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# Specific matrix metalloproteinase 9 (MMP-9) haplotype affect the circulating MMP-9 levels in women with migraine

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ABSTRACT

We investigated whether three relevant polymorphisms (C-1562T, microsatellite −90(CA)$_{14-24}$, and Q279R) in the MMP-9 gene, or MMP-9 haplotypes, are associated with migraine and affect MMP-9 and tissue inhibitor of MMPs (TIMP)-1 levels in patients with migraine. We studied 102 healthy women (controls) and 187 women with migraine (141 without aura — MWA, and 46 with aura — MA). Patients with MWA had higher plasma MMP-9 concentrations than patients with MA. Patients with MA had the highest TIMP-1 and lowest MMP-9/TIMP-1 ratios. The MMP-9 "C L Q" haplotype was associated with higher plasma MMP-9 concentrations in migraine patients.



## 1. Introduction

Migraine is a chronic, intermittent, neurological disorder associated with a complex combination of cerebral and vascular events (Moskowitz, 2007; Goadsby et al., 2009; Markus Schürks et al., 2009). In this context, a neurovascular hypothesis has been proposed to explain the pathophysiology of migraine (Schurks et al., 2009), although the precise event responsible for migraine attacks still remains incompletely understood (Pietrobon and Striessnig, 2003). In this respect, the disruption of the blood–brain barrier (BBB) has been implicated during a migraine attack (Leira et al., 2007; Imamura et al., 2008). Despite no clear evidence of BBB proteolysis during migraine attacks (Edvinsson and Tfelt-Hansen, 2008), experimental induction of cortical spreading depression showed that breakdown of BBB may result of matrix metalloproteinases (MMP)-dependent mechanisms (Gursoy-Ozdemir et al., 2004).

MMPs are a large family of zinc-dependent enzymes involved in the degradation of a wide spectrum of extracellular matrix proteins. Their activation and activity is modulated by endogenous inhibitors, the tissue inhibitors of MMP (TIMPs), and imbalanced MMP activities have been suggested as relevant pharmacological targets in many disease conditions (Castro et al., 2011; Fontana et al., 2012; Marson et al., 2012; Romi et al., 2012). Importantly, MMP-9 has been implicated as a major mediator of BBB disruption in neuroinflammatory conditions that may promote migraine (Montaner et al., 2001; Castellanos et al., 2003; Gursoy-Ozdemir et al., 2004; Gurney et al., 2006). Indeed, experimental studies with MMP-9 knockout mice showed reduced BBB leakage and edema formation (Asahi et al., 2001), thus strongly indicating that MMP-9 is very important for this process. Moreover, previous studies showed increased circulating MMP-9 levels in migraine patients (Leira et al., 2007; Imamura et al., 2008), and it is possible that circulating MMP-9 levels may reflect migraine attacks (Leira et al., 2007; Imamura et al., 2008; Martins-Oliveira et al., 2009). However, it is not known whether genetic variations in the MMP-9 gene may affect the susceptibility to migraine and whether there are subgroups of patients that are genetically exposed to increased MMP-9 levels as a result of genetic differences. Such patients would potentially benefit from MMPs inhibitors.

Mounting evidence suggests that MMP-9 gene polymorphisms may affect MMP-9 levels, the progression of disease conditions and therapeutic responses (Demacq et al., 2009; Jacob-Ferreira et al., 2010b; Lacchini et al., 2010; Belo et al., 2012; Palei et al., in press). Relevant functional MMP-9 polymorphisms include the C(−1562)T polymorphism (Zhang et al., 1999), a microsatellite −90(CA)$_n$ (13–25 repeats) in promoter region (Shimajiri et al., 1999), and the Q279R in exon 6 (Allan et al., 1995). Therefore, in the present study, we examined

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whether these polymorphisms and their combinations (haplotypes) are associated with migraine or with altered MMP-9 concentrations in migraine patients. We have also studied the TIMP-1 concentrations and MMP-9/TIMP-1 ratio, which may provide a better index of net MMP-9 activity.

## 2. Materials and methods

### 2.1. Subjects

This study was approved by the Ethics Committee at Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Brazil. After complete description of nature of the study, each participant gave written informed consent.

We enrolled a total of 187 women with migraine and 102 healthy women without history of migraine. Among them, 141 women were diagnosed with migraine without aura (MWA) and 46 with aura (MA). Migraine patients were enrolled at the Headache Clinic of the Neurology Department, University Hospital of the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo. Diagnosis of migraine was made according to the International Classification of Headache Disorders criteria Anon (2004).

All study subjects were of Brazilian origin and underwent a complete medical history and physical examination. Furthermore, we excluded patients with preexistent diseases, pregnant as well as other kinds of headache. The control group included healthy women without headache were randomly selected from the local population visiting our University for non-medical reasons and unrelated to the patients.

### 2.2. Biochemical measurements

After written, informed consent was obtained, venous blood samples were collected into vacutainer plastic tubes (Becton-Dickinson, Brazil) containing sodium/potassium EDTA. We collected blood samples from patients either in the interictal phase or under migraine attack because previous results showed no differences in MMPs, TIMPs, or MMPs/TIMPs ratios when MA or MWA patients with headache attack were compared with asymptomatic MA or MWA patients, respectively (Martins-Oliveira et al., 2009). The blood samples were centrifuged at $1000 \times g$ for 10 min. The plasma samples were separated and immediately stored at −70 °C until used to measure plasma MMP-9 and TIMP-1 concentrations. In addition, aliquots of whole blood were separated and stored at −20 °C for genomic DNA extraction.

### 2.3. DNA isolation and genotype analyses

Genomic DNA was obtained from the cellular component of 1 mL of whole blood by a salting-out method and stored at −20 °C until analysis. To determine the genotypes for the −90(CA)14–24 (rs2234681) polymorphism, a PCR was carried out using the primers: 5′-GAC TTG GCA GTG GAG ACT GCG GGC A-3′ (sense) e 5′-GAC CCC ACC CCT CCT TGA CAG GCA A-3′ (antisense) (Demacq et al., 2008). The PCR conditions were performed as previously described (Demacq et al., 2008) and the amplified products were separated in a 7% polyacrylamide-urea gel and visualized by silver staining. Differences in molecular weight (or number of bases), from 146 bp (CA 14 repeats) to 166 bp (CA 24 repeats) were determined by comparison with migration of a 10 bp DNA ladder (Invitrogen, Carlsbad, CA, USA) and with some samples from homozygotes that were sequenced. The alleles for the microsatellite −90(CA) 14–24 polymorphism were classified as "low" (L) count when the number of CA repeats was less than 21, and as "high" (H) when the number of CA repeats was 21 or more (Demacq et al., 2009).

Genotypes for the (C-1562T (rs3918242)) polymorphism of MMP-9 were determined by polymerase chain reaction (PCR) amplification using the primers 5′-GCC TGG CAC ATA GTA GGC CC-3′ (sense) and 5′-CTT CCT AGC CAG CCG GCA TC-3′ (antisense) and PCR conditions as previously described (Demacq et al., 2006, 2008). The amplified products were digested with *Sph I* (New England Biolabs, Ipswich, MA, USA) overnight at 37 °C, producing fragments of 247 bp and 188 bp in the case of a polymorphic variant (allele T), or an undigested 435 bp band in the case of a wild type allele (allele C) (Jacob-Ferreira et al., 2010b). Fragments were separated by electrophoresis in 12% polyacrylamide gels and visualized by silver staining.

Genotypes for Q279R (rs17576) were determined using TaqMan® Allele Discrimination assay (Applied Biosystems, Foster City, CA). The PCR conditions were performed according to appointed by manufacturer's instructions.

### 2.4. Determination of plasma MMP-9 and TIMP-1 levels

To investigate the effects of MMP-9 polymorphisms or haplotypes on the circulating levels of MMP-9, we measured the plasma MMP-9 concentrations using a commercially available enzyme-linked immunosorbent assay (R&D Systems, Inc., Minneapolis MN) according to manufacturer's instructions. Moreover, we measured the plasma concentrations of TIMP-1 using commercially available enzyme-linked immunosorbent assays (R&D Systems, Inc., Minneapolis MN) and calculated the MMP-9/TIMP-1 ratio because this ratio may be a better index of net MMP-9 activity (Belo et al., 2009).

### 2.5. Haplotype inference

Haplotypes were inferred using the Bayesian statistical based program PHASE version 2.1 (http://www.stat.washington.edu/stephens/software.html) (Stephens et al., 2001) to estimate the haplotype frequencies in the population and the most likely pairs of haplotypes for each individual (Table 4). The possible haplotypes including genetic variants for three MMP-9 polymorphisms studied (H or L variants for −90(CA) 14–24, C or T variants for the C-1562T and Q or R variants for Q279R) were: H1 (CLR), H2 (CHR), H3 (CHQ), H4 (THQ), H5 (TLR), H6 (CLQ), H7 (THR) and H8 (TLQ). Therefore, we evaluated whether MMP-9 haplotypes modulate circulating MMP-9, TIMP-1 and MMP-9/TIMP-1 ratio among studied groups. However, due to low frequency of the H7 and H8 haplotypes, we excluded them of analysis. To assess differences in haplotype frequency distributions was used chi-square test, and to compare haplotype frequencies in controls and migraine a value of $p<0.00625$ (0.05/number of haplotypes) was considered significant to correct for the number of comparisons made.

### 2.6. Statistical analysis

The clinical data of study participants were compared by Kruskall-Wallis test followed, by Dunn's multiple comparison test for continuous variables and were expressed as mean ± standard deviation. Categorical variables were compared by Fisher's exact test or $\chi^2$ test and expressed as frequencies and percentages (StatView, Cary, NC, USA).

The distribution of genotypes for each polymorphism was examined for deviation from the Hardy-Weinberg equilibrium by using chi-squared tests (StatView, Cary, NC, USA). Differences in the genotype and allele frequencies of each polymorphism among the groups were analyzed using chi-squared tests. In function of the relatively low frequency of the TT genotype, we combined both TT and CT genotypes (CT + TT group) to compare the effect of the genotypes on MMP-9 plasma levels. A value of $p<0.05$ was considered the minimum level of statistical significance.

## 3. Results

The data regarding clinical characteristics of the individuals enrolled in the present study population are summarizes in Table 1. There were no significant differences in the clinical parameters among groups ($p>0.05$).

We found higher plasma MMP-9 concentrations in MWA patients compared with MA patients ($p<0.05$; Table 1). In addition, we found higher plasma TIMP-1 concentrations in the MA group of women with MA compared with those found in MWA and the control group ($p<0.05$; Table 1). Interestingly, we found that women with MA had lower MMP-9/TIMP-1 ratios when compared with MWA and with the control group ($p<0.05$; Table 1).

Table 2 shows the genotype and allele distributions in migraine and control groups. The distribution of genotypes for the three polymorphisms evaluated in this study showed no deviation from Hardy-Weinberg equilibrium in both case and control groups. We found no significant difference in the genotype and allelic distribution for the three MMP-9 polymorphisms when patients and controls were compared ($p>0.05$).

We examined the effects of MMP-9 polymorphisms on plasma MMP-9 concentrations in the three study grups (Table 3). While we found no major effects of genotypes on plasma MMP-9 levels in the three study groups ($p>0.05$; Table 3), we found lower MMP-9 levels in patients with the CT or TT genotypes for the C-1562T polymorphism in the MA group than in controls or in MWA patients ($p<0.05$; Table 3).

We estimated haplotype frequencies in the three groups (Table 4). We found no significant differences in the distribution of MMP-9 haplotype frequencies when the three groups were compared ($p>0.05$; Table 4).

Interestingly, the analysis of how MMP-9 haplotypes affect MMP-9 levels in plasma showed the slightly higher MMP-9 levels in healthy subjects carrying the H4 (T H Q) haplotype compared with the other haplotypes ($p<0.05$; Fig. 1A). While no significant effects were found for MMP-9 haplotypes on plasma MMP-9 in the MWA and in the MA groups ($p>0.05$; Fig. 1C and D, respectively), the H6 haplotype was associated with the highest MMP-9 levels when all migraine patients were combined ($p<0.05$; Fig. 1C).

Finally, the analysis of TIMP-1 levels and MMP-9/TIMP-1 ratios showed no effects of MMP-9 haplotypes in the three groups ($p>0.05$; Figs. 2 and 3, respectively).

**Table 1**
Clinical characteristics of study participants.

| Variables | Controls | MWA | MA | p value |
|---|---|---|---|---|
| N | 102 | 141 | 46 | – |
| Age (years) | 36.3 ± 11.2 | 38.4 ± 11.3 | 39.2 ± 10.2 | 0.188 |
| BMI (kg/m$^2$) | 26.4 ± 5.3 | 27.2 ± 5.8 | 26.2 ± 5.7 | 0.617 |
| Smoking (%) | 20.4 | 24.0 | 17.3 | 0.620 |
| Family history (%) | – | 68.2 | 82.6 | 0.084 |
| Frequency (%) | | | | |
| 1 to 3 per month | – | 10.8 | 15.2 | 0.436 |
| 3 to 5 per month | – | 18.6 | 8.7 | 0.159 |
| 5 to 10 per month | – | 11.6 | 19.6 | 0.212 |
| 10 to 15 per month | – | 24.1 | 19.6 | 0.683 |
| > 15 days per month | – | 34.9 | 36.9 | 0.858 |
| Intensity of attacks (%) | | | | |
| Mild | – | – | 4.3 | 0.068 |
| Moderate | – | 32.6 | 26.1 | 0.461 |
| Severe | – | 67.4 | 69.6 | 0.855 |
| Pain free (%) | – | 48.8 | 45.6 | 0.734 |
| Under migraine attack (%) | – | 51.2 | 54.4 | |
| Ethnicity (%) | | | | |
| Whites | 69.3 | 62.5 | 58.7 | 0.444 |
| Non-whites | 30.7 | 37.5 | 41.3 | |
| MMP-9 (ng/ml) | 213.5 ± 168.0 | 219.2 ± 157.0* | 159.3 ± 118.1 | <0.05 |
| TIMP-1 (ng/ml) | 394.2 ± 158.7 | 374.8 ± 145.9 | 515.1 ± 155.7** | <0.05 |
| MMP-9/TIMP-1 ratio | 0.641 ± 0.595 | 0.665 ± 0.501 | 0.374 ± 0.399** | <0.05 |

BMI, body mass index; MWA, migraine without aura; MA, migraine with aura. Values are the mean ± S.D. or % number of subjects.
* $p<0.05$ vs. MA.
** $p<0.05$ vs. controls and MWA.

**Table 2**
MMP-9 genotype and allele distributions.

| Polymorphism | Genotypes | Controls (n = 102) | Migraine | | |
|---|---|---|---|---|---|
| | | | All (n = 187) | MWA (n = 141) | MA (n = 46) |
| −90(CA)$_n$ | LL | 0.196 (20) | 0.246 (46) | 0.248 (35) | 0.239 (11) |
| | HL | 0.510 (52) | 0.487 (91) | 0.482 (68) | 0.500 (23) |
| | HH | 0.294 (30) | 0.267 (50) | 0.270 (38) | 0.261 (12) |
| C-1562T | CC | 0.794 (81) | 0.839 (157) | 0.851 (120) | 0.804 (37) |
| | CT | 0.177 (18) | 0.150 (28) | 0.142 (20) | 0.174 (8) |
| | TT | 0.029 (3) | 0.011 (2) | 0.007 (1) | 0.022 (1) |
| R(279)Q | RR | 0.480 (49) | 0.476 (89) | 0.490 (69) | 0.435 (20) |
| | RQ | 0.402 (41) | 0.406 (76) | 0.411 (58) | 0.391 (18) |
| | QQ | 0.118 (12) | 0.118 (22) | 0.099 (14) | 0.174 (8) |
| | Alleles | | | | |
| −90(CA)$_n$ | L | 0.451 (92) | 0.489 (183) | 0.489 (138) | 0.489 (45) |
| | H | 0.549 (112) | 0.511 (191) | 0.511 (144) | 0.511 (47) |
| C-1562T | C | 0.882 (180) | 0.914 (342) | 0.922 (260) | 0.891 (82) |
| | T | 0.118 (24) | 0.086 (32) | 0.078 (22) | 0.109 (10) |
| R(279)Q | R | 0.681 (139) | 0.679 (254) | 0.695 (196) | 0.630 (58) |
| | Q | 0.319 (65) | 0.321 (120) | 0.305 (86) | 0.370 (34) |

MWA, migraine without aura; MA, migraine with aura.

## 4. Discussion

While most previous studies have focused on the possible predictive value of circulating MMP-9 levels in migraine attacks, this is the first study to investigate the possible association of functional MMP-9 polymorphisms with migraine. Moreover, no previous studies have examined how combinations of genetic markers (haplotypes) in the MMP-9 gene affect the circulating MMP-9 levels, or another relevant index of net MMP-9 activity (MMP-9/TIMP-1 ratio). The main finding of the present study is that a specific MMP-9 haplotype "C L Q" is associated with high MMP-9 concentrations in patients with migraine, although we found no significant associations between MMP-9 genetic polymorphisms and migraine. This finding may have relevant pharmacogenetic implications including the identification of a particular group of migraine patients that may benefit from the use of MMPs inhibitors.

Although we have not investigated the molecular mechanisms explaining how migraine patients carrying the "C L Q" haplotype have higher MMP-9 levels, we could speculate that enhanced MMP-9 levels associated with this haplotype may predispose these patients to increased vascular BBB permeability, thus promoting the development of an inflammatory environment in their central nervous systems, which contributes to migraine attacks (Gursoy-Ozdemir et al., 2004). Interestingly, we found that MMP-9/TIMP-1 tended ($p<0.10$) to be higher in subjects with migraine carrying the "C L Q" haplotype, thus suggesting that this haplotype associated with increased MMP-9 levels is possibly also associated with increased net MMP-9 activity (Belo et al., 2009; Fontana et al., 2011). It is possible that the increased MMP-9 levels found in these subjects may result in impaired BBB integrity because clinical (Castellanos et al., 2003; Rosell et al., 2006) and experimental (Sumii and Lo, 2002) studies suggest that MMP-9 is critically implicated in this alteration.

Previous studies reported enhanced plasma MMP-9 levels in migraine patients, either between or during migraine attacks (Leira et al., 2007; Imamura et al., 2008). Our findings reported here show increased MMP-9 levels in patients with MWA compared to patients with MA, and these findings are very similar to those previously shown in a smaller study (Martins-Oliveira et al., 2009). Interestingly, we found that MA patients, but not MWA patients, had lower MMP-9 and higher TIMP-1 concentrations than healthy controls, thus suggesting that higher TIMP-1 levels could be associated with MMP-9 plasma reduction in MA patients, although we have not determined the mechanisms possibly involved in these differences. Another study showed that MMP-9 levels were not correlated with the frequency or duration of

**Table 3**
Effects of genotype on plasma MMP-9 concentrations in controls and in migraine patients.

| Polymorphism | Genotype | Controls (n = 102) | Migraine | | | *P* |
|---|---|---|---|---|---|---|
| | | | All (n = 187) | MWA (n = 141) | MA (n = 46) | |
| $-90(CA)_n$ | LL | 150.9 (100.2–404.4) | 179.4 (126.2–275.6) | 181.3 (133.6–274.6) | 164.2 (73.8–434.6) | ns |
| | HL | 162.1 (115.7–229.0) | 155.3 (95.1–256.5) | 164.7 (99.9–265.5) | 105.6 (68.8–172.1) | ns |
| | HH | 176.3 (101.9–332.9) | 156.6 (100.2–267.1) | 180.8 (101.7–320.0) | 120.5 (85.7–162.4) | ns |
| C-1562T | CC | 153.8 (105.1–244.7) | 163.4 (100.1–258.0) | 166.6 (107.8–266.2) | 146.6 (73.2–212.5) | ns |
| | CT + TT | 183.2 (130.0–348.5) | 151.2 (103.1–292.3) | 240.6 (121.5–382.8) | 103.3* (86.0–140.8) | 0.016 |
| R(279)Q | RR | 170.6 (113.5–336.8) | 171.8 (102.7–250.1) | 177.6 (116.2–250.1) | 124.4 (61.2–261.7) | ns |
| | RQ | 144.8 (91.2–191.2) | 152.2 (95.6–260.2) | 162.4 (97.7–282.8) | 113.8 (71.8–169.4) | ns |
| | QQ | 182.9 (124.5–408.8) | 161.5 (114.7–368.8) | 302.7 (137.4–467.3) | 140.8 (94.0–162.4) | ns |

MWA, migraine without aura; MA, migraine with aura. Values are expressed as median (interquartile range).
* $p<0.05$ vs. control group and MWA (Kruskall–Wallis test).

**Table 4**
Estimated MMP-9 haplotype frequencies in the control group and in migraine patients.

| | Haplotypes | Controls | Migraine | MWA | MA |
|---|---|---|---|---|---|
| H1 | CLR | 0.429 (87) | 0.453 (170) | 0.442 (125) | 0.467 (43) |
| H2 | CHR | 0.249 (51) | 0.226 (84) | 0.253 (71) | 0.163 (15) |
| H3 | CHQ | 0.186 (38) | 0.203 (76) | 0.184 (52) | 0.240 (22) |
| H4 | THQ | 0.112 (23) | 0.082 (31) | 0.073 (21) | 0.108 (10) |
| H5 | TLR | – | – | – | – |
| H6 | CLQ | 0.021 (4) | 0.033 (12) | 0.043 (12) | 0.021 (2) |
| H7 | THR | 0.003 (1) | – | – | – |
| H8 | TLQ | – | 0.003 (1) | 0.005 (1) | – |

MWA, migraine without aura; MA, migraine with aura.

migraine attacks (Ashina et al., 2010). It is highly probable that some differences between studies may explain differences in MMP-9 levels. For example, we included only women in our present study, and this is not the case in many other studies which included both men and women. Moreover, it is possible that many environmental factors may also affect MMP-9 levels (Jacob-Ferreira et al., 2009, 2010a).

Another potential implication of the present findings is that migraine patients carrying the "C L Q" haplotype may be exposed at increased cardiovascular risk. Indeed, it is widely acknowledged that migraine affects the risk of cardiovascular events (Kurth et al., 2006; Bigal et al., 2009). Whether patients with migraine carrying the "C L Q" haplotype are exposed to increased cardiovascular risk associated with this specific haplotype is unknown. However, this suggestion is supported by growing evidence indicating that increased circulating MMP-9 levels are proportionally associated with increased cardiovascular risk (Garvin et al., 2008). Indeed, a complex long term study would be required to test this hypothesis. However, if this hypothesis is proven true, migraine patients with the "C L Q" haplotype would clearly benefit from the use of MMPs inhibitors.

Some limitations of the present study should be considered. Firstly, there were differences in the number of subjects in each group, and this may have decreased the power to detect significant differences

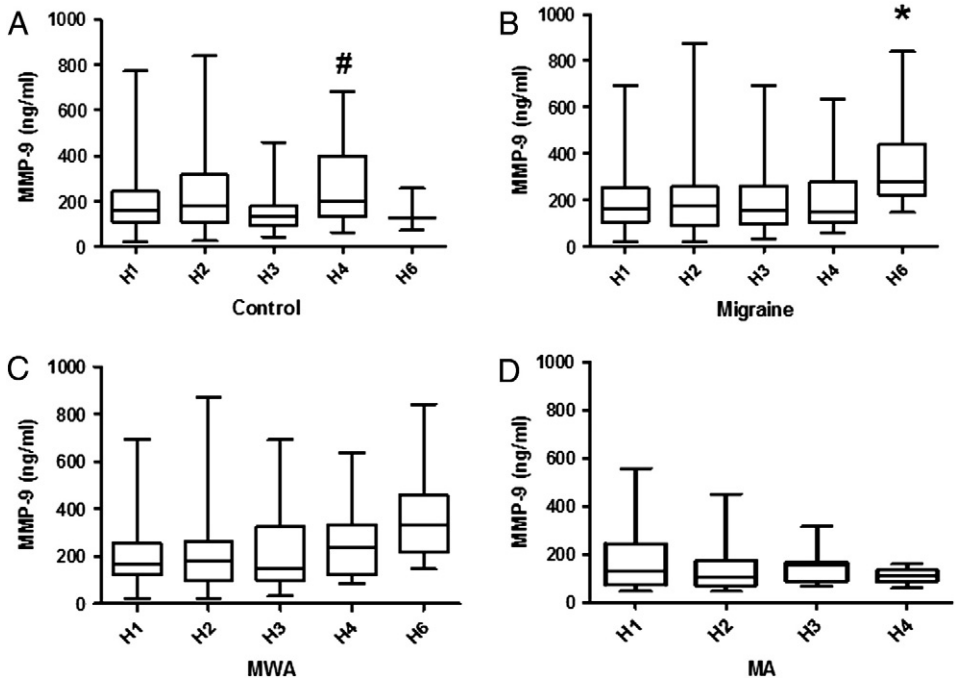


**Fig. 1.** Effects of MMP-9 haplotypes on plasma MMP-9 concentrations in the control (Panel A), migraine (Panel B), migraine without aura (MWA; Panel C) and migraine with aura (MA; Panel D) groups. The box and whisker plots show range and quartiles. The boxes extend from the 25th percentile to the 75th percentile, with a line at the median. The whiskers show the highest and the lowest values. #$p<0.05$ for H4 vs. H3 (panel A). *$p<0.05$ for H6 vs. H1, H2, H3 (panel B).

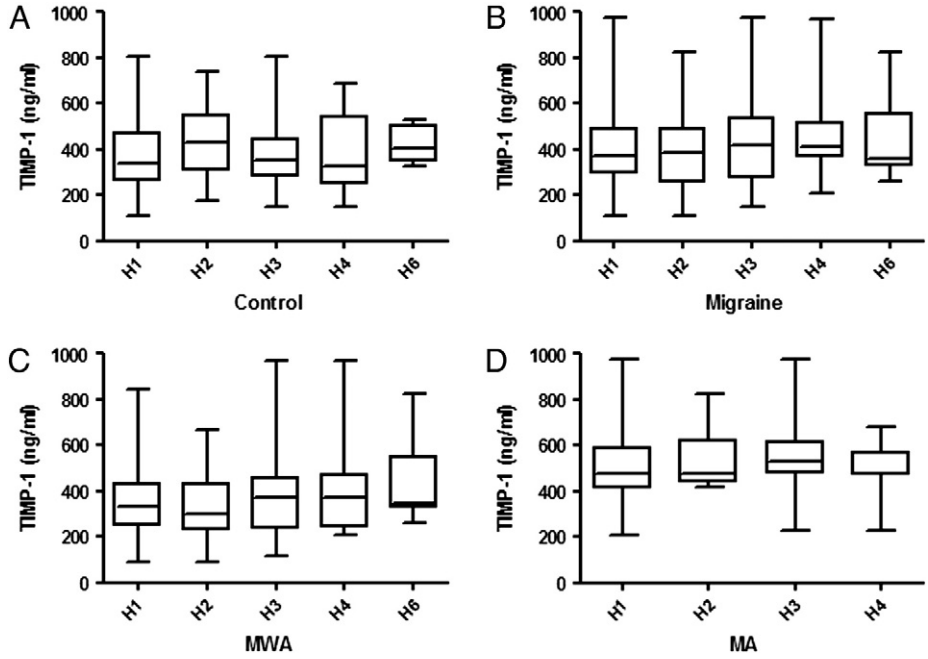


**Fig. 2.** Effects of MMP-9 haplotypes on plasma TIMP-1 CONCENTRATIONS in the control (Panel A), migraine (Panel B), migraine without aura (MWA; Panel C) and Migraine with aura (MA; Panel D) groups. The box and whisker plots show range and quartiles. The boxes extend from the 25th percentile to the 75th percentile, with a line at the median. The whiskers show the highest and the lowest values.

between groups; secondly, we studied only women. We decided to include only women in our study because the prevalence of migraine is higher in women than in men (Jensen and Stovner, 2008). The male:female ratio for migraine among adults varies from 1:2 to 1:3, thus facilitating the recruitment of patients for the study. Because we included only women in the present study, we can not necessarily extrapolate our findings to men, especially because there are many differences between man and women, particularly with respect to hormonal issues; thirdly, we have not evaluated clinical cardiovascular events in the present study or other parameters of the insulin and lipid metabolism that may affect MMPs in migraine patients (Bernecker et al., 2011). However, these limitations require additional studies.

In conclusion, our findings suggest that the "C L Q" haplotype is associated with highest MMP-9 levels in migraine patients, possibly

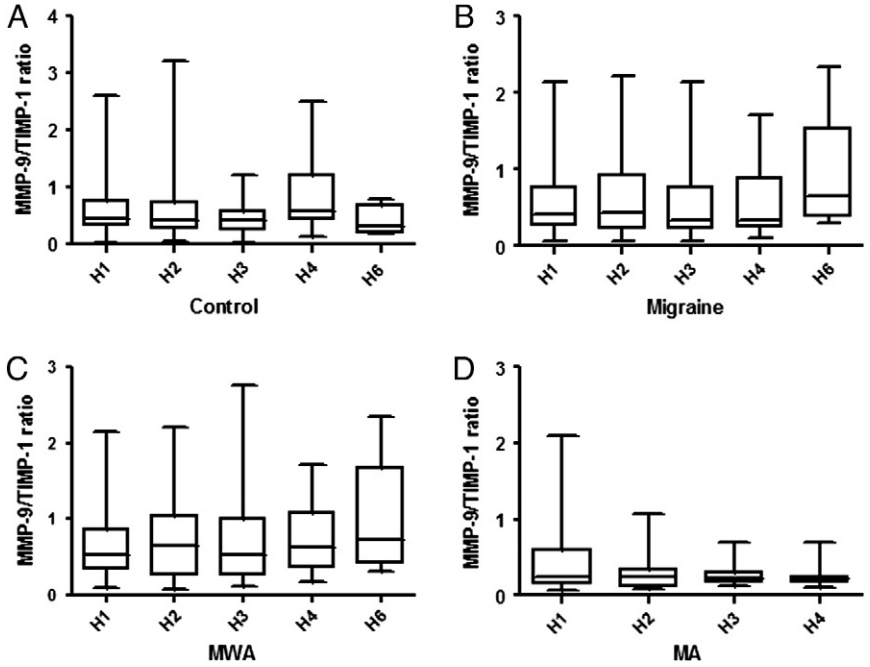


**Fig. 3.** Effects of MMP-9 haplotypes on MMP-9/TIMP-1 ratio in the control (Panel A), migraine (Panel B), migraine without aura (MWA; Panel C) and migraine with aura (MA; Panel D) groups. The box and whisker plots show range and quartiles. The boxes extend from the 25th percentile to the 75th percentile, with a line at the median. The whiskers show the highest and the lowest values.

contributing to proteolytic breakdown of the BBB. Patients with this specific haplotype may benefit from the use of MMPs inhibitors.

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