructions.

The +34A>C intron 3 *SOD1* polymorphism (rs2234694) and the exon 2 *SOD2* Val16Ala polymorphism (rs4880) were determined by polymerase chain reaction (PCR) amplification on the basis of the Ensembl sequences ENSG00000142168 and ENSG00000112096, respectively, for *SOD1* and *SOD2* Genes.

Standard PCR was performed using the following primers: F5'-TATCCAGAAAACACGGTGGGCC-3' R5'-TCCTGTATTAGTTCCTCCCTTTGGCAC-3' (*SOD1*), and F5'-TCTCGTCTTCAGCACCAGCAGG-3' R5'-TGGTACTTCTCCTC GGTGACG-3' (*SOD2*) in a GeneAmp PCR System 9700 (Life Technologies, Carlsbad, CA) using HotStarTaq Master Mix (QIAGEN, Inc., Chatsworth, CA). All Sanger sequencing analyses were carried out in order to exclude pre-analytical and analytical errors on both strands using Big Dye Terminator v3.1 Cycle Sequencing kit (Life Technologies), run on an ABI 3130 Genetic Analyzer (Life Technologies) and repeated on PCR products obtained from new nucleic acid extractions.

Statistical analysis

Accordance of allelic frequencies with the Hardy-Weinberg equilibrium was assessed by Chi-square goodness-of-fit test. Data are presented as mean $\pm$ SD for normally distributed variables. Mean differences were assessed by two-way analysis of variance; while for nominal ones, association was assessed by the Chi-squared test or Fisher-Freeman-Halton exact test where needed. ES was estimated by Cramer's V. Statistical significance was assessed for a *p*-value less than 0.05. For a minimal observed ES of 0.24 and a sample of 490 (six degrees of freedom), the achieved power of association study was 99.09% for an error probability of 5%.

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Abbreviations Used

BioBIM = Interinstitutional Multidisciplinary BioBank
 CM = chronic migraine
 CSD = cortical spreading depression
 ES = effect size
 MwA = migraine with aura
 MwoA = migraine without aura
 NSAID = nonsteroidal anti-inflammatory drug
 ROS = reactive oxygen species
 SD = standard deviation
 SOD1 = superoxide dismutase 1
 SOD2 = superoxide dismutase 2
 UAs = unilateral cranial autonomic symptoms