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SHORT COMMUNICATION

Lack of association of hormone receptor polymorphisms with migraine

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Background and purpose: Previous studies concerning the role of hormone receptor genetic variants in migraine have provided conflicting results. The aim of this study was to investigate the role of common polymorphisms in the estrogen receptor gene (*ESR1*) and the progesterone receptor gene (*PGR*) in the risk for migraine in a Spanish population. *Methods:* In a case–control study, including 210 Caucasoid migraine patients and 210 controls, we examined association between three single nucleotide polymorphisms in the coding region of *ESR1*, rs2077642, rs1801132, and rs2228480, and an *Alu* insertion in *PGR*, and migraine, migraine without aura or migraine with aura. Genotypic, allelic and reconstructed haplotype distributions were compared. *Results:* We found no significant differences between cases and controls in the distribution of genotypes or alleles for either polymorphism. No haplotype was over-represented in patients. *Conclusions:* Our study does not support a major contribution of *ESR1* and *PGR* to the pathogenesis of migraine.

Introduction

Migraine is a common, disabling, primary neurovascular disorder characterized by abnormal modulation and expression of head pain and other sensory modalities. The two most frequent migraine presentations are migraine without aura (MO) and migraine with aura (MA) [1]. Epidemiological observations suggest a connection between sexual hormones and migraine: between puberty and menopause there is a clear pre-dominance of migraine in women. Clinical studies have shown that estrogen withdrawal after sustained exposure may trigger MO episodes whilst high estrogen concentrations may prompt MA attacks [2,3]. Additionally, estrogen and progesterone modulate neurotransmitter networks relevant to head pain pathophysiology [3–5].

Common forms of migraine are complex disorders, where interaction of multiple genes and environmental factors delineates the phenotype. Case–control association studies have tried to pinpoint these pre-disposing genetic factors. Positive association between estrogen receptor 1 gene (*ESR1*) or progesterone receptor gene (*PGR*) and migraine has been reported [6–11]. We here sought to replicate these findings in a Spanish population.

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Methods

Two hundred and ten migraine patients (mean age 29 ± 14 years, 153 women) of Caucasoid origin were recruited from Catalonia, Spain. Migraine was classified as MO (n = 102), MA (n = 86) or migraine with hemiplegic aura (HM; n = 22) based on ICHD-II criteria [1]. The control group was composed of 210 Caucasoid sex-matched non-migraneurs (mean age 47 ± 15 years). No age-matching was performed, to avoid inclusion of pre-symptomatic subjects as controls of affected children. Written informed consent from participants and approval from the Vall d'Hebron University Hospital Ethics Committee were obtained according to the guidelines of the Helsinki Declaration. DNA was extracted from peripheral blood (n = 405) or saliva following standard procedures.

Three synonymous exonic polymorphisms, rs2077647 (c.30T > C; p.S10S), rs1801132 (c.975C > G; p.P325P) and rs2228480 (c.1782G > A; p.T594T) in *ESR1* (RefSeq NM_000125.2) and an intronic *Alu* insertion in *PGR* that contains a transcriptional enhancer [12], were selected. PCR primers and protocols used for genotyping are available upon request. The rs2077647 and rs2228480 genotypes were determined by PCR-RFLP using *BstF5* and *BtgI* enzymes; rs1801132 was genotyped by single strand conformation polymorphism (SSCP) analysis. Alleles containing the *PGR Alu* insertion were detected by agarose electrophoresis of the PCR product.

Statistical power was calculated using the Genetic Power Calculator software (statgen.iop.kcl.ac.uk/gpc)

with $\alpha = 0.05$, a lifetime risk of 1.8 and the minimal allele frequency (MAF) found in controls. Analyses were performed in the migraine group and in MO or MA independently. Each gender was analyzed independently, as were cases with onset before or after age 10 years. For linkage disequilibrium (LD) calculations, r^2 values between the three *ESR1* polymorphisms were obtained in controls using HAPLOVIEW 3.32 (www.broad.mit.edu/haploview/haploview-downloads). Genotype and allele frequency comparisons as well as Hardy–Weinberg equilibrium calculation were performed using the SNPassoc R library (likelihood ratio test). Nominal significance threshold ($P < 0.05$) was lowered to $P < 0.0031$ after the multiple comparison correction of Bonferroni. Haplotype estimations from genotype data and the test of overall association were performed using the UNPHASED software (www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased) with a three-marker window.

Results

Polymorphisms of *ESR1* and the *Alu* insertion in *PGR* were in Hardy–Weinberg equilibrium in both controls and patients. MAF ranged from 0.14 (rs2228480) to 0.455 (rs2077647). Statistical power was 65% for rs2077647, 89% for rs1801132 and the *Alu* insertion, and 88% for rs2228480. For migraine subtypes, using MAF = 0.14, power estimates were 74% for MO and 70% for MA. Single nucleotide polymorphisms (SNPs) in *ESR1* were in weak LD ($r^2 < 0.006$).

To evaluate the contribution of *ESR1* and *PGR* polymorphisms to migraine susceptibility, genotype and allele distributions in patients and controls were compared. No significant differences were noticed (Table 1). Independent analysis of MO or MA groups did not disclose significant differences either (Table 1). To uncover specific genetic susceptibility in subgroups with distinct hormonal influences, patients were separated by gender or age of onset (≤10 years, $n = 74$ and >10 years, $n = 120$), but analysis of genotype and allele distributions revealed no significant differences (data not shown). Finally, UNPHASED did not show over-representation of any *ESR1* haplotype in patients (Table 2).

Discussion

In the present study, no significant association between three synonymous polymorphisms in *ESR1* plus an *Alu* insertion in *PGR* and migraine in a Caucasoid population was found. Hormone receptor genes have been involved in migraine susceptibility [7–11]. The *ESR1* SNP rs2228480 was analyzed in two independent Australian groups and an increased frequency of A allele carriers was detected in MO, MA as well as in female migraineurs [6]. In the same samples, *Alu* insertion carriers were 1.8 times more probably to

Table 1 Genotype and allele distributions of *ESR1* rs2077647, rs1801132, and rs2228480 and *PGR Alu* insertion polymorphisms in patient and control groups

	Genotypes *n* (%)					Alleles *n* (%)		
	11	12	22	Sum	*P*	1	2	*P*
rs2077647								
Migraine	52 (24.8)	109 (51.9)	49 (23.3)	210	0.4234	213 (50.7)	207 (49.3)	0.2688
MO	27 (26.5)	54 (52.9)	21 (20.6)	102	0.6948	108 (52.9)	96 (47.1)	0.7099
MA	20 (23.3)	44 (51.2)	22 (25.6)	86	0.4198	84 (48.8)	88 (51.2)	0.2085
Controls	64 (30.5)	101 (48.1)	45 (21.4)	210		229 (54.5)	191 (45.5)	
rs1801132								
Migraine	140 (66.7)	58 (27.6)	12 (5.7)	210	0.3610	338 (80.5)	82 (19.5)	0.9305
MO	72 (70.6)	25 (24.5)	5 (4.9)	102	0.3528	169 (82.8)	35 (17.2)	0.5191
MA	55 (64.0)	25 (29.1)	6 (7.0)	86	0.3948	135 (78.5)	37 (21.5)	0.5405
Controls	136 (64.8)	67 (31.9)	7 (3.3)	210		339 (80.7)	81 (19.3)	
rs2228480								
Migraine	154 (73.3)	52 (24.8)	4 (1.9)	210	0.7102	360 (85.7)	60 (14.3)	0.9212
MO	72 (70.6)	27 (26.5)	3 (2.9)	102	0.7258	171 (83.8)	33 (16.2)	0.4845
MA	65 (75.6)	20 (23.3)	1 (1.2)	86	0.6455	150 (87.2)	22 (12.8)	0.6845
Controls	157 (74.8)	47 (22.4)	6 (2.9)	210		361 (86.0)	59 (14.0)	
Alu								
Migraine	142 (67.6)	62 (29.5)	6 (2.9)	210	0.8524	346 (82.4)	74 (17.6)	0.857
MO	72 (70.6)	28 (27.5)	2 (2.0)	102	0.6323	172 (84.3)	32 (15.7)	0.4525
MA	56 (65.1)	27 (31.4)	3 (3.5)	86	0.8878	174 (80.6)	42 (19.4)	0.6794
Controls	142 (67.6)	60 (28.6)	8 (3.8)	210		344 (81.9)	76 (18.1)	

rs2077647: 1 = T, 2 = C; rs1801132: 1 = C, 2 = G; rs2228480: 1 = G, 2 = A.

Table 2 Haplotype estimation of *ESR1* SNPs

rs2077647–rs1801132–rs2228480	Cases *n* (%)	Controls *n* (%)
T-C-G	138 (34.4)	132 (32.1)
T-C-A	23 (5.7)	26 (6.4)
T-G-G	40 (9.9)	29 (7.1)
C-C-G	147 (36.5)	152 (36.8)
C-C-A	26 (6.4)	27 (6.6)
C-G-G	29 (7.1)	46 (11.0)

Overall association $\chi^2 = 3.829$; d.f. = 5; $P = 0.5743$.

suffer migraine and interaction analysis between the *Alu* insertion and rs2228480 revealed that carrying at least one copy of both risk alleles increased migraine relative risk to 3.2 [7]. A study in a Spanish population failed to replicate these results but revealed that women carrying the C allele at rs1801132 had a 1.6 higher risk of migraine [8]. In turn, this association was not replicated in the Australian population [9]. Using wider genetic coverage, *ESR1* associations were not replicated in a sample of MA Finnish patients: nominal associations for five SNPs did not remain significant after Bonferroni correction for multiple testing [10]. Finally, positive association of *PGR*, but not *ESR1* SNPs, with migraine-associated vertigo was reported [11]. The variants we have used, including the previously unanalyzed rs2077647, are exonic SNPs with minimum allele frequencies (MAF) >0.1 and putative functional effects, perhaps as splicing enhancers. However, our results failed to replicate any of the positive results obtained in previous studies. Replication is considered an important step in establishing the validity of significant results. Thus, the present findings do not corroborate the biological influence of *ESR1* or *PGR* in migraine pathophysiology, albeit potential pitfalls leading to false negative results are sample size and genetic and clinical heterogeneity inherent to migraine.

Hormone-related genetic susceptibility might conceivably augment in subgroups such as women or cases with onset after puberty. In our population, however, genotype, allele and haplotype distributions showed no differences between any of these categories and controls (data not shown). Amongst the still not fully validated migraine categories, the revised ICHD-II classification Appendix includes three MO subtypes: pure menstrual MO, menstrually-related MO, and non-menstrual MO [1]. It would seem reasonable to consider these menstrually-defined subgroups in future MO association studies involving hormone receptor genes.

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