Gene 512 (2013) 35–40

Contents lists available at SciVerse ScienceDirect

Gene

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

Matrix metalloproteinase (MMP)-2 gene polymorphisms affect circulating MMP-2 levels in patients with migraine with aura

Flavia M. Gonçalves [a], Alisson Martins-Oliveira [b], Riccardo Lacchini [b], Vanessa A. Belo [b], Jose G. Speciali [c], Fabíola Dach [c], Jose E. Tanus-Santos [b,*]

[a] Department of Pharmacology, State University of Campinas, Campinas, SP, Brazil
[b] Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, SP, Brazil
[c] Department of Neuroscience, Division of Neurology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, SP, Brazil

ARTICLE INFO

Article history:
Accepted 29 September 2012
Available online 5 October 2012

Keywords:
Migraine
Matrix metalloproteinase-2
Polymorphisms
Haplotype
TIMP-2

ABSTRACT

Matrix metalloproteinases (MMP) are involved in the disruption of blood–brain barrier (BBB) during migraine attacks. In the present study, we hypothesized that two functional polymorphisms ($C^{-1306}T$ and $C^{-735}T$) in *MMP-2* gene and *MMP-2* haplotypes are associated with migraine and modify MMP-2 and tissue inhibitor of MMP (TIMP)-2 levels in migraine. Genotypes for *MMP-2* polymorphisms were determined by real time-PCR using Taqman allele discrimination assays. Haplotypes were inferred using the PHASE program. Plasma MMP-2 and TIMP-2 concentrations were measured by gelatin zymography and ELISA, respectively, in 148 healthy women without history of migraine and in 204 women with migraine (153 without aura; MWA, and 51 with aura; MA). Patients with MA had higher plasma MMP-2 concentrations and MMP-2/TIMP-2 ratios than patients with MWA and controls ($P<0.05$). While *MMP-2* genotype and haplotype distributions for the polymorphisms were similar among the groups ($P>0.05$), we found that the CC genotype for $C^{-735}T$ polymorphism and the CC haplotype were associated with higher plasma MMP-2 concentrations in MA group ($P<0.05$). Our findings may help to understand the role of MMP-2 and its genetic variants in the pathophysiology of migraine and to identify a particular group of migraine patients with increased MMP-2 levels that would benefit from the use of MMP inhibitors.

1. Introduction

Migraine is a common, complex, disabling, neurovascular disorder affecting approximately 10% of the adult population worldwide, especially women (Jensen and Stovner, 2008). Special attention should be given to this clinical disorder because women with migraine, particularly those with aura, are exposed to an increased risk of cardiovascular diseases including ischemic stroke (Bigal et al., 2010; Rist et al., 2010). Although the underlying etiology of this condition remains unknown, cortical spreading depression (CSD), plasma protein extravasation, and neurogenic inflammation are mechanisms involved in the pathophysiology of migraine (Galletti et al., 2009; Pietrobon and Striessnig, 2003). In this respect, mounting evidence suggests that matrix metalloproteinases (MMP) may alter the vascular permeability of cerebral vessels and disrupt blood–brain barrier (BBB), thus leading to a migraine attacks (Ashina et al., 2010; Bernecker et al., 2011; Gursoy-Ozdemir et al., 2004; Martins-Oliveira et al., 2009). Importantly, increased circulating MMP-2 concentrations were shown in patients with migraine (Martins-Oliveira et al., 2009).

MMPs are a large family of zinc-dependent endopeptidases involved in the degradation of many components of the extracellular matrix (Sbardella et al., 2012). Accordingly, MMP activity is regulated at the levels of gene transcription, post-translational modifications, and by interactions with their endogenous inhibitors, the tissue inhibitors of MMPs (TIMPs) (Fontana et al., 2012). Because an imbalance between MMP-2 and its endogenous inhibitor (TIMP-2) contributes to several pathologic conditions affecting both the cardiovascular and the central nervous systems (Ceron et al., 2012; Heo et al., 1999; Marson et al., 2012; Palei et al., 2012; Rosenberg, 2009), MMP inhibition has been suggested as an attractive therapeutic target in prevention of such diseases (Castro et al., 2011; Romi et al., 2012). Importantly, TIMP-2 is the most relevant MMP-2 inhibitor (Raffetto and Khalil, 2008) and therefore the study of circulating MMP-2/TIMP-2 ratio may be useful to assess reflect net MMP-2 activity in clinical studies, and therefore changes in TIMP-2 levels may increase MMP-2 activity favoring disease conditions.

Functional single nucleotide polymorphisms in the promoter region of the *MMP-2* gene (the $C^{-1306}T$/rs243865 and the $C^{-735}T$/

Abbreviations: MMPs, Matrix metalloproteinases; BBB, Blood–brain barrier; TIMPs, Tissue inhibitor of MMPs; CSD, Cortical spreading depression; MWA, Migraine without aura; MA, Migraine with aura; RT-PCR, Real time-polymerase chain reaction; ELISA, Enzyme-linked immunosorbent assay.

* Corresponding author at: Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Av. Bandeirantes, 3900, 14049-900 Ribeirao Preto, SP, Brazil. Tel.: +55 16 3602 3163; fax: +55 16 3602 0220.
E-mail addresses: tanus@fmrp.usp.br, tanussantos@yahoo.com (J.E. Tanus-Santos).

0378-1119
http://dx.doi.org/10.1016/j.gene.2012.09.109

rs2285053) affect MMP-2 expression or activity (Price et al., 2001; Yu et al., 2004) and may predispose to disease conditions (Lacchini et al., 2012; Saracini et al., 2012; Zhou et al., 2005), especially in those subjects carrying *MMP-2* variants associated with increased MMP-2 concentrations (Jacob-Ferreira et al., 2011; Marson et al., 2012). However, no previous study has examined how *MMP-2* gene polymorphisms affect MMP-2 concentrations in patients with migraine.

This study aimed at determining whether functional *MMP-2* polymorphisms are associated with migraine, and whether *MMP-2* polymorphisms affect MMP-2 concentrations in patients. Furthermore, because the analysis of combinations of genetic markers in a region of interest (haplotypes) may provide an improved genetic information (Crawford and Nickerson, 2005), the possibility that *MMP-2* haplotypes affect the circulating MMP-2 and TIMP-2 levels, and MMP-2/TIMP-2 ratio (a better index of net MMP-2 activity) in migraine patients was also examined.

2. Materials and methods

2.1. Study population

This study was approved by the Ethics Committee at Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Brazil, and each subject provided written informed consent.

This study included 204 women with migraine enrolled at the Headache Clinic of the Division of Neurology, University Hospital of the Faculty of Medicine of Ribeirao Preto, and 148 healthy women without history of migraine. Among patients, 153 women were diagnosed with migraine without aura (MWA) and 51 with migraine with aura (MA). All study subjects underwent a complete medical history and physical examination. Diagnosis of migraine was made according to the International Classification of Headache Disorders criteria (2004). The control group included healthy women without headache randomly selected from the local population, unrelated to the patients. Furthermore, women with other diseases including inflammatory diseases, pregnant, or with other kinds of headache were excluded from the study.

After written, informed consent was obtained, venous blood samples were collected into vacutainer plastic tubes containing sodium/potassium EDTA. Blood samples were centrifuged at 1000 ×*g* for 10 min. Plasma samples were separated and immediately stored at −70 °C until used to measure plasma MMP-2 and TIMP-2 concentrations. In addition, aliquots of whole blood were separated and stored at −20 °C for genomic DNA extraction.

2.2. DNA isolation and genotype determination

Genomic DNA was extracted from the cellular component of 1 mL of whole blood through salting-out method and stored at −20 °C until analyzed.

Genotypes for the $C^{-1306}T$ (rs243865) and the $C^{-735}T$ (rs2285053) polymorphisms in the 5'-flanking region of *MMP-2* gene were determined by real time-polymerase chain reaction (RT-PCR), using Taqman Allele Discrimination assays (Applied Biosystems, Carlsbad, CA, USA). Probes and primers used for the $C^{-1306}T$ genotyping assay were customized as follows: forward 5′-GCCATTGTCAATGTTCCCTAAAACA-3′, reverse 5′-TGACTTCTGAGCTGAGACCTGAA-3′ and probes 5′-CAGC ACTC[T/C]ACCTCT-3′. TaqMan PCR was performed in a total volume of 12 μl (3 ng of DNA, 1× TaqMan master mix, 1× assay mix) placed in 96-well PCR plates. Fluorescence from PCR amplification was detected using Chromo 4 Detector (Bio-Rad Laboratories, Hercules, CA, USA) and analyzed with manufacturer's software. Probes and primers used in *MMP-2* $C^{-735}T$ assay were designed by Applied Biosystems (ID: C_26734093-20). TaqMan PCR and fluorescence reading were performed as described above for the $C^{-1306}T$ polymorphism (Lacchini et al., 2012).

2.3. Determination of plasma MMP-2 by SDS-PAGE gelatin zymography

To investigate the effects of *MMP-2* polymorphisms or haplotypes on the circulating levels of MMP-2, gelatin zymography of MMP-2 from plasma samples was performed as previously reported (Gerlach et al., 2007). Briefly, plasma samples were diluted in sample buffer (2% SDS, 125 mM Tris–HCl; pH 6.8, 10% glycerol and 0.001% bromophenol blue) and subjected to electrophoresis on 7% SDS-PAGE co-polymerized with gelatin (1%) as the substrate. After electrophoresis the gel was incubated for 1 h at room temperature in a 2% Triton X-100 solution, and incubated at 37 °C for 16 h in Tris–HCl buffer, pH 7.4, containing 10 mM $CaCl_2$. Gels were stained with 0.05% Coomassie brilliant blue G-250, and then unstained with 30% methanol and 10% acetic acid. Gelatinolytic activities were detected as unstained bands against the background of Coomassie blue-stained gelatin, by densitometry using ImageJ Program (National Institutes of Health, USA). MMP-2 was identified as a band at 72 kDa by direct comparison with relative mobility of Sigma SDS-PAGE LMW marker proteins. The intensity value for the MMP-2 band was calculated as relative activity according to the intensity of related MMP-2 standard (Gerlach et al., 2007).

2.4. Enzyme immunoassay of TIMP-2

Plasma TIMP-2 concentrations were measured in EDTA-plasma, using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) according to manufacturer's instructions. The reaction was evaluated using a μQuant™ microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA). Moreover, we calculated the MMP-2/TIMP-2 ratio because this ratio may be a better index of net MMP-2 activity (Martins-Oliveira et al., 2009).

2.5. Haplotype inference

Haplotypes were inferred using the Bayesian statistical based program PHASE version 2.1 (Stephens et al., 2001) (http://www.stat.washington.edu/stephens/software.html) to estimate the haplotype frequencies in the population and the most likely pairs of haplotypes for each individual. The possible haplotypes including genetic variants of two polymorphisms in the *MMP-2* gene studied, $C^{-1306}T$ and $C^{-735}T$ were: H1 (CC); H2 (CT); H3 (TC); H4 (TT). However, due to low frequency of the H4 haplotype, we excluded it from the analysis.

To assess differences in haplotype frequency distributions was used χ^2 test, and to compare haplotype frequencies in controls and migraine a value of $P<0.0125$ (0.05/4 — number of possible haplotypes) was considered significant to correct for the number of comparisons made.

2.6. Statistical analyses

The clinical data of study participants were compared by the Kruskall–Wallis test followed by the Dunn's multiple comparison test. The results were expressed as mean ± standard deviation. The categorical variables were compared by Fisher's exact test or χ^2 test and expressed as frequencies and percentages (StatView, Cary, NC, USA). A value of $P<0.05$ was considered to be statistically significant.

The distribution of genotypes for each polymorphism was assessed for deviation from the Hardy–Weinberg equilibrium, and differences in the genotypic and alleles frequencies of each polymorphism between the groups were also assessed using χ^2 tests. Due to the relatively low frequency of the TT genotype for both MMP-2 polymorphisms, the CT and TT genotypes (CT + TT group) were combined. To compare the effect of *MMP-2* genotypes on circulating levels of MMP-2, TIMP-2, and MMP-2/TIMP-2 ratios, the unpaired