

Progesterone Receptor Gene (PROGINS) Polymorphism Correlates with Late Onset of Migraine

Raffaello Palmirotta,^{1,*} Piero Barbanti,^{2,*} Cristiano Ialongo,³ Maria Laura De Marchis,¹
Jhessica Alessandrini,¹ Gabriella Egeo,² Cinzia Aurilia,² Luisa Fofi,² Maria Giovanna Valente,⁴
Patrizia Ferroni,¹ David Della-Morte,^{1,5} and Fiorella Guadagni¹

Progesterone influences central neuronal excitability, a key event in migraine pathophysiology. Progesterone receptor gene (*PGR*) rs1042838 (G/T - Val660Leu) variant is indicative of PROGINS haplotype and associated to a reduced *PGR* activity. With the aim of investigating whether any type of association existed between this genetic variant and migraine pathophysiology, genotyping was performed in 380 consecutive migraine patients and 185 age-, sex-, and race-ethnicity-matched healthy controls from Interinstitutional Multidisciplinary Bio-Bank (BioBIM) of IRCCS San Raffaele Pisana, Rome, Italy. rs1042838 genotypes did not correlate with demographics or clinical migraine features. However, TT (Leu) genotype was significantly associated with a later age of migraine onset: Patients affected by migraine with aura showed a linear relationship between copy number of the T allele carried by the individual and the age of migraine onset. Our data suggest that the PROGINS *PGR* polymorphism does not directly predispose to migraine but significantly delays migraine onset probably via a reduction in brain neuronal excitability.

Introduction

MIGRAINE IS THE MOST COMMON disabling neurological disorder involving sensory sensitivity and characterized by recurrent episodes of headache (Barbanti *et al.*, 2011). Despite the prevalence and social consequences of this condition, the molecular and genetic mechanisms underlying the pathophysiology of migraine are still partly unknown (Goadsby *et al.*, 2009).

Various clinical and epidemiological observations indicate a strong correlation between female gender, sex female hormones, and migraine susceptibility: (1) The disease is predominant in women after puberty (Gupta *et al.*, 2007; Corominas *et al.*, 2009); (2) the clinical course is modified by reproductive events such as menarche, pregnancy, and menopause (Mac Gregor, 1997; Brandes, 2006; Martin and Behbehani, 2006a); and (3) the use of hormonal contraceptives and hormone replacement may influence migraine occurrence (Massiou and Mac Gregor, 2000; Martin and Behbehani, 2006b; Nappi *et al.*, 2013).

According to the significant female hormone involvement in migraine, some authors have postulated that genes encoding hormone receptors and causing changes in neurotransmitter systems may play a role in the molecular pathogenesis of

migraine headache (Colson *et al.*, 2004, 2005; Martin and Behbehani, 2006a; Lee *et al.*, 2007; Joshi *et al.*, 2010; Schürks *et al.*, 2010; Rodriguez-Acevedo *et al.*, 2013).

However, while the correlation between migraine and estrogen receptors appears to be fairly assessed, the association studies with progesterone receptor are less numerous and more controversial (Schürks *et al.*, 2010).

Progesterone receptor gene (*PGR*), located on chromosome 11q22 (OMIM# 607311), encodes a steroid receptor that principally mediates the effect of progesterone on the establishment and maintenance of reproductive events (UniProt# P06401).

PGR is characterized by the presence of three common variants consisting of a 306-bp ALU insertion/deletion in intron 7/G, a missense Val660Leu (G/T substitution) (rs1042838) in exon 4, and a silent His770His (C/T substitution) (rs1042839) in exon 5. All these three variants are in linkage disequilibrium; therefore, the variation of a single polymorphism is indicative of the simultaneous variation of the other two (Rockwell *et al.*, 2012).

On the basis of such biological evidence, several authors define these complex variants with the name of “PROGINS haplotype” (Alu insertion and T alleles for the other two single nucleotide polymorphisms [SNPs]) determining a

¹Interinstitutional Multidisciplinary Biobank (BioBIM), Biomarker Discovery and Advanced Technologies (BioDAT), Rome, Italy.

²Headache and Pain Unit, Department of Neurological, Motor and Sensorial Sciences, IRCCS San Raffaele Pisana, Rome, Italy. Departments of ³Internal Medicine and ⁵Systems Medicine, University of Rome “Tor Vergata,” Rome, Italy.

⁴Fondazione San Raffaele, Ceglie Messapica, Italy.

*These authors contributed equally.

reduced amount of gene transcripts and decreased protein activity (Romano *et al.*, 2007; Rockwell *et al.*, 2012).

Several studies have investigated a possible association between *PGR* polymorphisms and migraine (Colson *et al.*, 2004, 2005; Lee *et al.*, 2007; Corominas, *et al.*, 2009; Joshi *et al.*, 2010; Schürks *et al.*, 2010; Rodriguez-Acevedo *et al.*, 2013), providing conflicting results due to several biases, including a poor clinical patients' characterization.

Therefore, to shed more light on this topic, we investigated the association between *PGR* rs1042838 polymorphism and migraine in a large cohort of Mediterranean Caucasian migraine patients from the Interinstitutional Multidisciplinary BioBank (BioBIM) of IRCCS San Raffaele Pisana, Rome, extensively investigating and carefully detailing their demographic and clinical features.

Materials and Methods

Patients' recruitment, inclusion and exclusion criteria

Since January 2008, 380 Mediterranean Caucasian unrelated individuals affected by migraine without aura (MwoA), migraine with aura (MwA), and chronic migraine (CM) (Headache Classification Committee of the International Headache Society, 2013) were consecutively recruited at the Headache and Pain Unit, IRCCS San Raffaele Pisana, Rome, Italy. Specifically trained neurologists have performed a complete physical examination on migraine patients, including a neurological and fundoscopic examination and anthropometric measurements. Detailed information on clinical migraine features, concomitant diseases and treatments, sociodemographics, and lifestyle was gathered with face-to-face interviews using a structured questionnaire, as previously described (Palmirotta *et al.*, 2013a). Comorbidities diagnoses were based on analysis of medical charts and documents, symptoms, treatments, and the patient's self-reported medical history. Patients with significant metabolic, renal, endocrine, hepatic, pulmonary, cardiovascular, or other systemic disease were excluded from the study.

We enrolled in our Biobank 185 healthy controls from subjects in a state of good health with no significant medical diseases and not-pharmaceutical therapies. They were screened for sociodemographics, lifestyle, and concomitant diseases and treatments by a direct interview using a structured questionnaire. Healthy subjects with a family history of migraine or other neurological diseases were excluded.

Informative and consent forms, approved by the institutional Ethics Committee of San Raffaele Scientific Institute, were provided to both groups, along with the permission to obtain blood samples for research purposes (Palmirotta *et al.*, 2013a).

Genotyping

All biological samples were stored at the BioBIM of IRCCS San Raffaele Pisana following the biobanking standard operative procedures (Palmirotta *et al.*, 2011). DNA extraction, PCRs, and sequencing analysis were performed as previously described (Palmirotta *et al.*, 2013b). *PGR* polymorphism rs1042838 (G/T - Val660Leu) was determined by a 135-bp PCR amplification on the basis of the *PGR* Ensembl (Ensembl accession number ENSG00000082175) using the following primers: F5'-AGTTGTGAGAGCACTGGATGC-3' and R5'-AACAGGTTGATCAGTGGTGG-3'.

Statistical analysis

An association between categorical variables was assessed by a generalized Cochran–Mantel–Haenszel test to control for gender as a confounder. For continuous variables, mean differences were evaluated through a univariate general linear model (GLM) for fixed main effects. Particularly, to avoid an excessive stratification, the effects of familiarity for migraine and the kind of migraine were controlled, considering them as factors in the GLM besides the *PGR* genotype.

Tukey's HSD *post hoc* test was used for pairwise comparisons that were possible. Normality was assessed through the Kolmogorov–Smirnov test, and homogeneity of variance was assessed through the Levene test. Logistic regression was used to estimate odds ratio (OR). Statistical models were corrected for the most significant covariates found in this study (familiarity for migraine). Statistical significance was assessed for $p < 0.05$ (two-tailed probability). All calculations were done by IBM SPSS 20 (IBM Corp., Armonk, NY), except for power analysis done by G*Power 3.1.3 free software (Heinrich Heine Universität, Dusseldorf, Germany). Data are represented as mean ± standard deviation or mean ± standard error of the mean.

Results

Demographic characteristics and the age of migraine onset of the study populations are reported in Table 1. Genotypes and the allele frequencies of the rs1042838 polymorphism between migraineurs and controls did not significantly differ from those predicted by the Hardy–Weinberg equilibrium in controls (chi-squared=3.71, $p=0.054$) as well as in MwoA (chi-squared=0.03, $p=0.820$), MwA (chi-squared=0.95, $p=0.329$), and CM (chi-squared=0.8, $p=0.370$) patients (Table 2). When controlling for gender, no significant statistical association was found between the *PGR* genotype and the kind of migraine ($p=0.666$), laterality ($p=0.242$), unilateral autonomic symptoms ($p=0.229$), prophylaxis

TABLE 1. BASELINE CHARACTERISTICS OF THE MIGRAINE PATIENTS AND CONTROLS

Variable	All patients with migraine (380)				
	Controls (180)	MwA (64)	MwoA (191)	CM (125)	
Sex, F/M (F%)	124/56 (68.9)	49/15 (76.6)	156/35 (81.7)	112/13 (89.6)	
Age at observation, mean (SD)	38.9 (12.0)	39.3 (12.7)	40.4 (11.8)	47.5 (13.3)	
Age at migraine onset, mean (SD)	—	22.1 (12.7)	20.5 (11.6)	21.0 (12.0)	

CM, chronic migraine; F, female; M, male; MwA, migraine with aura; MwoA, migraine without aura; SD, standard deviation.

TABLE 2. DISTRIBUTIONS OF GENOTYPE AND ALLELE FREQUENCIES OF *PGR* rs1042838 POLYMORPHISM OBSERVED IN PATIENTS AND CONTROLS

	n	PGR genotypes (%)			PGR alleles (%)		HW (p)
		GG	GT	TT	G	T	
Controls	180	139 (77.2)	35 (19.4)	6 (3.3)	313 (86.9)	47 (13.1)	0.054
Male (%)	56	36 (64.3)	15 (26.8)	5 (8.9)	87 (77.7)	25 (22.3)	0.089
Female (%)	124	103 (83.1)	20 (16.1)	1 (0.8)	226 (91.1)	22 (8.9)	0.978
All patients with migraine (%)	380	274 (72.1)	95 (25.0)	11 (2.9)	643 (84.6)	117 (15.4)	0.432
Male (%)	63	46 (73.0)	16 (25.4)	1 (1.6)	108 (85.7)	18 (14.3)	0.769
Female (%)	317	228 (71.9)	79 (24.9)	10 (3.2)	535 (84.4)	99 (15.6)	0.333
MwA (%)	64	45 (70.3)	16 (25.0)	3 (4.7)	106 (82.8)	22 (17.2)	0.330
Male (%)	15	11 (73.3)	4 (26.7)	0 (0.0)	26 (86.7)	4 (13.3)	0.551
Female (%)	49	34 (69.4)	12 (24.5)	3 (6.1)	80 (81.6)	18 (18.4)	0.199
MwoA (%)	191	137 (71.7)	50 (26.2)	4 (2.1)	324 (84.8)	58 (15.2)	0.821
Male (%)	35	27 (77.1)	8 (22.9)	0 (0.0)	62 (88.6)	8 (11.4)	0.445
Female (%)	156	110 (70.5)	42 (26.9)	4 (2.6)	262 (84.0)	50 (16.0)	0.997
CM (%)	125	92 (73.6)	29 (23.2)	4 (3.2)	213 (85.2)	37 (14.8)	0.371
Male (%)	13	8 (61.5)	4 (30.8)	1 (7.7)	20 (76.9)	6 (23.1)	0.631
Female (%)	112	84 (75.0)	25 (22.3)	3 (2.7)	193 (86.2)	31 (13.8)	0.498

Values are given as *n* (%).

HW, Hardy-Weinberg equilibrium; *PGR*, progesterone receptor gene.

($p=0.124$), abuse of drug ($p=0.748$), and kind of drug abused ($p=0.732$).

PGR was not a factor for frequency of attacks per month (males $p=0.702$, females $p=0.773$), for the quantity of drug abused (males p =not calculable, females $p=0.143$), and for the duration of abuse (males p =not calculable, females $p=0.896$).

The small number of male patients did not allow a reliable analysis of variance to be performed.

With regard to female patients, the type of migraine ($p=0.749$) and the familiarity for migraine ($p=0.158$) were not factors for mean onset age nor for considering each subgroup of migraineurs patients (interaction term: type of migraine*familiarity $p=0.295$). Conversely, *PGR* genotype was a significant factor ($p=0.014$, effect size: partial $\eta^2=0.027$), with no difference in each group of migraine type ($p=0.092$).

However, in the sole MwA female patients, a significant difference was found between GG carriers and GT carriers ($p=0.04$), and between GG carriers and TT carriers ($p<0.01$) (Fig. 1A). Notably, a significantly slight linear relation ($r^2=0.221$; slope= 9.08 ± 2.49 , $p<0.01$; intercept= 18.34 ± 1.74 , $p<0.01$) was found between the number of T alleles possessed and mean shift in onset age. Hence, it was as follows: Mean onset age in MwA females= 18.34 years + 9.08 *(number of T alleles) years (Fig. 1B).

However, in the same group, the *PGR* TT genotype failed to give a statistically significant OR with regard to an onset age greater than the average for women seen in this study (20.9 ± 11.9 years).

The power reached for the ANOVA model was 92.2% to detect and an effect size like a Cohen's d 1.0; for the logistic regression, it was 82.3% to detect and an effect size like an OR of 1.2.

No correlations were found between mean onset age of migraine and age of menarche ($p=0.232$), nor were they found between *PGR* genotype and age of menarche ($p=0.84$).

Discussion

In this study, we found that the rs1042838 *PGR* polymorphism, indicative of PROGENS haplotype, is not associated with migraine risk or clinical features but correlates with the age of migraine onset in female patients.

The frequency of this genotype in our study is similar to that observed in normal Caucasian subjects (Schürks *et al.*, 2010). Previous association studies on the link between PROGENS polymorphism and migraine yielded conflicting results: A positive correlation was found in two independent Caucasian populations (Colson *et al.*, 2005): a protective

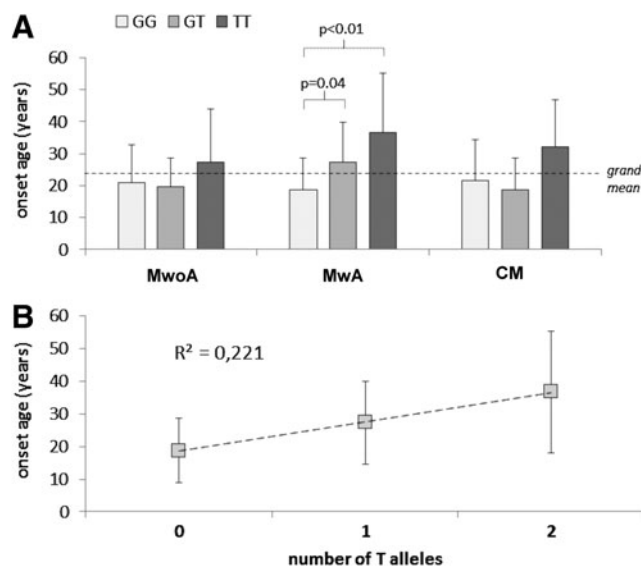


FIG. 1. (A) Mean onset age in different migraine kind groups according to the progesterone receptor gene (*PGR*) genotype; (B) linear relation between mean onset age and number of T alleles in MwA females. CM, chronic migraine; MwA, migraine with aura; MwoA, migraine without aura.

association in an Indian population (Joshi *et al.*, 2010) and no association in a Caucasian and in a mixed population (Corominas *et al.*, 2009; Rodriguez-Acevedo *et al.*, 2013). A meta-analysis study also confirmed the lack of association between *PGR* gene variants and migraine (Schürks *et al.*, 2010).

Our data support the lack of association between this variant, migraine risk, or migraine clinical features. However, a new clinically useful finding from this study is the evidence of an association between rs1042838 TT genotype, indicative of the “PROGINS variant haplotype,” and a delayed age of migraine onset compared with GT and GG genotypes, with a linear relationship in the MWA female patient group.

Interestingly, in a precedent study performed on 150 migraine patients selected for the presence of concomitant vertigo (migraine-associated vertigo [MAV]), Lee *et al.* (2007) reported that the *PGR* PROGINS variant allele T of rs1042838 was significantly associated with MAV. We could not test this effect in our study, as only a few patients had vertigo, a symptom that is more frequent in young migraineurs. It should be emphasized that the mean age of our patients (42 ± 12.9 years) is much higher than that of subjects analyzed in previous studies (Corominas *et al.*, 2009; Joshi *et al.*, 2010).

Our data support the hypothesis that the *PGR* gene is not a direct migraine risk factor but is more a disease-modifier gene whose own variants result in a more delayed disease onset.

How the PROGINS variants might influence the age of migraine onset is a matter of speculation. We hypothesize that it could alter brain cortical excitability. In fact, the ALU insertion of PROGINS haplotype results in a reduction of gene transcript and the missense Val600Leu leads to different *PGR* phosphorylation and degradation on ligand binding (Romano *et al.*, 2007). Consequently, the subjects carrying the PROGINS polymorphism show a reduced progesterone responsiveness due to the decreased receptor bioactivity (Romano *et al.*, 2007; D’Amora *et al.*, 2009).

Sex steroids influence neuronal firing through steroid receptors dispersed throughout the brain (Noseda and Burstein, 2013). In the rat, progesterone may enhance neuronal excitability as estrogens (Choudhuri *et al.*, 2002; Chauvel *et al.*, 2013), although its exact molecular mechanisms are unclear. Central neuronal excitability plays a crucial role in the predisposition to develop migraine (Aurora and Wilkinson, 2007) as inferred by cortical spreading depression (CSD), the electrophysiological substrate of MWA and possibly MwoA. Usually, migraine starts in puberty, when sex hormones contribute toward lowering the neuronal response threshold to trigger factors in predisposed individuals. Ovarian hormones have a complex role in CSD susceptibility. In ovariectomized animals, CSD frequency is markedly decreased compared with normal cycling rats. Estrogens can increase the susceptibility to CSD through different mechanisms encompassing effects on glutamate neurotransmission, as well as through an increase of dendritic spines and synaptic density (Eikermann-Haerter *et al.*, 2012). Interestingly, it has been recently demonstrated that progesterone also significantly increases CSD frequency and restores the CSD inhibition by L-kynurenine (Chauvel *et al.*, 2013).

We, therefore, suggest that a lower progesterone receptor functionality in rs1042838 T allele carriers might reduce progesterone excitatory effects on neurons, raising their

response threshold to migraine triggers and, hence, leading to a delayed onset of the disease. Considering the correlation between estrogens, CSD susceptibility, and glutamate neurotransmission, it is important to note the linear correlation we found between the copy number of T allele and the age at migraine onset in female patients affected by MWA, the migraine type most clearly characterized by a glutamate-dependent cortical hyperexcitability (Eikermann-Haerter *et al.*, 2012). In addition, it is interesting to report that previous genome-wide analysis and association studies suggested a significant association between “PROGINS” variant and the increase of age for menarche (Guo *et al.*, 2006; Taylor *et al.*, 2010). However, our data did not allow us to find any correlation between age of menarche, PROGINS genotype, and age of migraine onset.

The main strength of this study is the detailed demographic and clinical characterization of migraine patients that allowed for clinical correlations neglected in previous studies (Palmirotta *et al.*, 2013b). Limitation to acknowledge is the presence of possible artifacts, as the number of MWA patients might be too low for sub-analysis.

Conclusion

PROGINS *PGR* polymorphism does not influence migraine risk and features but is positively correlated with a later age of migraine. Further genetic association studies are imperative to understand the role of *PGR* in migraine pathophysiology that may be useful as a biomarker for detecting asymptomatic individuals at an increased risk for migraine and for developing effective treatment strategies as well (Eikermann-Haerter *et al.*, 2012; Nappi *et al.*, 2013).

Acknowledgments

This study was partially supported by the Grant MERIT RBNE08NKH7 to San Raffaele Foundation Ceglie Messapica (www.fondazioneanraffaele.com/) and partially by the grant PO FESR 2007/2013 Linea di Intervento 4.1.1.1–SIASOP.

Disclosure Statement

No competing financial interests exist.

References

- Aurora, S.K., and Wilkinson, F. (2007). The brain is hyperexcitable in migraine. *Cephalalgia* **27**, 1442–1453.
- Barbanti, P., Aurilia, C., Egeo, G., and Fofi, L. (2011). Migraine prophylaxis: what is new and what we need? *Neurol Sci* **32 Suppl 1**, S111–S115.
- Brandes, J.L. (2006). The influence of estrogen on migraine: a systematic review. *JAMA* **295**, 1824–1830.
- Chauvel, V., Schoenen, J., and Multon, S. (2013). Influence of ovarian hormones on cortical spreading depression and its suppression by L-kynurenine in rat. *PLoS One* **8**, e82279.
- Choudhuri, R., Cui, L., Yong, C., Bowyer, S., Klein, R.M., Welch, K.M., and Berman, N.E. (2002). Cortical spreading depression and gene regulation: relevance to migraine. *Ann Neurol* **51**, 499–506.
- Colson, N.J., Lea, R.A., Quinlan, S., Mac Millan, J., and Griffiths, L.R. (2004). The estrogen receptor 1 G594A polymorphism is associated with migraine susceptibility in two independent case/control groups. *Neurogenetics* **5**, 129–133.

- Colson, N.J., Lea, R.A., Quinlan, S., Mac Millan, J., and Griffiths, L.R. (2005). Investigation of hormone receptor genes in migraine. *Neurogenetics* **6**, 17–23.
- Corominas, R., Ribasés, M., Cuenca-León, E., Cormand, B., and Macaya, A. (2009). Lack of association of hormone receptor polymorphisms with migraine. *Eur J Neurol* **16**, 413–415.
- D'Amora, P., Maciel, T.T., Tambellini, R., Mori, M.A., Pesquero, J.B., Sato, H., Girão, M.J., Guerreiro da Silva, I.D., and Schor, E. (2009). Disrupted cell cycle control in cultured endometrial cells from patients with endometriosis harboring the progesterone receptor polymorphism PROGINS. *Am J Pathol* **175**, 215–224.
- Eikermann-Haerter, K., Can, A., and Ayata, C. (2012). Pharmacological targeting of spreading depression in migraine. *Expert Rev Neurother* **12**, 297–306.
- Goadsby, P.J., Charbit, A.R., Andreou, A.P., Akerman, S., and Holland, P.R. (2009). Neurobiology of migraine. *Neuroscience* **161**, 327–341.
- Guo, Y., Shen, H., Xiao, P., Xiong, D.H., Yang, T.L., Guo, Y.F., Long, J.R., Recker, R.R., and Deng, H.W. (2006). Genomewide linkage scan for quantitative trait loci underlying variation in age at menarche. *J Clin Endocrinol Metab* **91**, 1009–1014.
- Gupta, S., Mehrotra, S., Villalon, C.M., Perusquia, M., Saxena, P.R., and Maassen Van Den Brink, A. (2007). Potential role of female sex hormones in the pathophysiology of migraine. *Pharmacol Ther* **113**, 321–340.
- Headache Classification Committee of the International Headache Society (IHS). (2013). The International Classification of Headache Disorders, 3rd Edition (beta version). *Cephalalgia* **33**, 629–808.
- Joshi, G., Pradhan, S., and Mittal, B. (2010). Role of the oestrogen receptor (ESR1 PvuII and ESR1 325 C->G) and progesterone receptor (PROGINS) polymorphisms in genetic susceptibility to migraine in a North Indian population. *Cephalalgia* **30**, 311–320.
- Lee, H., Sininger, L., Jen, J.C., Cha, Y.H., Baloh, R.W., and Nelson, S.F. (2007). Association of progesterone receptor with migraine-associated vertigo. *Neurogenetics* **8**, 195–200.
- Mac Gregor, E.A. (1997). Menstruation, sex hormones, and migraine. *Neurol Clin* **15**, 125–141.
- Martin, V.T., and Behbehani, M. (2006a). Ovarian hormones and migraine headache: understanding mechanisms and pathogenesis-part I. *Headache* **46**, 3–23.
- Martin, V.T., and Behbehani, M. (2006b). Ovarian hormones and migraine headache: understanding mechanisms and pathogenesis-part 2. *Headache* **46**, 365–386.
- Massiou, H., and Mac Gregor, E.A. (2000). Evolution and treatment of migraine with oral contraceptives. *Cephalalgia* **20**, 170–174.
- Nappi, R.E., Merki-Feld, G.S., Terreno, E., Pellegrinelli, A., and Viana, M. (2013). Hormonal contraception in women with migraine: is progestogen-only contraception a better choice? *J Headache Pain* **14**, 66.
- Noseda, R., and Burstein, R. (2013). Migraine pathophysiology: anatomy of the trigeminovascular pathway and associated neurological symptoms, CSD, sensitization and modulation of pain. *Pain* **154 Suppl 1**, S44–S53.
- Palmirotta, R., Barbanti, P., Ludovici, G., Egeo, G., Aurilia, C., Fofi, L., De Marchis, M.L., Spila, A., Ferroni, P., Della-Morte, D., and Guadagni, F. (2013a). Establishment of a biorepository for migraine research: the experience of Inter-institutional Multidisciplinary BioBank (BioBIM). *Neurol Sci* **34**, 1659–1663.
- Palmirotta, R., Ludovici, G., De Marchis, M.L., Savonarola, A., Leone, B., Spila, A., De Angelis, F., Della-Morte, D., Ferroni, P., and Guadagni, F. (2011). Pre-Analytical procedures for DNA studies: the experience of the Interinstitutional Multidisciplinary BioBank (BioBIM). *Biopreserv Biobank* **9**, 35–45.
- Palmirotta, R., Ludovici, G., Egeo, G., Ialongo, C., Aurilia, C., Fofi, L., De Marchis, M.L., Della-Morte, D., Barbanti, P., and Guadagni, F. (2013b). Prion protein gene M129V polymorphism and variability in age at migraine onset. *Headache* **53**, 540–545.
- Rockwell, L.C., Rowe, E.J., Arnson, K., Jackson, F., Froment, A., Nduabe, P., Seck, B., Jackson, R., and Lorenz, J.G. (2012). Worldwide distribution of allelic variation at the progesterone receptor locus and the incidence of female reproductive cancers. *Am J Hum Biol* **24**, 42–51.
- Rodriguez-Acevedo, A.J., Maher, B.H., Lea, R.A., Benton, M., and Griffiths, L.R. (2013). Association of oestrogen-receptor gene (ESR1) polymorphisms with migraine in the large Norfolk Island pedigree. *Cephalalgia* **33**, 1139–1147.
- Romano, A., Delvoux, B., Fischer, D.C., and Groothuis, P. (2007). The PROGINS polymorphism of the human progesterone receptor diminishes the response to progesterone. *J Mol Endocrinol* **38**, 331–350.
- Schürks, M., Rist, P.M., and Kurth, T. (2010). Sex hormone receptor gene polymorphisms and migraine: a systematic review and meta-analysis. *Cephalalgia* **30**, 1306–1328.
- Taylor, K.C., Small, C.M., Epstein, M.P., Sherman, S.L., Tang, W., Wilson, M.M., Bouzyk, M., and Marcus, M. (2010). Associations of progesterone receptor polymorphisms with age at menarche and menstrual cycle length. *Horm Res Paediatr* **74**, 421–427.

Address correspondence to:
 Raffaele Palmirotta, MD, PhD
 Interinstitutional Multidisciplinary Biobank (BioBIM)
 Biomarker Discovery and Advanced
 Technologies (BioDAT)
 IRCCS San Raffaele Pisana
 Via di Val Cannuta 247
 Rome 00166
 Italy

E-mail: raffaele.palmirotta@sanraffaele.it

Received for publication May 22, 2014; received in revised form October 17, 2014; accepted November 2, 2014.