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Research Submission

Diamine Oxidase rs10156191 and rs2052129 Variants Are Associated With the Risk for Migraine

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Background.—Histamine has been implicated in the pathogenesis of migraine. We investigated the possible association between functional single nucleotide polymorphisms (SNPs) in the *diamine oxidase* gene (*DAO;* chromosome 7q36.1, involved in histamine metabolism) and the risk for migraine.

Methods.—We studied the frequency of the rs2052129, rs10156191, rs1049742, and rs1049793 genotypes and allelic variants in 197 patients with migraine and 245 healthy controls using a *TaqMan*-based qPCR Assay.

Results.—The DAO SNP rs10156191, which is related to decreased DAO enzyme activity, is associated with the risk of developing migraine, particularly in women. The odds ratio (OR) for the defect allele positivity is 1.61 (95% confidence interval 1.31-2.37) for overall migraine patients and 2.08 (1.29-3.36) for women suffering from migraine. The association was not influenced by confounders such as the age at onset, the presence of aura, positivity of alcohol as a triggering factor, positive family history of aura, or family history of allergy. Multiple regression analyses did not confirm association with the rest of genetic factors.

Conclusion.—Our findings, which should be framed as hypothesis generating, suggest that *DAO* genotypes and allelic variants are associated with the risk for migraine in Caucasian Spanish people, especially in women.

Key words: migraine, genetics, histamine, *diamine oxidase* gene, risk factor, biomarker

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Migraine is a frequent disorder (10-18%) with a male:female ratio of 1:2-3. Despite the high positivity of family history (50-70%) and the increased risk for suffering both migraine with aura (MWA) and migraine without aura (MWoA) by the first-degree relatives of patients, migraine genetics is not well known. Only a number of genes (*CACNA1A*, *ATP1A2*, *SCN1A*) have been identified for familial hemiplegic migraine.[1] In recent years, there has been an increasing interest in the possible relationship between genetic polymorphisms and the risk for migraine, mainly (but not exclusively) related with serotonergic and dopaminergic systems, with variable and inconsistent results (a detailed revision of these studies is out of the scope for the present work). These genetic studies include hypothesis-driven candidate gene association studies and well as hypothesis-free genome-wide association studies (GWAS). The findings obtained in GWAS are so far inconclusive. The first migraine GWAS established association between 2 genes, *MTDH* and *PGCP*, and the risk for migraine.[2] This finding was confirmed in 2 replication studies,[3,4] whereas other studies showed lack of association.[5,6] The putative associations of migraine with diverse SNPs have been refined in some studies.[7-9] Ligthart et al[3] reported association of one SNP in *NGFR* gene and the risk for migraine, but they could not replicate this finding in 3 cohorts. Finally, Chasman et al[7] described association between SNPs in the *PRMD16*, *TRPM8*, and *LRP1* genes. Recent studies identified association between an SNP in the *SLC39A12* gene and the risk for migraine[4,5,10] or MWoA[4,11] in different populations, but failed to find association with SNPs in the *GALNT16* gene.[4,5,10,11] In addition, interethnic variability in the associations exists, as *LRP1* was found to be associated with the risk for migraine in Indians,[4] but not in Chinese[10,11] or Swedish individuals.[5]

Regarding genetic studies based on mechanistic hypothesis, histamine seems to be a plausible candidate involve in the etiology or in the clinical presentation of migraine. Histamine has been implicated in the pathogenesis of migraine headaches in several ways, as summarized in Table 1. Krajewska and Rydzewski[12] reported increased serum DAO activity in 28 patients with migraine (that was even higher during migraine attacks) compared with 19 controls. In contrast with these findings, a Spanish group claimed, in nonscientific newspapers, that 90% of patients with migraine show DAO deficiency, which could be improved with the ingestion of a DAO capsule before the meals, leading to migraine improvement (http://www.lavanguardia.com/salud/20130306/54369041093/cientificos-demuestran-que-una-enzima-sirve-para-prevenir-el-90-de-migranas.html) Histamine acts through four metabotropic histamine receptors, which are all G-protein-coupled (GPCR)

Table 1.—Clinical, Epidemiological, Biochemical, Experimental, and Pharmacological Data on the Possible Relationship Between Histamine and Migraine

Clinical-epidemiological data
Higher frequency of frequency of migraine in patients with allergic (histamine-driven) diseases.[35,36]
Biochemical data
Increased plasma/serum histamine levels in patients with migraine, both during headache and symptom-free periods.[37-40]
Increased plasma and cerebrospinal fluid levels of histidine (the amino acid precursor of histamine) in patients with migraine during attacks, in comparison with controls.[40]
Increased spontaneous histamine release by leukocytes of migraine patients compared with controls.[39,41-44]
Experimental data
Relationship between the activation of dural mast cells, rich in histamine, and migraine pathogenesis shown in experimental models.[45]
Pharmacological data
Intravenous infusion of histamine precipitates immediate and delayed headache in patients with tension-type headache and in migraine patients (more severe and pulsatile in the migraine group), but not in controls without headache. This pharmacological effect is most likely through activation of H_1 receptors since pretreatment with histamine HRH1 antagonists, but not with nitric oxide synthase inhibitors, abolishes both immediate and delayed headache induced by intravenous infusion of histamine.[46,47] HRH2 antagonists are much less effective than HRH1 antagonists in abolishing the headache, but they are significantly better than placebo.[46]
N-alpha-methyl-histamine (HRH3 agonist and main metabolite of histamine via histamine N-methyl-transferase) administered subcutaneously has shown efficacy in the prophylaxis of migraine in double-blind, placebo-controlled studies.[48]
Subcutaneous histamine has shown similar efficacy to botulinum toxin type A in migraine prophylaxis in a randomized, double-blind study,[49] and has been considered as probably effective for migraine prevention (level B) in evidence-based guidelines.[23]

(HRH1, HRH2, HRH3, and HRH4) transducer extracellular signals via Gq, Gs, and Gi/o proteins, respectively. In general, histamine modulates inflammatory and allergic responses via HRH1, gastric acid secretion through HRH2, neurotransmitter release in the central nervous system (CNS) via HRH3, and chemotaxis and inflammatory mediators release via HRH4.[13,14] The brain stores and releases histamine from mast cells and histaminergic neurons of the tuberomammillary nucleus of the posterior basal hypothalamus. Histaminergic fibers project widely to most regions of the CNS, including thalamus, hippocampus, striatum, amygdale, and cerebral cortex.[14-17]

Histamine is synthesized by decarboxylation of its precursor histidine by the enzyme hystidine-decarboxylase (E.C. 4.1.1.22), and degraded through 2 enzymes, histamine N-methyltransferase (HNMT, EC. 2.1.1.8) responsible for inactivating histamine in the brain and diamine oxidase (DAO, EC 1.4.3.6), which is responsible for scavenging extracellular histamine after mediator release.[17-19] DAO activity is expressed in peripheral tissues, mainly in the kidney and colon, and in the thymus, and placenta as well.[16] DAO enzyme is codified by the human *amiloride-binding protein 1* gene (also named as *ABP1*, *ABP*, *diamine oxidase DAO* or *DAO1* gene) (chromosome 7q36.1, gene identity 26, MIM 104610). Three common nonsynonymous single nucleotide polymorphisms (SNPs), which bring about 3 amino acid substitutions, namely Thr16Met (rs10156191), Ser332Phe (rs1049742), and His645Asp (rs1049793), have been identified in Caucasian individuals. The functional effects of these SNPs in DAO enzyme activity have been studied in detail.[20,21] Recently, an additional SNP rs2052129 (G4586T), located in the gene promoter, and which seems to cause decreased transcriptional activity, has been described.[22] Individuals carrying rs1049793,[20] rs10156191,[20] and rs2052129[22] minor alleles have shown lower serum DAO activity when compared with noncarriers.

The aim of the present study is to investigate the possible association between the functional SNPs rs2052129, rs10156191, rs1049742, and rs1049793 in the *DAO* gene (which are associated with decreased DAO activity) with the risk of developing migraine in Caucasian Spanish people. This is the first case–control study analyzing the putative role of functional *DAO* SNPs and the risk of developing migraine.

PATIENTS AND METHODS

Patients and Controls.—We studied 197 patients with diagnostic criteria for migraine, and not for other headache types, according with the classification of the International Headache Society[23] (61 men, 136 women, mean age 37.5 ± 12.8 years, mean age at onset of migraine 16.8 ± 10.3 years), and 245 controls (97 men, 148 women, mean age 38.9 ± 15.3 years). Patients were recruited from those who made their first visit or a follow-up visit to the general neurological clinics of 3 hospitals between September 2006 and August 2007 (113 patients began with migraine episodes under age 15 years, 147 had positive family history for migraine, and 98 had MWA). All eligible patients were invited to participate and all of them agreed to do so. These patients participated in other genetic association studies by our group.[24-28]

Controls were healthy unrelated Caucasian Spanish individuals, most of them students or professors from the University of Extremadura, who did not have either personal or familial positive history of migraine and did not suffer from other headache types. Controls matching gender and age with patients in the study were invited to participate. Control individuals under 18 years were not included in the study. Over 80% of control individuals invited to participate agreed to do so.

All the participants gave written informed consent. The work was done according to the principles of the Declaration of Helsinki. The study protocol was approved by the ethics committees of the University Hospitals "Príncipe de Asturias" (Alcalá de Henares, Madrid, Spain) and "Infanta Cristina" (Badajoz, Spain).

Genotyping.—Genotyping was performed in genomic DNA obtained from venous blood samples of participants using TaqMan assays (supplied by Life Technologies, Alcobendas, Madrid, Spain) designed to detect the following SNPs: rs2052129 (C__11630976_1) a promoter gene variant, rs10156191 (C__25593951_10) a nonsynonymous variant causing the amino acid substitution Thr 16 Met, rs1049742 (C___7599782_20) a nonsynonymous variant causing

the amino acid substitution Ser 332 Phe, and rs1049793 (C___7599774_10) a nonsynonymous variant causing the amino acid substitution His 664 Asp. Detection was carried out by real-time PCR (qPCR) by using an Eppendorf realplex thermocycler (Eppendorf Iberica SLU, San Sebastian de los Reyes, Madrid, Spain) using fluorescent probes. The amplification conditions were: after a denaturation time of 10 minutes at 96°C, 45 cycles of 92°C 15 seconds 60°C 90 seconds were carried out. Fluorescence was measured at the end of each cycle and at endpoint. All samples analyzed were successfully genotyped for all SNPs analyzed. All amplification reactions were determined in triplicate. Genotypes were assigned by the gene identification software (RealPlex 2.0, Eppendorf) and by analysis of the reference cycle number for each fluorescence curve, calculated using the CalQPlex algorithm (Eppendorf). Laboratory methods were exactly the same for migraine sufferers and controls.

Statistical Analysis.—Statistical analyses were performed using the SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The sample size was determined from the allele frequencies reported for South-European Caucasian individuals in the 1000 genomes catalog (http://browser.1000genomes.org), with a genetic model analyzing the frequency for the minor allele with an odds ratio (OR) value = 1.5 ($P = .05$), as recommended for pharmacogenomic studies.[29,30] For genotype comparisons, data were adjusted to dominant, recessive, and allelic models. The best fit was obtained with the dominant model for the minor allele, and this model was used to calculate the OR and P values. Correction for multiple testing was done according to the false discovery rate (FDR) procedure as described elsewhere.[31] Two comparisons were made in the FDR analyses. In the first one, we analyzed the putative role of factors known to influence DAO activity (genotypes and gender) in the risk of developing migraine (24 comparisons including all genotypes and alleles). In the second comparison, we analyzed putative confounders: age at onset, history of migraine, the presence of aura and antecedents of allergy (36 comparisons including all genotypes and alleles). In addition, logistic analyses under the standard additive model including all genotypes plus gender, age at onset, alcohol as a triggering factor, family history of migraine, family history of allergy, and presence of aura were carried out in a single model. The Hardy–Weinberg equilibrium was confirmed by means of Arlequin software Ver. 2.000 (CMPG Zoological Institute, University of Berne, Berne, Switzerland).

RESULTS

The frequencies of *DAO* rs2052129, rs10156191, rs1049742, and rs1049793 genotypes and allelic variants were in Hardy–Weinberg's equilibrium, both in migraine patients and control groups.

The frequencies of genotypes carrying the rs10156191T allele were significantly higher, according to the crude P values, in migraine patients than in controls (Table 2), and the frequency of rs2052129G allele was significantly higher in migraine patients, although the statistical significance disappeared after multiple comparison analyses. The frequency of the rest of the genotypes did not differ significantly between migraine patients and controls (Table 2).

Regarding the possible influence of gender, carrying the rs2052129G in men and carrying rs10156191T allele in women were associated with increased risk for migraine (Table 3) and the statistical significance remained after correction for multiple comparisons. The frequencies of *DAO* rs2052129, rs10156191, rs1049742, and rs1049793 genotypes and allelic variants were not influenced by age at onset of migraine (Table 4), family history of migraine (Table 4), and presence of aura (Table 4). The frequency of carriers of rs10156191 T was higher in migraine patients with previous history of allergic diseases when compared with that of migraine patients without history of allergy, whereas the frequency of the other studied SNPs was not influenced by the previous history of allergy (Table 5).

Mean ± standard deviation age at onset for patients with genotypes rs2052129G/G, GT, and TT were, respectively, 16.5 ± 10.3, 17.5 ± 10.3, and 17.4 ± 13.3 years; for patients with genotypes rs10156191C/C/, C/T, and T/T, 17.3 ± 10.9, 16.2 ± 9.6, and 16.7 ± 9.9 years; for patients with genotypes rs1049742C/C, C/T, and T/T, 17.0 ± 10.5, 16.4 ± 3.5, and 8.0 ± 0.5 years; and for patients with genotypes rs1049793C/C, CG, and GG, 16.9 ± 9.7, 18.3 ± 11.7 and

Table 2.—*DAO* Genotype and Allelic Variants of Patients With Migraine and Healthy Volunteers

	Migraine Patients (N = 197, 394 Alleles)	Controls (N = 245, 490 Alleles)	Allele Positivity OR (95% CI); P; Pc
Genotypes			
rs2052129 G/G	120 (60.9; 54.1-67.7)	130 (53.1; 46.8-59.3)	
G/T	70 (35.5; 28.8-42.2)	97 (39.6; 33.5-45.7)	0.73 (0.50-1.06); .099; .297
T/T	7 (3.6; 1.0-6.1)	18 (7.3; 4.1-10.6)	
rs10156191 C/C	108 (54.8; 47.9-61.8)	162 (66.1; 60.2-72.0)	
C/T	77 (39.1; 32.3-45.9)	71 (29.0; 23.3-34.7)	1.61 (1.309-2.37); .015; .072
T/T	12 (6.1; 2.8-9.4)	12 (4.9; 2.2-7.6)	
rs1049742 C/C	174 (88.3; 83.8-92.8)	210 (85.7; 81.3-90.1)	
C/T	21 (10.7; 6.4-15.0)	33 (13.5; 9.2-17.7)	0.79 (0.45-1.39); .419; .864
TT	2 (1.0; 0.4 to 2.4)	2 (0.8; 0.3 to 1.9)	
rs1049793 C/C	109 (55.3; 48.4-62.3)	129 (52.7; 46.4-58.9)	
C/G	69 (35.0; 28.4-41.7)	98 (40.0; 33.9-46.1)	0.90 (0.62-1.31); .575; .864
G/G	19 (9.6; 5.5-13.8)	18 (7.3; 4.1-10.6)	
			Allele Frequency Difference OR (95% CI); P; Pc
Alleles			
rs2052129 G	310 (78.7; 74.6-82.7)	357 (72.9; 68.9-76.8)	0.73 (0.53-0.99); .046; .158
rs2052129 T	84 (21.3; 17.3-25.4)	133 (27.1; 23.2-31.1)	
rs10156191 C	293 (74.4; 70.1-78.7)	395 (80.6; 77.1-84.1)	1.43 (1.04-1.97); .026; .104
rs10156191 T	101 (25.6; 21.3-29.9)	95 (19.4; 15.9-22.9)	
rs1049742 C	369 (93.7; 91.2-96.1)	453 (92.4; 90.1-94.8)	0.83 (0.49-1.40); .485; .864
rs1049742 T	25 (6.3; 3.9-8.8)	37 (7.6; 5.2-9.9)	
rs1049793 C	287 (72.8; 68.5-77.2)	356 (72.7; 68.7-76.6)	0.99 (0.74-1.33); .950; .959
rs1049793 G	107 (27.2; 22.8-31.5)	134 (27.3; 23.4-31.3)	

The values in each cell represent: number (percentage) and (95% confidence intervals). Allele positivity compares the presence of the minor allele either in heterozygosity or homozygosity vs the absence of the minor allele. P, crude P value; Pc: corrected P value according the false discover rate procedure as described within the methods section.
CI = confidence interval; OR = odds ratio.

11.5 ± 6.9 years (nonsignificant differences for the comparison of carriers vs noncarriers of variant alleles). Logistic regression including in a single model all genotypes, gender, age at onset, alcohol as a triggering factor, the presence of family history of aura, family history of allergy, and the presence of aura revealed that in this study group, only 2 factors were related to the risk of developing migraine, namely the rs10156191 C/C genotype (P = .021), and the rs10156191 C/T genotype (P = .040). The rest of putative genetic associations were discarded in the multiple analysis. The association of both rs10156191 genotypes with the risk of developing migraine was influenced by gender (P = .047 and P = .031 for the interaction with the C/C and C/T genotypes, respectively). No interaction of age at onset, alcohol as a triggering factor, the presence of family history of aura, family history of allergy, and the presence of aura with the genetic associations identified in this study was observed.

DISCUSSION

Despite the fact that clinical and experimental data suggest the possible involvement of histamine in the pathogenesis of migraine (Table 1), the possible contribution of genetic polymorphisms related with histamine in the risk of developing migraine is not well established.

Table 3.—*DAO* Genotype and Allelic Variants of Patients With Migraine and Healthy Volunteers Distributed by Gender

	Migraine Women (N = 136, 272 Alleles)	Control Women (N = 148, 296 Alleles)	Allele Positivity OR (95% CI); P; Pc	Migraine Men (N = 61, 122 Alleles)	Control Men (N = 97, 194 Alleles)	Allele Positivity OR (95% CI); P; Pc
Genotypes						
rs2052129 G/G	73 (53.7; 45.3-62.1)	79 (53.4; 45.3-61.4)		47 (77.0; 66.5-87.6)	51 (52.6; 42.6-62.5)	
G/T	59 (43.4; 35.1-51.7)	58 (39.2; 31.3-47.1)	0.99 (0.62-1.58); .959; .959	11 (18.0; 8.4-27.7)	39 (40.2; 30.4-50.0)	0.33 (0.16-0.68); .002; .036
T/T	4 (2.9; 0.1-5.8)	11 (7.4; 3.2-11.7)		3 (4.9; -0.5-10.3)	7 (7.2; 2.1-12.4)	
rs10156191 C/C	66 (48.5; 40.1-56.9)	98 (66.2; 58.6-73.8)		42 (68.9; 57.2-80.5)	64 (66.0; 56.6-75.4)	
C/T	62 (45.6; 37.2-54.0)	43 (29.1; 21.7-36.4)	2.08 (1.29-3.36); .003; .036	15 (24.6; 13.8-35.4)	28 (28.9; 19.8-37.9)	0.88 (0.44-1.74); .708; .864
T/T	8 (5.9; 1.9-9.8) (2)	7 (4.7; 1.3-8.1)		4 (6.6; 0.3-12.8)	5 (5.2; 0.8-9.6)	
rs1049742 C/C	119 (87.5; 81.9-93.1)	127 (85.8; 80.2-91.4)		55 (90.2; 82.7-97.6)	83 (85.6; 78.6-92.6)	
C/T	16 (11.8; 6.3-17.2)	20 (13.5; 8.0-19.0)	0.86 (0.44-1.72); .676; .864	5 (8.2; 1.3-15.1)	13 (13.4; 6.6-20.2)	0.65 (0.23-1.79); .397; .864
T/T	1 (0.7; –0.7 to 2.2)	1 (0.7; –0.6 to 2.0)		1 (1.6; –1.5 to 4.8)	1 (1.0;–1.0 to 3.0)	
rs1049793 C/C	75 (55.1; 46.8-63.5)	79 (53.4; 45.3-61.4)		34 (55.7; 43.3-68.2)	50 (51.5; 41.6-61.5)	
C/G	53 (39.0; 30.8-47.2)	59 (39.9; 32.0-47.8)	0.93 (0.58-1.49); .765; .874	16 (26.2; 15.2-37.3)	39 (40.2; 30.4-50.0)	0.85 (0.44-1.61); .607; .864
G/G	8 (5.9; 1.9-9.8)	10 (6.8; 2.7-10.8)		11 (18.0; 8.4-27.7)	8 (8.2; 2.8-13.7)	
			Allele Frequency Difference OR (95% CI); P; Pc			Allele Frequency Difference OR (95% CI); P; Pc
Alleles						
rs2052129 G	205 (75.4; 70.2-80.5)	216 (73.0; 67.9-78.0)	0.88 (0.61-1.29); .515; .864	105 (86.1; 79.9-92.2)	141 (72.7; 66.4-79.0)	0.43 (0.24-0.79); .005; .040
rs2052129 T	67 (24.6; 19.5-29.8)	80 (27.0; 22.0-32.1)		17 (13.9; 7.8-20.1)	53 (27.3; 21.0-33.6)	
rs10156191 C	66 (48.5; 40.1-56.9)	239 (80.7; 76.3-85.2)	1.69 (1.14-2.49); .008; .048	99 (81.1; 74.2-88.1)	156 (80.4; 74.8-86.0)	0.95 (0.54-1.70); .872; .951
rs10156191 T	62 (45.6; 37.2-54.0)	57 (19.3; 14.8-23.7)		23 (18.9; 11.9-25.8)	38 (19.6; 14.0-25.2)	
rs1049742 C	119 (87.5; 81.9-93.1)	274 (92.6; 89.6-95.6)	0.88 (0.46-1.68); .705; .864	115 (94.3; 90.1-98.4)	179 (92.3; 88.5-96.0)	0.73 (0.29-1.84); .498; .864
rs1049742 T	16 (11.8; 6.3-17.2)	22 (7.4; 4.4-10.4)		7 (5.7; 1.6-9.9)	15 (7.7; 4.0-11.5)	
rs1049793 C	203 (74.6; 69.5-79.8)	217 (73.3; 68.3-78.4)	0.93 (0.64-1.36); .720; .864	84 (68.9; 60.6-77.1)	139 (71.6; 65.3-78.0)	1.14 (0.70-1.87); .595; .864
rs1049793 G	69 (25.4; 20.2-30.5)	79 (26.7; 21.6-31.7)		38 (31.1; 22.9-39.4)	55 (28.4; 22.0-34.7)	

The values in each cell represent: number (percentage) and (95% confidence intervals). Allele positivity compares the presence of the minor allele either in heterozygosity or homozygosity vs the absence of the minor allele. P, crude P value; Pc: corrected P value according the false discover rate procedure as described within the methods section. CI = confidence interval; OR = odds ratio.

Table 4.—*DAO* Genotype and Allelic Variants of Patients With Migraine Distributed by Age, Family History, and the Presence of Aura

	Age at Onset ≤ 16 Years (*N* = 113; 226 Alleles)	Age at Onset ≥ 16 Years (*N* = 84; 168 Alleles)	Allele Positivity OR (95% CI); P; Pc	Positive Family History of Migraine (*N* = 147, 294 Alleles)	Negative Family History of Migraine (*N* = 50, 100 Alleles)	Allele Positivity OR (95% CI); P; Pc	Migraine With Aura (*N* = 98; 196 Alleles)	Migraine Without Aura (*N* = 99, 198 Alleles)	Allele Positivity OR (95% CI); P; Pc
Genotypes									
rs2052129 G/G	72 (63.7; 54.9-72.6)	48 (57.1; 46.6-67.7)		89 (60.5; 52.6-68.4)	31 (62.0; 48.5-75.5)		57 (58.2; 48.4-67.9)	63 (63.6; 54.2-73.1)	
G/T	37 (32.7; 24.1-41.4)	33 (39.3; 28.8-49.7)	0.76 (0.43-1.35); .349; .934	53 (36.1; 28.3-43.8)	17 (34.0; 20.9-47.1)	1.07 (0.55-2.06); .855; .934	37 (37.8; 28.2-47.4)	33 (33.3; 24.0-42.6)	1.26 (0.71-2.23); .431; .934
T/T	4 (3.5; 0.1-6.9)	3 (3.6; −0.4 to 7.5)		5 (3.4; 0.5-6.3)	2 (4.0; −1.4 to 9.4)		4 (4.1; 0.2-8.0)	3 (3.0; −0.3 to 6.4)	
rs10156191 C/C	63 (55.8; 46.6-64.9)	45 (53.6; 42.9-64.2)		82 (55.8; 47.8-63.8)	26 (52.0; 38.2-65.8)		49 (50.0; 40.1-59.9)	59 (59.6; 49.9-69.3)	
C/T	45 (39.8; 30.8-48.8)	32 (38.1; 27.7-48.5)	0.92 (0.52-1.62); .761; .934	56 (38.1; 30.2-45.9)	21 (42.0; 28.3-55.7)	0.86 (0.45-1.63); .642; .934	43 (43.9; 34.1-53.7)	34 (34.3; 25.0-43.7)	1.48 (0.84-2.59); .176; .934
T/T	5 (4.4; 0.6-8.2)	7 (8.3; 2.4-14.2)		9 (6.1; 2.2-10.0)	3 (6.0; 0.-6-12.6)		6 (6.1; 1.4-10.9)	6 (6.1; 1.4-10.8)	
rs1049742 C/C	99 (87.6; 81.5-93.7)	75 (89.3; 82.7-95.9)		130 (88.4; 83.3-93.6)	44 (88.0; 79.0-97.0)		87 (88.8; 82.5-95.0)	87 (87.9; 81.4-94.3)	
C/T	12 (10.6; 4.9-16.3)	9 (10.7; 4.1-17.3)	1.18 (0.48-2.87); .717; .934	15 (10.2; 5.3-15.1)	6 (12.0; 3.0-21.0)	0.96 (0.36-2.59); .934; .934	10 (10.2; 4.2-16.2)	11 (11.1; 4.9-17.3)	0.92 (0.38-2.19); .845; .934
TT	2 (1.8; −0.7 to 4.2)	0 (0.0; 0.0-0.0)		2 (1.4; −0.5 to 3.2)	0 (0.0; 0.0-0.0)		1 (1.0; −1.0 to 3.0)	1 (1.0; −1.0 to 3.0)	
rs1049793 C/C	62 (54.9; 45.7-64.0)	47 (56.0; 45.3-66.6)		81 (55.1; 47.1-63.1)	28 (56.0; 42.2-69.8)		55 (56.1; 46.3-65.9)	54 (54.5; 44.7-64.4)	
36	38 (33.6; 24.9-42.3)	31 (36.9; 26.6-47.2)	1.05 (0.59-1.84); .880; .934	49 (33.3; 25.7-41.0)	20 (40.0; 26.4-53.6)	1.04 (0.54-1.98); .912; .934	35 (35.7; 26.2-45.2)	34 (34.3; 25.0-43.7)	0.94 (0.54-1.65); .824; .934
G/G	13 (11.5; 5.6-17.4)	6 (7.1; 1.6-12.7)		17 (11.6; 6.4-16.7)	2 (4.0; −1.4 to 9.4)		8 (8.2; 2.7-13.6)	11 (11.1; 4.9-17.3)	
			Allele Frequency Difference OR (95% CI); P; Pc			Allele Frequency Difference OR (95% CI); P; Pc			Allele Frequency Difference OR (95% CI); P; Pc
Alleles									
rs2052129 G	181 (80.1; 74.9-85.3)	129 (76.8; 70.4-83.2)	0.82 (0.51-1.34); .428; .934	231 (78.6; 73.9-83.3)	79 (79.0; 71.0-87.0)	1.03 (0.59-1.79); .928; .934	151 (77.0; 71.2-82.9)	159 (80.3; 74.8-85.8)	1.22 (0.75-1.97); .429; .934
rs2052129 T	45 (19.9; 14.7-25.1)	39 (23.2; 16.8-29.6)		63 (21.4; 16.7-26.1)	21 (21.0; 13.0-29.0)		45 (23.0; 17.1-28.8)	39 (19.7; 14.2-25.2)	
rs10156191 C	171 (75.7; 70.1-81.3)	122 (72.6; 65.9-79.4)	0.85 (0.54-1.35); .494; .934	220 (74.8; 69.9-79.8)	73 (73.0; 64.3-81.7)	0.91 (0.54-1.52); .717; .934	141 (71.9; 65.6-78.2)	152 (76.8; 70.9-82.7)	1.29 (0.82-2.03); .272; .934
rs10156191 T	55 (24.3; 18.7-29.9)	46 (27.4; 20.6-34.1)		74 (25.2; 20.2-30.1)	27 (27.0; 18.3-35.7)		55 (28.1; 21.8-34.4)	46 (23.2; 17.3-29.1)	
rs1049742 C	210 (92.9; 89.6-96.3)	159 (94.6; 91.2-98.0)	1.35 (0.58-3.13); .487; .934	275 (93.5; 90.7-96.3)	94 (94.0; 89.3-98.7)	1.08 (0.42-2.79); .870; .934	184 (93.9; 90.5-97.2)	185 (93.4; 90.0-96.9)	0.93 (0.41-2.09); .857; .934
rs1049742 T	16 (7.1; 3.7-10.4)	9 (5.4; 2.0-8.8)		19 (6.5; 3.7-9.3)	6 (6.0; 1.3-10.7)		12 (6.1; 2.8-9.5)	13 (6.6; 3.1-10.0)	
rs1049793 C	162 (71.7; 65.8-77.6)	125 (74.4; 67.8-81.0)	1.15 (0.73-1.80); .548; .934	211 (71.8; 66.6-76.9)	76 (76.0; 67.6-84.4)	1.25 (0.74-2.11); .411; .934	145 (74.0; 67.8-80.1)	142 (71.7; 65.4-78.0)	0.89 (0.57-1.39); .614; .934
rs1049793 G	64 (28.3; 22.4-34.2)	43 (25.6; 19.0-32.2)		83 (28.2; 23.1-33.4)	24 (24.0;15.6-32.4)		51 (26.0; 19.9-32.2)	56 (28.3; 22.0-34.6)	

The values in each cell represent: number (percentage) and (95% confidence intervals). Allele positivity compares the presence of the minor allele either in heterozygosity or homozygosity vs the absence of the minor allele. P, crude *P* value; Pc: corrected *P* value according the false discover rate procedure as described within the methods section.
CI = confidence interval; OR = odds ratio.

Table 5.—*DAO* Genotype and Allelic Variants of Patients With Migraine Distributed According to Antecedents of Allergy

	Antecedents of Allergy (*N* = 84; 168 Alleles)	No Antecedents (*N* = 113; 226 Alleles)	Allele Positivity OR (95% CI); P; Pc
Genotypes			
rs2052129 G/G	48 (57.1; 46.6-67.7)	72 (63.7; 54.9-72.6)	
G/T	33 (39.3; 28.8-49.7)	37 (32.7; 24.1-41.4)	1.32 (0.74-2.35); .349; .934
T/T	3 (3.6; –0.4 to 7.5)	4 (3.5; 0.1-6.9)	
rs10156191 C/C	39 (46.4; 35.8-57.1)	69 (61.1; 52.1-70.1)	
C/T	42 (50.0; 39.3-60.7)	35 (31.0; 22.4-39.5)	1.81 (1.02-3.21); .041; .934
T/T	3 (3.6; –0.4-7.5)	9 (8.0; 3.0-13.0)	
rs1049742 C/C	72 (85.7; 78.2-93.2)	102 (90.3; 84.8-95.7)	
C/T	12 (14.3; 6.8-21.8)	9 (8.0; 3.0-13.0)	1.55 (0.65-3.70); .325; .934
TT	0 (0.0; 0.0–0.0)	2 (1.8; –0.7-4.2)	
rs1049793 C/C	45 (53.6; 42.9-64.2)	64 (56.6; 47.5-65.8)	
C/G	31 (36.9; 26.6-47.2)	38 (33.6; 24.9-42.3)	1.13 (0.64-2.00); .668; .934
G/G	8 (9.5; 3.2-15.8)	11 (9.7; 4.3-15.2)	
			Allele Frequency Difference OR (95% CI); P; Pc
Alleles			
rs2052129 G	129 (76.8; 70.4-83.2)	181 (80.1; 74.9-85.3)	1.22 (0.75-1.97); .429; .934
rs2052129 T	39 (23.2; 16.8-29.6)	45 (19.9; 14.7-25.1)	
rs10156191 C	120 (71.4; 64.6-78.3)	173 (76.5; 71.0-82.1)	1.31 (0.83-2.06); .250; .934
rs10156191 T	48 (28.6; 21.7-35.4)	53 (23.5; 17.9-29.0)	
rs1049742 C	156 (92.9; 89.0-96.8)	213 (94.2; 91.2-97.3)	1.26 (0.56-2.84); .575; .934
rs1049742 T	12 (7.1; 3.2-11.0)	13 (5.8; 2.7-8.8)	
rs1049793 C	121 (72.0; 65.2-78.8)	166 (73.5; 67.7-79.2)	1.08 (0.69-1.68); .753; .934
rs1049793 G	47 (28.0; 21.2-34.8)	60 (26.5; 20.8-32.3)	

The values in each cell represent: number (percentage) and (95% confidence intervals). Allele positivity compares the presence of the minor allele either in heterozygosity or homozygosity vs the absence of the minor allele. P, crude *P* value; Pc: corrected *P* value according the false discover rate procedure as described within the methods section.
CI = confidence interval; OR = odds ratio.

Our group, in an association study involving 197 migraine patients and 245 healthy controls, reported lack of association between Thr105Ile SNP (rs1801105) polymorphism in the *HNMT* gene (located at chromosome 2q22.1, gene identity 3176, MIM 605238) and the risk for migraine.

In the present study, we identified association of the risk of developing migraine with the SNP rs10156191 as well as gender. In addition, subgroup analyses suggested that male homozygotes for rs2052129G and women carrying the rs10156191T allele showed increased risk for migraine. However, subgroup analyses are unreliable because of the sample size and therefore these findings should be interpreted cautiously. The frequency of DAO genotypes and their association with the risk of developing migraine were unrelated with age at onset, alcohol as a triggering factor, the presence of family history of aura, family history of allergy, and the presence of aura.

It is to be noted that the rs10156191 and the rs2052129 SNPs cause altered enzyme activity *in vivo*.[20,22] The allele with the rs10156191 T sequence encodes a variant with the amino acid substitution Met in the position 16, instead of Thr in the wild-type protein. This amino acid substitution reduces the enzyme intrinsic activity, thus decreasing the ability to metabolize circulating histamine.[19,20]

Because the rs10156191 T is more common among migraineurs (Table 2) and particularly in women (Table 3), it is to be expected that the genetic predisposition to a decreased metabolic clearance of circulating histamine may be involved in the development of migraine. The functional effect of the allele rs2052129 T is a decrease in the enzyme expression.[22] Because among migraineurs there is an increase in the frequency of the rs2052129 G allele, our results point against the hypothesis of decreased histamine metabolism. However, a previous study failed to find clinical associations of the rs2052129 T allele in study groups with positive association for rs10156191 T, thus suggesting that these 2 variant alleles may have quantitatively different clinical impact.[32]

It has been shown that gender is a major factor on DAO enzyme activity *in vivo*, with women displaying higher enzyme activity and higher interindividual variability than men,[21] and therefore, it is not surprising that the association of *DAO* SNPs with migraine would show association with gender. Nevertheless, considering the limited sample size in this study, and that the most significant association was observed in women, the findings obtained in this study would require replication to obtain more support to the proposed association. The *DAO* SNPs studied here have been related with other histamine-related disorders such as drug-induced hypersensitivity,[32] rhinitis,[33] or ulcerative colitis.[34] Aside from the SNPs analyzed in the present study, no additional nonsynonymous SNPs have been reported to occur in Caucasian subjects with a significant minor allele frequency in the genes analyzed according to public databases (http://browser.1000genomes.org).

CONCLUSION

In summary, *DAO* alleles related with decreased DAO enzyme activity seem to be associated with migraine risk in Caucasian Spanish people. These findings, however, should be framed as hypothesis generating. Further studies combining genotyping for *DAO* allelic variants with measurement of DAO serum activity in the same migraine patient and control groups are needed.

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REFERENCES

1. Mateos V, Pareja JA, Pascual J. *Tratado de cefaleas*. Madrid: Luzán 5 S.A. Ediciones; 2009.
2. Anttila V, Stefansson H, Kallela M, et al. Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1. *Nat Genet*. 2010;42:869-873.
3. Ligthart L, de Vries B, Smith AV, et al. Meta-analysis of genome-wide association for migraine in six population-based European cohorts. *Eur J Hum Genet*. 2011;19:901-907.
4. Ghosh J, Pradhan S, Mittal B. Genome-wide-associated variants in migraine susceptibility: A replication study from North India. *Headache*. 2013;53:1583-1594.

5. Ran C, Graae L, Magnusson PK, Pedersen NL, Olson L, Belin AC. A replication study of GWAS findings in migraine identifies association in a Swedish case–control sample. *BMC Med Genet*. 2014;15:38.
6. Sintas C, Carreño O, Fernández-Morales J, et al. A replication study of a GWAS finding in migraine does not identify association in a Spanish case–control sample. *Cephalalgia*. 2012;32:1076-1080.
7. Chasman DI, Schürks M, Anttila V, et al. Genome-wide association study reveals three susceptibility loci for common migraine in the general population. *Nat Genet*. 2011;43:695-698.
8. Freilinger T, Anttila V, de Vries B, et al. Genome-wide association analysis identifies susceptibility loci for migraine without aura. *Nat Genet*. 2012;44:777-782.
9. Anttila V, Winsvold BS, Gormley P, et al. Genome-wide meta-analysis identifies new susceptibility loci for migraine. *Nat Genet*. 2013;45:912-917.
10. Fan X, Wang J, Fan W, et al. Replication of migraine GWAS susceptibility loci in Chinese Han population. *Headache*. 2014;54:709-715.
11. An XK, Ma QL, Lin Q, Zhang XR, Lu CX, Qu HL. PRDM16 rs2651899 variant is a risk factor for Chinese common migraine patients. *Headache*. 2013;53:1595-1601.
12. Krajewska M, Rydzewski W. Histaminase activity in serum of patients with migraine. *Neurol Neurochir Pol*. 1973;7:55-58.
13. Weaber C. Serotonin and other biogenic amines. In: Olesen J, Goadsby PJ, Ramadan NM, Tfelt-Hansen P, Welch KMA, eds. *The Headaches*. 3rd edn. Philadelphia, PA: Lippincot Williams & Wilkins; 2006:143-149.
14. Alstadhaug KB. Histamine in migraine and brain. *Headache*. 2014;54:246-259.
15. Panula P, Yang HY, Costa E. Histamine-containing neurons in the rat hypothalamus. *Proc Natl Acad Sci U S A*. 1984;81:2572-2576.
16. Watanabe T, Taguchi Y, Shiosaka S, et al. Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. *Brain Res*. 1984;295:13-25.
17. García-Martín E, Ayuso P, Martínez C, Blanca M, Agúndez JAG. Histamine pharmacogenomics. *Pharmacogenomics*. 2009;10:867-883.
18. Kim SH, Krapfenbauer K, Cheon MS, Fountoulakis M, Caims NJ, Lubec G. Human brain cytosolic histamine-N-methyltranferase is decreased in down syndrome and increased in Pick's disease. *Neurosci Lett*. 2002;321:169-172.
19. Obata T. Semicarbazide-sensitive amine oxidase (SSAO) in the brain. *Neurochem Res*. 2002;27:263-268.
20. Ayuso P, Garcia-Martin E, Martinez C, Agundez JA. Genetic variability of human diamine oxidase: Occurrence of three nonsynonymous polymorphisms and study of their effect on serum enzyme activity. *Pharmacogenet Genomics*. 2007;17:687-693.
21. Garcia-Martin E, Ayuso P, Martinez C, Agundez JA. Improved analytical sensitivity reveals the occurrence of gender-related variability in diamine oxidase enzyme activity in healthy individuals. *Clin Biochem*. 2007;40:1339-1341.
22. Maintz L, Yu CF, Rodriguez E, et al. Association of single nucleotide polymorphisms in the diamine oxidase gene with diamine oxidase serum activities. *Allergy*. 2011;66:893-902.
23. Headache Classification Committee of the International Headache Society. The International Classification of Headache Disorders, 2nd ed. *Cephalalgia*. 2004;24(Suppl. 1):1-160.
24. García-Martín E, Martínez C, Serrador M, et al. l Histamine-N-methyl transferase polymorphism and risk for migraine. *Headache*. 2008;48:1343-1348.
25. García-Martín E, Martínez C, Serrador M, et al. Alcohol dehydrogenase 2 genotype and risk for migraine. *Headache*. 2010;50:85-91.
26. García-Martín E, Martínez C, Serrador M, et al. Dopamine receptor 3 (DRD3) polymorphism and risk for migraine. *Eur J Neurol*. 2010;17:1220-1223.
27. García-Martín E, Martínez C, Serrador M, et al. Paraoxonase 1 (PON 1) polymorphisms and risk for migraine. *J Neurol*. 2010;257:1482-1485.
28. García-Martín E, Martínez C, Serrador M, et al. SLC1A2 rs3794087 variant and risk for migraine. *J Neurol Sci*. 2014;338:92-95.
29. Daly AK, Day CP. Candidate gene case–control association studies: Advantages and potential pitfalls. *Br J Clin Pharmacol*. 2001;52:489-499.
30. Brennan P. Gene-environment interaction and aetiology of cancer: What does it mean and how can we measure it? *Carcinogenesis*. 2002;23:381-387.

31. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Statist Soc B*. 1995;1:289-300.
32. Agúndez JA, Ayuso P, Cornejo-García JA, et al. The diamine oxidase gene is associated with hypersensitivity response to non-steroidal anti-inflammatory drugs. *PLoS ONE*. 2012;7:e47571.
33. García-Martín E, García-Menaya J, Sánchez B, Martínez C, Rosendo R, Agúndez JA. Polymorphisms of histamine-metabolizing enzymes and clinical manifestations of asthma and allergic rhinitis. *Clin Exp Allergy*. 2007;37:1175-1182.
34. García-Martin E, Mendoza JL, Martínez C, et al. Severity of ulcerative colitis is associated with a polymorphism at diamine oxidase gene but not at histamine N-methyltransferase gene. *World J Gastroenterol*. 2006;12:615-620.
35. Aamodt AH, Stovner LJ, Langhammer A, Hagen K, Zwart JA. Is headache related to asthma, hay fever, and chronic bronchitis? The Head-HUNT Study. *Headache*. 2007;47:204-212.
36. Heatley RV, Denburg JA, Bayer N, Bienenstock J. Increased plasma histamine levels in migraine patients. *Clin Allergy*. 1982;12:145-149.
37. Haimart M, Pradalier A, Launay JM, Dreux C, Dry J. Whole blood and plasma histamine in common migraine. *Cephalalgia*. 1987;7:39-42.
38. Gazerani P, Pourpak Z, Ahmadiani A, Hemmati A, Kazemnejad A. A correlation between migraine, histamine and immunoglobulin E. *Scand J Immunol*. 2003;57:286-290.
39. Kemper RH, Meijler WJ, Korf J, Ter Horst GJ. Migraine and function of the immune system: A metaanalysis of clinical literature published between 1966 and 1999. *Cephalalgia*. 2001;21:549-557.
40. Castillo J, Martínez F, Corredera E, Lema M, Noya M. Migraña e histamina: Determinación de histidina en plasma y líquido cefalorraquídeo durante crisis de migraña. *Rev Neurol*. 1995;23:749-751.
41. Selmaj K. Histamine release from leucocytes in migraine. *Cephalalgia*. 1983;3:37-40.
42. Selmaj K. Histamine release from leucocytes during migraine attack. *Cephalalgia*. 1984;4:97-100.
43. Launay JM, Pradalier A, Haimart M, Artigou C, Dreux C, Dry J. Histaminoliberation et migraine. *Rev Med Interne*. 1987;8:273-275.
44. Reuter U, Bolay H, Jansen Olesen I, et al. Delayed inflammation in rat meninges: Implications for migraine pathophysiology. *Brain*. 2001;124:2490-2502.
45. Krabbe AA, Olesen J. Headache provocation by continuous intravenous infusion of histamine. Clinical results and receptor mechanisms. *Pain*. 1980;8:253-259.
46. Lassen LH, Thomsen LL, Olesen J. Histamine induces migraine via the H1 receptor. Support for the NO hypothesis of migraine. *Neuroreport*. 1995;6:1475-1479.
47. Millán-Guerrero RO, Isais-Millán R, Benjamín TH, Tene CE. N-alpha-methyl histamine safety and efficacy in migraine prophylaxis: Phase III study. *Can J Neurol Sci*. 2006;33:195-199.
48. Millán-Guerrero RO, Isais-Millán S, Barreto-Vizcaíno S, Rivera-Castaño L, Rios-Madariaga C. Subcutaneous histamine versus botulinum toxin type A in migraine prophylaxis: A randomized, double-blind study. *Eur J Neurol*. 2009;16:88-94.
49. Holland S, Silberstein SD, Freitag F, et al. Evidence-based guideline update: NSAIDs and other complementary treatments for episodic migraine prevention in adults: Report of the Quality Standards Subcommittee of the American Academy of Neurology and the American Headache Society. *Neurology*. 2012;78:1346-1353.