

Matrix metalloproteinase 1 promoter polymorphisms and risk of myocardial infarction: a case-control study in a Spanish population

Running head: MMP1 and myocardial infarction risk

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This work was supported by the Spanish Fondo de Investigaciones Sanitarias-Fondos FEDER-European Union (FIS 07/0659), and Red de Investigación Renal REDinREN (RD06/0016). PRG was supported by grants from Fundación Mapfre Medicina-Universidad de Oviedo, Fundación Renal Iñigo Álvarez de Toledo and FICYT

Abstract

Inherited and acquired risk factors contribute to the development of the atherosclerotic lesion and its most common clinical manifestation, myocardial infarction (MI). Multiple studies have suggested a role for matrix metalloproteinase (MMPs) in atherosclerosis, and several functional polymorphisms in the *MMP-1* gene have been linked to the risk of MI. The aim of this study was to evaluate the association between *MMP-1* promoter polymorphisms and early MI in a Spanish cohort.

Methods: We performed a case-control study with 261 unrelated patients who had suffered an MI before 55 years and 194 healthy controls, all male and smokers. The genotypes for the three *MMP-1* promoter polymorphisms -1607 1G/2G, -519 A/G, and -340 T/C were determined through PCR-RFLP. Allelic, genotypic, and haplotypic frequencies were statistically compared between groups.

Results: Frequencies of the three polymorphisms did not differ between patients and controls. The -1607 1G/2G and -519 A/G variants were in linkage disequilibrium. Analysis of the haplotype frequencies showed significant associations of the 2G₋₁₆₀₇-G₋₅₁₉-T₋₃₄₀ ($p < 0.006$; OR=2.40; 95% CI=1.27-4.55) and 1G₋₁₆₀₇-G₋₅₁₉-T₋₃₄₀ ($p < 0.05$, OR=0.68; 95% CI=0.50-0.94) haplotypes with the risk of early MI.

Conclusion: *MMP-1* promoter polymorphisms are associated with the risk of early MI in a Spanish population of smoking males.

Key words: myocardial infarction, matrix metalloproteinase 1, polymorphisms.

Condensed Abstract.

Myocardial infarction (MI) is caused by several inherited and acquired risk factors that predispose to the development of atherosclerotic lesions and plaque rupture. Matrix metalloproteinases (MMPs), especially MMP-1, plays an important role in plaque rupture and the posterior remodeling of the vessel wall.

The aim of this study was to evaluate the association between *MMP-1* functional promoter polymorphisms and early MI in a Spanish cohort.

Our data suggest that these *MMP-1* promoter polymorphisms, grouped in haplotypes, have significant associations with early MI in smoking males, in a Northern Spanish population where MI is strongly linked to this habit. The results suggested that the associations are mainly driven by the -1607 1G/2G polymorphism and the -519 A/G and -340 T/C should be modifiers of the relationship.

Introduction.

Myocardial infarction (MI) is caused by several inherited and acquired risk factors that predispose to the development of atherosclerotic lesions and plaque rupture [1]. Degradation of the supporting elements of a coronary atherosclerotic plaque weakens its fibrous cap and thereby increases susceptibility to rupture. Matrix metalloproteinases (MMPs) disrupt extracellular matrix, are present in atherosclerotic plaques, and appear to be more active in unstable lesions. Therefore, MMPs may play an important role in plaque rupture and the posterior remodeling of the vessel wall [2]. In particular, MMP-1 (collagenase-1) has been found in the shoulder of the atherosclerotic plaque, suggesting that an excess of collagenolytic activity could lead to an early plaque rupture [3]. Moreover, ApoE knockout mice expressing human MMP-1 had less advanced atherosclerotic lesions compared to their littermates that do not express MMP-1 [4]. In this way, MMP-1 could play a dual role in coronary artery disease (CAD): on one hand it favors the remodeling of the atherosclerotic lesions, but on the other, enhanced MMP-1 expression promotes plaque rupture [5].

The MMP-1 is a candidate to modify the risk of atherosclerosis and MI in humans, and several polymorphisms have been analyzed through case-control studies. Most of them were focused on the 1G/2G insertion-deletion polymorphism at nucleotide position -1607. It has been reported that the insertion allele (2G) has increased gene transcription, compared to the deletion allele [6]. Homozygosity for the 2G allele has been associated with a lower risk for coronary heart disease in a Caucasian population [7] and haplotypes of 1G/2G MMP-1 (1G allele) and 5A/6A MMP-3 have been associated with increased risk of CAD but not with MI [8]. Other studies neither could find any association of this polymorphism with MI [9].

Other genetic variants in the promoter have also been studied. Associations between haplotypes of the -519 A/G and -340 T/C polymorphisms and MI have been reported among British, Swedish, and Chinese populations [10, 11], and the -519 A/G polymorphism has been associated with hypertension [12], a conventional risk

factor for CAD. Moreover, concrete haplotypes of these polymorphisms have been linked to increased promoter activities and higher levels of MMP-1 [10], suggesting a relationship between these variants and the increased presence of this protein in atherosclerotic plaques.

Here, we analyzed the effect of these three functional promoter polymorphisms on MI risk in a group of Spanish male patients, all smokers, and who have suffered an early event of MI.

Methods

Patients and controls.

We studied 261 unrelated male patients who have survived to a first episode of acute MI before their 55 years. All these patients were diagnosed between 1998 and 2006 at our Cardiology Department, and MI was defined according to the World Health Organization (WHO) criteria [13]. These patients underwent coronary angiography for diagnostic purposes, and had at least one atherosclerotic-diseased coronary vessel (a narrowing of >30% of the vessel diameter). All the patients were recruited for a research project on the genetic factors involved in MI [14, 15].

The control group consisted of 194 healthy male individuals, between 30 and 60 years, recruited through the Blood Bank at our Institution. These controls did not have suffered episodes of cardiovascular disease but they were not angiographically evaluated to exclude the presence of diseased coronary vessels, because coronary angiography is an invasive and potentially dangerous technique, only used for diagnostic/therapeutic purposes among patients with symptoms of CAD.

Because early MI is strongly associated with smoking in our population, all the patients and controls were current or former smokers. Those with history of hypertension or a blood pressure >140/90 mm Hg in at least four determinations were considered as hypertensives. Those with a total cholesterol value >200 mg/dL were considered as hypercholesterolemics. Patients with a history of diabetes or a basal glycemia >120 mg/dL were excluded. Table 1 shows the main clinical, biochemical, and anthropometric characteristics of patients and controls.

All the participants were Caucasian males from the same region (Asturias, Northern Spain, total population 1 million), and gave their written informed consent to participate in the study, that was approved by the Ethical Committee of Clinical Investigation of Asturias.

MMP-1 polymorphisms genotyping.

DNA was obtained from 10 mL of peripheral blood following a salting-out method [16]. The three polymorphisms [-1607 1G/2G (rs11292517), -519 A/G (rs1144393), and -340 T/C (rs514921)] were genotyped through PCR-RFLP with the following conditions: 1 denaturing cycle at 95 °C for 3 min and 30 cycles at 95 °C for 30 s, annealing temp (58, 62, and 60 °C, respectively) for 30 s, and 72 °C for 30 s. For -519 A/G, the fragment of 399 bp obtained with primers forward 5' TTA CAG AGA TGG GGT CTC AC 3' and reverse 5' CCA TGG AGT ACT CTT TGA CC 3', was digested with *Kpn* I. For -1607 1G/2G and -340 T/C, primers with a mismatch were designed: forward 5' AAG TGT TCT TTG GTC TCT GC 3', reverse 5' GGA TTG ATT TGA GAT AAG TCA GAT C 3' and forward 5' TGT GTG GAG AAA CCT GTA GGA C 3', reverse 5' TCA CTT GGT GTT GCA ATG TC 3', pairs for both SNPs respectively (underlined are mismatched bases). The fragments of 176 and 209 bp were digested with *Bgl* II and *Sau* 96I, respectively, followed by electrophoresis on 3% agarose gels and ethidium bromide staining to visualize the fragments.

Statistical analysis.

The SPSS Statistical Package (v.12.0) was used for the standard statistical comparisons. Continuous baseline characteristics were compared between the two groups using a Student's t-test, and categorical data with a χ^2 test, which was also used to compare allele and genotype frequencies for each polymorphism and the deviation from Hardy-Weinberg equilibrium. Linkage disequilibrium coefficients (D') between polymorphisms were calculated with the SHEsis online program [17], that was also used to estimate haplotype frequencies in both groups.

Results and Discussion

Several studies suggested that MMP-1 prevents or delays the progression of atherosclerotic plaques. Null mice for TIMP-1 + ApoE and overexpressing human MMP-1 have less atherosclerotic lesions than expected, suggesting that MMP-1 could play a protective role in the progression of lesions [4]. But the role of MMP-1 in the subsequent events of CAD, like MI, or in carotid artery intima-media thickness, remains unclear [12].

Our case-control study included 455 individuals, among which 261 were unrelated patients and 194 healthy controls, sex and smoking status matched, with no differences in levels of total cholesterol, LDL-C, and triglycerides. However, we found statistically significant differences in some conventional risk factors like age, number of hypertensives and hypercholesteroleemics, and HDL-C levels (Table 1). All the participants were genotyped for the -1607 1G/2G, -519 A/G, and -340 T/C *MMP-1* polymorphisms, that were selected because they have been described to affect the transcription of the gene. The three polymorphisms were in Hardy-Weinberg equilibrium in patients and controls and we found a weak linkage disequilibrium between -1607 1G/2G and -519 A/G ($D'=0.57$) but not with the -340 C/T polymorphism.

We did not find significant differences in allelic or genotypic frequencies between both groups, for the three polymorphisms taken separately (Table 2). These results are in agreement with previous studies on 1G/2G polymorphism and MI risk, that reported no association of any genotype of this polymorphism and MI [10], although some groups have found a reduced risk of coronary heart disease for carriers of the 2G₋₁₆₀₇ allele [7] or a increased risk of CAD for the 1G₋₁₆₀₇ allele [8]. Another study have found an association between the single presence of the A allele

of the -519 A/G polymorphism and the risk of acute coronary syndrome (ACS) [11], but we did not find the same result.

There are also described haplotypic effects of the -519 A/G and -340 T/C polymorphisms on MI risk, with the A₋₅₁₉-C₋₃₄₀ and G₋₅₁₉-T₋₃₄₀ haplotypes being protective for MI in Caucasian population [10], but A₋₅₁₉-C₋₃₄₀ being of risk for ACS in Chinese population [11]. In our study, we could not find any effect for the G₋₅₁₉-T₋₃₄₀ haplotype (Table 3) but we found a borderline protective effect of the A₋₅₁₉-C₋₃₄₀ haplotype (OR=0.73; 95% CI=0.53-0.99; p=0.045), supporting the previous data on the Caucasian population and, although we found a trend to increase the risk of MI for the G₋₅₁₉-C₋₃₄₀ haplotype as is described in British and Swedish patients [10] (OR=1.45; 95% CI=0.81-2.58), it does not reach statistical significance (Table 3). In addition, Han et al reported that A₋₅₁₉-T₋₃₄₀ haplotype significantly increased the risk of ACS, but we do not found any statistically significant protective effect for this combination (Table 3).

The -1607 1G/2G is the most studied polymorphism in the *MMP-1* gene, in part because the 2G₋₁₆₀₇ allele has over 20 fold higher transcriptional activity than 1G₋₁₆₀₇ [6]. In our population, the G₋₅₁₉-T₋₃₄₀ haplotype, that has been described as protective for MI [10], is also protective, but only if it is forming a haplotype with the 1G₋₁₆₀₇ allele (1G₋₁₆₀₇-G₋₅₁₉-T₋₃₄₀,: OR=0.68; 95% CI=0.50-0.94; p=0.02) (Table 4). If the haplotype is with the 2G₋₁₆₀₇ variant (the more active), it confers a significant risk of suffer MI (OR=2.40; 95% CI=1.27-4.55; p<0.005). This effect is not only due to the -1607 1G/2G polymorphism, since nor the allelic neither the genotypic frequencies are statistically different in patients and controls (Table 2). Therefore, it is necessary a specific haplotypic background with the -519 A/G and -340 T/C polymorphisms to have an effect on MI risk.

Different studies showed that alleles linked to higher MMP-1 transcriptional activities would be associated with increased risk of MI and alleles with less activity would confer a protective effect [7, 10]. In our study, the protective effect of the

haplotype is given when the three alleles with lower transcriptional activity are together, but the presence of a more active allele (2G₋₁₆₀₇) and the less active haplotype G₋₅₁₉-T₋₃₄₀ confers an increased risk of MI, suggesting that the -1607 1G/2G polymorphism is the main driver in the associations and the -519 A/G and -340 T/C polymorphisms should be modifiers of the relationship. We can not determine the effect of the haplotype with the more active alleles (2G₋₁₆₀₇-G₋₅₁₉-C₋₃₄₀) because of its little frequency in our population (2 %; Table 4) that not allow the program to analyze it.

Finally, our work has some limitations. First, it was based on a limited number of cases and controls, although we analyzed a homogeneous population: Caucasian, male, smoker, non diabetic, and lower than 60 years, younger than populations of other studies. Compared to other papers, hypercholesterolemics and hypertensives were underrepresented in our patients, and even these variables did not match in our groups, so we can not exclude that this mask the effect of the *MMP-1* polymorphisms on MI risk. In addition, the control group did not include individuals angiographically evaluated to confirm the absence of diseased vessels, although this could act underestimating our findings.

In conclusion, our data showed that concrete haplotypes of these three *MMP-1* promoter polymorphisms have significant associations with early MI in smoking males, in a Northern Spanish population where MI is strongly linked to this habit. The results suggest that this association is mainly driven by the -1607 1G/2G polymorphism, although with a background of a specific haplotype of -519 A/G and -340 T/C polymorphisms.

Acknowledgements.

We want to acknowledge the Spanish Fondo de Investigaciones Sanitarias-Fondos FEDER-European Union and Red de Investigación Renal REDinREN (RD06/0016).

We also want to thank Fundación Mapfre Medicina-Universidad de Oviedo, Fundación Renal Iñigo Álvarez de Toledo, and Fundación para el Fomento en Asturias de la Investigación Científica Aplicada y la Tecnología (FICYT) for supporting PRG.

References

- 1.- Lusis, AJ. Atherosclerosis. *Nature* 2000. **407**:233.
- 2.- Katsuda, S, and T Kaji. Atherosclerosis and extracellular matrix. *J Atheroscler Thromb* 2003. **10**:267.
- 3.- Sukhova, GK, U Schonbeck, E Rabkin, FJ Schoen, AR Poole, RC Billingham, et al. Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atheromatous plaques. *Circulation* 1999. **99**:2503.
- 4.- Lemaitre, V, TK O'Byrne, AC Borczuk, Y Okada, AR Tall, and J D'Armiento. ApoE knockout mice expressing human matrix metalloproteinase-1 in macrophages have less advanced atherosclerosis. *J Clin Invest* 2001. **107**:1227.
- 5.- Newby, AC. Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. *Physiol Rev* 2005. **85**:1.
- 6.- Rutter, JL, TI Mitchell, G Buttice, J Meyers, JF Gusella, LJ Ozelius, et al. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res* 1998. **58**:5321.
- 7.- Ye, S, CR Gale, and CN Martyn. Variation in the matrix metalloproteinase-1 gene and risk of coronary heart disease. *Eur Heart J* 2003. **24**:1668.
- 8.- Horne, BD, NJ Camp, JF Carlquist, JB Muhlestein, MJ Kolek, ZP Nicholas, et al. Multiple-polymorphism associations of 7 matrix metalloproteinase and tissue inhibitor metalloproteinase genes with myocardial infarction and angiographic coronary artery disease. *Am Heart J* 2007. **154**:751.
- 9.- Nojiri, T, H Morita, Y Imai, K Maemura, M Ohno, K Ogasawara, et al. Genetic variations of matrix metalloproteinase-1 and -3 promoter regions and their associations with susceptibility to myocardial infarction in Japanese. *Int J Cardiol* 2003. **92**:181.
- 10.- Pearce, E, DA Tregouet, A Samnegard, AR Morgan, C Cox, A Hamsten, et al. Haplotype effect of the matrix metalloproteinase-1 gene on risk of myocardial infarction. *Circ Res* 2005. **97**:1070.
- 11.- Han, Y, Z Wu, X Zhang, C Yan, S Xi, Y Yang, et al. Impact of matrix metalloproteinase-1 gene variations on risk of acute coronary syndrome. *Coron Artery Dis* 2008. **19**:227.
- 12.- Armstrong, C, S Abilleira, M Sitzer, HS Markus, and S Bevan. Polymorphisms in MMP family and TIMP genes and carotid artery intima-media thickness. *Stroke* 2007. **38**:2895.
- 13.- Tunstall-Pedoe, H, K Kuulasmaa, P Amouyel, D Arveiler, AM Rajakangas, and A Pajak. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation* 1994. **90**:583.
- 14.- Rodriguez, I, E Coto, JR Reguero, P Gonzalez, V Andres, I Lozano, et al. Role of the CDKN1A/p21, CDKN1C/p57, and CDKN2A/p16 genes in the risk of atherosclerosis and myocardial infarction. *Cell Cycle* 2007. **6**:620.
- 15.- Gonzalez, P, A Diez-Juan, E Coto, V Alvarez, JR Reguero, A Batalla, et al. A single-nucleotide polymorphism in the human p27kip1 gene (-838C>A) affects basal promoter activity and the risk of myocardial infarction. *BMC Biol* 2004. **2**:5.
- 16.- Miller, SA, DD Dykes, and HF Polesky. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988. **16**:1215.
- 17.- Shi, YY, and L He. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res* 2005. **15**:97.
- 18.- Friedewald, WT, RI Levy, and DS Fredrickson. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972. **18**:499.

Table 1. Characteristics of patients and controls.

| | MI N=261 | Controls N=194 | p-value |
|--------------------------|-----------------|-------------------|---------|
| Age (y) | 46.05 ± 5.97 | 43.49 ± 7.48 | <0.0001 |
| Hypertensives, % | 39 | 18 | <0.0001 |
| Hypercholesterolemics, % | 24.7 | 6.1 | <0.0001 |
| Cholesterol (mg/dL) | 216.11 ± 51.15 | 213.31 ± 37.26 | 0.12 |
| LDL-C (mg/dL)* | 145.29 ± 45.86 | 134.43 ± 36.78 | 0.35 |
| HDL-C (mg/dL) | 34.60 ± 9.96 | 51.03 ± 14.79 | <0.0001 |
| Triglycerides (mg/dL) | 181.78 ± 105.66 | 139.42 ± 95.58 | 0.78 |

Data are mean ± standard deviation. HDL-C: high density lipoproteins- cholesterol; LDL-C: low density lipoproteins- cholesterol. * Calculated with the Friedewald formula [18].

Table 2. Allelic and genotypic frequencies for the -1607 1G/2G, -519 A/G, and -340 T/C MMP-1 individual polymorphisms in cases and controls.

| Position | Allele | MI N=522 | Controls N=388 | Genotype | MI N=261 | Controls N=194 |
|----------|-----------|-------------|-------------------|-------------|-------------|-------------------|
| -1607 | 2G | 0.56 | 0.54 | 2G2G | 0.33 | 0.28 |
| | 1G | 0.44 | 0.46 | 2G1G | 0.46 | 0.51 |
| | | | | 1G1G | 0.21 | 0.21 |
| -519 | A | 0.67 | 0.68 | AA | 0.47 | 0.45 |
| | G | 0.33 | 0.32 | AG | 0.41 | 0.45 |
| | | | | GG | 0.12 | 0.10 |
| -340 | T | 0.73 | 0.70 | TT | 0.54 | 0.48 |
| | C | 0.27 | 0.30 | TC | 0.39 | 0.43 |
| | | | | CC | 0.07 | 0.09 |

Table 3: Comparison of haplotype frequencies of the -519 A/G and -340 T/C polymorphisms in cases and controls.

| | MI (freq) | Controls (freq) | χ^2 | Pearson's p | Odds Ratio [95%CI] |
|-----|---------------|-----------------|----------|-------------|--------------------|
| A C | 104.46 (0.20) | 99.36 (0.26) | 4.010 | 0.045 | 0.73 [0.53~0.99] |
| A T | 246.54 (0.47) | 162.64 (0.42) | 2.538 | 0.11 | 1.24 [0.95~1.62] |
| G C | 35.54 (0.07) | 18.64 (0.05) | 1.596 | 0.21 | 1.45 [0.81~2.58] |
| G T | 135.46 (0.26) | 107.36 (0.28) | 0.336 | 0.56 | 0.92 [0.68~1.23] |

Table 4: Comparison of haplotype frequencies of the -1607 1G/2G, -519 A/G, and -340 T/C polymorphisms in cases and controls.

| | MI (freq) | Control (freq) | χ^2 | Pearson's p | Odds Ratio[95%CI] |
|--------|---------------|----------------|----------|-------------|-------------------|
| 1G A C | 36.19 (0.07) | 34.24 (0.09) | 1.169 | 0.28 | 0.76 [0.47~1.24] |
| 1G A T | 73.54 (0.14) | 40.97 (0.11) | 2.433 | 0.12 | 1.38 [0.92~2.08] |
| 1G G C | 27.61 (0.05) | 10.59 (0.03) | 3.568 | 0.058 | 1.98 [0.96~4.07] |
| 1G G T | 94.67 (0.18) | 94.20 (0.24) | 5.327 | 0.02 | 0.68 [0.50~0.94] |
| 2G A C | 68.05 (0.13) | 65.05 (0.17) | 2.595 | 0.11 | 0.74 [0.51~1.07] |
| 2G A T | 173.22 (0.33) | 121.74 (0.31) | 0.273 | 0.6 | 1.08 [0.81~1.43] |
| 2G G C | 8.15 (0.02) | 8.12 (0.02) | NA | NA | NA |
| 2G G T | 40.57 (0.08) | 13.09 (0.03) | 7.667 | 0.006 | 2.40 [1.27~4.55] |

NA= no analyzed