Gene 607 (2017) 36–40

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Investigation of polymorphisms in genes involved in estrogen metabolism in menstrual migraine

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ARTICLE INFO

Article history:
Received 27 September 2016
Received in revised form 23 December 2016
Accepted 12 January 2017
Available online 13 January 2017

Keywords:
Migraine
Menstrual migraine
Estrogen metabolism
Single nucleotide polymorphism
COMT
CYP genes

ABSTRACT

Migraine is a common, disabling headache disorder, which is influenced by multiple genes and environmental triggers. After puberty, the prevalence of migraine in women is three times higher than in men and >50% of females suffering from migraine report a menstrual association, suggesting hormonal fluctuations can influence the risk of migraine attacks. It has been hypothesized that the drop in estrogen during menses is an important trigger for menstrual migraine. Catechol-*O*-methyltransferase (COMT) and Cytochrome P450 (CYP) enzymes are involved in estrogen synthesis and metabolism. Functional polymorphisms in these genes can influence estrogen levels and therefore may be associated with risk of menstrual migraine. In this study we investigated four single nucleotide polymorphisms in three genes involved in estrogen metabolism that have been reported to impact enzyme levels or function, in a specific menstrual migraine cohort. 268 menstrual migraine cases and 142 controls were genotyped for rs4680 in *COMT* (Val158Met), rs4646903 and rs1048943 in *CYP1A1* (T3801C and Ile462Val) and rs700519 in *CYP19A1* (Cys264Arg). Neither genotype nor allele frequencies for the *COMT* and *CYP* SNPs genotyped were found to be significantly different between menstrual migraineurs and controls by chi-square analysis ($P > 0.05$). Therefore we did not find association of functional polymorphisms in the estrogen metabolism genes *COMT*, *CYP1A1* or *CYP19A1* with menstrual migraine. Further studies are required to assess whether menstrual migraine is genetically distinct from the common migraine subtypes and identify genes that influence risk.

1. Introduction

Migraine is a common neurological disorder typically characterized by recurring, incapacitating attacks of 1–3 days of severe headache, often accompanied by nausea, autonomic dysfunction, or other temporary neurological symptoms. It has clear social and economic burdens and significant negative impact on quality of life. The most common types of migraine which have been defined by the International Headache Society (IHS) criteria in the International Classification of Headache Disorders III beta version (ICHD-III β) are migraine without aura (MO), followed by migraine with aura (MA), which is distinguished by the presence of an aura preceding the headache in the early stages of the migraine (Headache Classification Committee of the International Headache Society, 2013). The aura lasts less than one hour and is typically visual, such as a scintillating scotoma. Sensory aura is less common and usually accompanies visual aura.

From large epidemiological studies the prevalence of migraine across populations has been shown to be approximately 12% (Lipton et al., 2001). Migraine affects both sexes equally until puberty, however after the menarche there is an increasing prevalence of migraine in women so that it is three times higher in women compared to men until menopause, when it decreases again (Victor et al., 2010). The higher rates in women are thought to be hormonally driven (Sacco et al., 2012). Aspects of the women's reproductive cycle, including the menstrual cycle, pregnancy, the postpartum period and menopause, are regulated through variations of the levels of

Abbreviations: χ^2, chi-square; COMT, catechol-*O*-methyltransferase; CYP, cytochrome P450; CYP1A1, CYP Family 1, Subfamily A, polypeptide 1; CYP19A1, CYP Family 19, Subfamily A, polypeptide 1; HRM, high resolution melt; HWE, Hardy-Weinberg Equilibrium; IHS, International Headache Society; ICHD-III β, International Classification of Headache Disorders III beta version; MA, migraine with aura; MAF, minor allele frequency; MM, menstrual migraine; MO, migraine without aura; MRM, menstrual related migraine, PCR polymerase chain reaction; PMM, pure menstrual migraine; RFLP, restriction-fragment length polymorphism; SNP, single nucleotide polymorphism.

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http://dx.doi.org/10.1016/j.gene.2017.01.008
0378-1119/

estrogen and progesterone and their feedback control. >50% of females suffering from MO report a menstrual association (Vetvik et al., 2014; Pavlovic et al., 2015), which has led to the further classification of menstrual migraine (MM) subtypes: Pure MM (PMM) is diagnosed in women who report MO attacks during days −2 to +3 of menstruation, with no attacks at other times in the cycle, in at least two of three menstrual cycles; menstrually-related migraine (MRM) is diagnosed when migraine attacks also occur at other times of the cycle as well as in conjunction with menstruation. Migraine attacks that take place during the menstrual period tend to be more disabling, more severe, longer in duration and more resistant to treatment than attacks at other times of the month (Pavlovic et al., 2015; Vetvik et al., 2015) (and references within). The drop in estrogen during the late luteal phase which causes menstrual bleeding is thought to be an important trigger for MM (Somerville, 1972; MacGregor et al., 2006). Furthermore, estrogen is a neurosteroid, and can influence the pain processing networks and vascular endothelium involved in the pathophysiology of migraine (Martin and Behbehani, 2006a).

Migraine can be triggered by various factors, but the disorder has a strong genetic component. Genome wide association studies (GWAS) of common migraine have identified single nucleotide polymorphisms (SNPs) at thirteen loci that have achieved genome-wide significance with respect to association with migraine (Anttila et al., 2013). While the implicated genes are involved in a variety of different pathways, many are known to have a role in synaptic or neuronal function. Hormonal-related genes have not been identified among loci reaching genome-wide significance in migraine GWAS to date. However, due to the prevalence of women with migraine a number of candidate gene studies have investigated the role of female hormone-related genes, including estrogen and progesterone receptors, with some positive associations reported (Schurks et al., 2010; Rodriguez-Acevedo et al., 2013; Li et al., 2015; Ghosh et al., 2014). The few genetic studies specifically of MM have also focused on hormone genes, in particular those related to estrogen, with mixed results (Sullivan et al., 2013; De Marchis et al., 2015; Rodriguez-Acevedo et al., 2014). Rodriguez-Acevedo et al. (2014) found that while variants in Estrogen Receptor 1 (*ESR1*) itself were not associated with MM, SNPs in tumour necrosis factor alpha (TNFα) and Spectrin Repeat Containing, Nuclear Envelope 1 (*SYNE1*), a gene neighbouring *ESR1*, were positively associated (Rodriguez-Acevedo et al., 2014).

Many genes are involved in estrogen biosynthesis and its metabolism, and a number of these have genetic variations that can affect the levels or function of the enzymes that they encode. Because hormones have been implicated in the risk of developing diseases such as cancer, e.g. particularly breast cancer in which estrogen plays a central role, SNPs in genes including catechol-*O*-methyltransferase (*COMT*), Cytochrome P450 (*CYP*) *CYP1A1* and *CYP19A1* have been well-studied with some showing a positive association with disease risk (He et al., 2012; Xiao et al., 2013; He et al., 2013; Daly, 2015; Haiman et al., 2003). COMT is the principal enzyme in the degradation of both catecholamines and estrogen. The *COMT* rs4680 (G>A) SNP causes a valine (Val) to a methionine (Met) substitution at amino acid position 158 (Val158Met), which affects the thermal stability and activity of the enzyme (Lotta et al., 1995), leading to a 30–50% decrease in COMT activity in Met homozygotes (Chen et al., 2004). CYP enzymes are essential in the synthesis and metabolism of hormones, including estrogen. CYP1A1 participates in estrogen metabolism in extrahepatic tissues by catalysing estrogen hydroxylation (Tsuchiya et al., 2005). *CYP1A1* rs1048943 results in an A>G transition at nucleotide position 2455, in exon 7 causing a substitution from isoleucine (Ile) to a valine (Val) at amino acid position 462 (Ile462Val). The CYP1A1 Ile462Val polymorphism locates in the heme-binding region of the enzyme and has been reported to result in a twofold increase in activity (Cosma et al., 1993; Crofts et al., 1994). rs4646903 (CYP1A1 T3801C) is a 3′ noncoding SNP that is important for translational efficiency and mRNA stability, and which has been associated with increased enzyme activity (Petersen et al., 1991; Landi et al., 1994). Cytochrome P450 aromatase (Cyp19) catalyses the formation of aromatic C18 estrogens from C19 androgens, which is the final and rate-limiting step of estrogen biosynthesis (Simpson et al., 1994). The *CYP19A1* rs700519 (C>T) SNP results in a substitution from a cysteine (Cys) to an Arginine (Arg) at amino acid position 264 (Cys264Arg) which been shown to result in a moderate decrease (25%) in enzyme activity (Ma et al., 2005).

In this study we focus on investigating whether functional polymorphisms in genes that encode enzymes required for estrogen synthesis and metabolism, and therefore have the potential, or have been shown to affect estrogen levels, are associated with risk of MM in a United Kingdom (UK) case-control population.

2. Materials and methods

2.1. Population

The population for this research consisted of 437 females recruited by the City of London Migraine Clinic, including 268 MM cases and 142 controls (median age 45; range 21–60 vs 40; 22–61 years). Migraine diagnosis was in accordance with ICHD-III β (Headache Classification Committee of the International Headache Society, 2013), however with respect to MM and its subtypes criteria B was not applied: 40 of the women reported their migraines to be predominantly associated with menstruation, however as detailed diary information was not available, an objective diagnosis of PMM or MRM could not be made (MacGregor, 2012). Therefore MM subtype analyses was not performed. Controls were women with no personal or family history of migraine, age and ethnicity matched to cases where possible. Saliva samples were collected and DNA isolated from Oragene saliva DNA extraction kits. Ethical approval for this study was obtained from the Queensland University of Technology (QUT) Ethics Committee. All participants provided consent to participate in this research.

2.2. Genotyping

The *COMT* rs4680, *CYP1A1* rs4646903 and rs1048943 polymorphisms were genotyped by polymerase chain reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP) analysis. For COMT rs4680 the primers 5′ ACTGTGGCTACTCAGCTGTG and 5′ GGAAAAAGGTCCAGACTGTT amplified a 169 bp PCR product, which was digested with *Nla*III (New England biolabs [NEB]), resulting in fragments of 114, 29 and 26 bp for the G allele and 96, 29, 26, and 12 bp for the minor A allele which were distinguished on 4% agarose gels. For *CYP1A1* rs4646903, the primers 5′ CAGTGAAGAGGTGTAGCCGCT and 5′ TAGGAGTCTTGTCTCATGCCT amplified a 343 bp PCR product, which was digested with *Msp*I (NEB). The T-allele is not cut, but the C-allele results in fragments of 207 and 136 bp. For *CYP1A1* rs1048943 the primers 5′ ACAGAGTCTAGGCCTCAGGG and 5′ CCCCTGATGGTGCTATCGAC amplified a 270 bp product which was digested with *Bsr*DI (NEB) to give fragments of 190 and 80 bp for the A allele, while the G allele is not cut. The latter two RFLPs were analysed on 3% agarose gels.

The *CYP19A1* rs700519 polymorphism was analysed by HRM (High Resolution Melt) analysis using a Rotor-Gene Q 2plex HRM System (QIAGEN). The primers 5′TCAACTCAGTGGCAAAGTCCA and 5′CAGCAAGGATTTGAAAGATGCC were used with a cycling profile of 45 cycles at 95 °C for 5 s, 58 °C for 10s and 72 °C for 20 s to generate a 109 bp PCR product which was subsequently subjected to melt analysis between 69 °C and 84 °C to produce three distinct curves corresponding to the CC, CT and TT genotypes. The TT genotype is rare, so none were detected in the MM case-control population, but we did detect a TT individual in an independent population, which was used as a positive control for this genotype in all HRM runs.