The role of genetic variants of matrix metalloproteinases in coronary and carotid atherosclerosis

Sonia Abilleira, Steve Bevan, Hugh S Markus

Current evidence suggests that matrix metalloproteinases (MMPs) have a role in early atherosclerosis, plaque rupture and myocardial infarction. Polymorphisms in MMP genes have been examined for associations with atherosclerosis, but interpretation is complicated by methodological issues. This article presents a systematic review of these association studies and a meta-analysis of available data for polymorphisms where a sufficient number of studies was available. The 5A allele of the MMP3 5A/6A polymorphism was associated with acute myocardial infarction (odds ratio (OR) 1.26, 95% confidence interval (CI) 1.1 to 1.4, \(p<0.001\)), suggesting its role in plaque rupture. There was no association with the functional MMP9 \(-1562C/T\) polymorphism (OR 1.11, 95% CI 1.0 to 1.3, \(p=0.18\)). Current data provide evidence for the role of MMP3 polymorphism in plaque destabilisation, but elucidation of the role of other MMP gene variants in atherosclerosis will depend on better study design, including a larger sample size, extensive screening of individual genes with haplotype analysis and replication of studies to avoid publication bias.

Atherosclerosis is the major cause of coronary artery disease (CAD) and stroke. Although much of the risk for this condition is explained by conventional risk factors, a great deal remains unexplained. Gene–environment interactions may be particularly relevant. Most studies have looked at associations between polymorphic variants in candidate genes and atherosclerosis, quantified by imaging or cardiovascular end points. A potential candidate gene system is the matrix metalloproteinase (MMP) family. The MMPs are proteolytic enzymes that degrade the extracellular matrix, leading to connective tissue remodelling during normal biological processes. Vascular remodelling is currently recognised as a determinant of major vascular pathologies including atherosclerosis and restenosis, and it is now widely accepted that deregulation of the MMP system has a pivotal role in vascular remodelling and atherosclerosis (table 1).

Studying genetic variants in the MMPs, which are associated with lifelong changes in MMP activity, offers the possibility of determining whether such relationships are really causal. Using genetic variants in this way is referred to as “mendelian randomisation”, and has recently been used to determine causality in cardiovascular and other complex diseases. In this article, we present a critical examination of the association between MMP polymorphisms and atherosclerosis, focusing on coronary and carotid atherosclerosis.

**Search strategy**

We performed a computer-based search using the PubMed database with the following search terms: “matrix metalloproteinases” and “atherosclerosis”, “coronary artery disease”, “carotid stenosis”, “intima-media thickness”, “polymorphism” and “genetics”. References from the retrieved articles were also searched for additional papers. Publications in English until November 2005 were included. Papers on aneurysms were not reviewed.

We reviewed all association studies on polymorphic variants in the MMP genes in patient groups with subclinical atherosclerosis or clinical coronary or carotid atherosclerosis. For each study, several markers of quality were determined, including details of clinical phenotyping, degree of controlling for ethnicity and other cardiovascular risk factors. Where data from \(>3\) similar studies were available, meta-analysis was performed using the Mantel–Haenszel method. This was possible for case–control studies of the MMP3 5A/6A and MMP9 \(-1562C/T\) polymorphisms. As MMPs may have different roles in early atherosclerosis and in late disease, we analysed studies with the end point of atherosclerotic stenosis on imaging separately from those with the end point of acute symptomatic events (myocardial infarction).

**POLYMORPHISMS OF MMPS TESTED FOR ASSOCIATIONS WITH CORONARY AND CAROTID ATHEROSCLEROSIS**

Genetic association studies with cardiovascular disease have used a range of phenotypes. Clinical end points such as myocardial infarction, angina or stroke, and intermediate phenotypes of subclinical atherosclerosis can both be used. The intermediate phenotypes have been measured using high-resolution duplex ultrasound of the carotid arteries or angiographic imaging of the coronary arteries. The use of intermediate phenotypes is statistically more powerful because continuous variables are used, and because the phenotypes overcome the problem of subclinical disease, in which a control, in a case–control study, may have presymptomatic atherosclerosis. It is important to remember that intermediate phenotypes deal with only part of the pathogenic process, and therefore associations may

**Abbreviations:** CAD, coronary artery disease; IMT, intima–media thickness; MMP, matrix metalloproteinase
In summary, some studies have shown associations between the low-activity 6A allele and both coronary and carotid atherosclerosis and increased IMT. This suggests that people with the 6A6A genotype, who produce less MMP3, may be at increased risk for atherosclerotic plaque development.

Carotid atherosclerosis

Four studies have examined the role of the 5A/6A polymorphism in carotid atherosclerosis, and all have shown association with the 6A allele. Three of them studied associations with IMT and one with degree of carotid stenosis. In healthy Caucasian men without major risk factors for atherosclerosis, the 6A allele was associated with increased carotid IMT, enlarged arterial lumen and a local reduction in wall shear stress.32 This association was strengthened in a further study on healthy, middle-aged men, and additive effects were found with the interleukin 6 (–174G/C) promoter polymorphism.34 Similar associations were found in 87 Hispanic people from the Northern Manhattan Prospective Cohort Study, who had had no stroke,35 and with degree of carotid stenosis in an Italian study.36 The combination of the MMP3 5A/6A genotype and the MMP9 −1652C/T polymorphism was an independent risk factor for internal carotid artery stenosis.32

In summary, some studies have shown associations between the low-activity 6A allele and both coronary and carotid atherosclerosis and increased IMT. This suggests that people with the 6A6A genotype, who produce less MMP3, may be at increased risk for atherosclerotic plaque development.

Table 1: Roles of matrix metalloproteinases in atherosclerosis

<table>
<thead>
<tr>
<th>Subclinical atherosclerosis</th>
<th>Clinical atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early migration and proliferation of SMCs</td>
<td>Carotid plaque instability</td>
</tr>
<tr>
<td>Infiltration of leucocytes</td>
<td>Coronary plaque rupture</td>
</tr>
<tr>
<td>Intimal thickening and growth of atherosclerotic lesions</td>
<td>Development of aneurysms</td>
</tr>
<tr>
<td>Delay of flow-limiting stenosis by expansive remodelling of plaques</td>
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</tr>
</tbody>
</table>

SMC, smooth-muscle cell.
However, heterogeneity was highly significant across studies (p<0.001). Heterogeneity is possibly suggested by the study of Yamada et al,\(^48\) where a large group of Japanese patients with myocardial infarction was investigated. In this study, an association was seen between the 6A allele and myocardial infarction only in women. Patients in Yamada et al’s study were older than those in the other studies. A possible explanation for the heterogeneity might be that in older women, myocardial infarction develops from severe flow-limiting stenoses rather than from complicated lesions.

**Other MMP3 polymorphisms**

Beyzade et al\(^{39}\) genotyped 1240 Caucasians for six MMP3 polymorphisms (–1986T/C, –1612 5A/6A, –1346A/C, –709A/C, –376G/C and +802A/G) and found none of them, except 5A/6A, significantly associated with CAD (p<0.05). A recent case–control study on three MMP3 polymorphisms (5A/6A, –376G/C and the coding Gly45Lys) through haplotype analysis showed an association between the 5A–G–Lys haplotype and an increased risk of myocardial infarction, although the effect of the haplotype was mainly due to the 5A/6A polymorphism.\(^{45}\)

**MMP9 polymorphisms**

Zhang et al\(^{59}\) described a functional –1562C/T polymorphism in the promoter region of MMP9. Transfection experiments and DNA–protein interaction assays indicated that the T allele had higher activity; an association with severity of coronary atherosclerosis measured by the number of coronary arteries showing >50% stenosis was reported, although no association was seen with myocardial infarction. Others have confirmed this association with CAD.\(^{60,66}\) The T allele has also been associated with complicated coronary lesions,\(^{62}\) and carriers of the T allele had higher levels of MMP9 mRNA and protein, and stiffer large arteries.\(^{63}\) In a prospective study,\(^{64}\) the T allele was associated with raised plasma MMP9 levels, which themselves predicted cardiovascular mortality in patients with CAD, although the T allele itself was not associated with mortality. Three published studies on 788 Caucasians, 248 Koreans and 2731 German men with angiographically documented CAD failed to confirm an association with the T allele.\(^{41,65,66}\) We carried out a meta-analysis of five studies\(^{41,60,61,65,66}\) and found no association between the T allele and angiographically documented coronary atherosclerosis (OR 1.11, 95% CI 1.0 to 1.3, \(p = 0.18\), fig 2).

### Table 1

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients with MI</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terashima et al(^{46}), 1999</td>
<td>161/330</td>
<td>108/330</td>
<td>1.96</td>
<td>1.4 to 2.7</td>
</tr>
<tr>
<td>Liu et al(^{46}), 2003</td>
<td>67/150</td>
<td>41/150</td>
<td>2.15</td>
<td>1.3 to 3.5</td>
</tr>
<tr>
<td>Beyzade et al(^{39}), 2003</td>
<td>112/133</td>
<td>586/810</td>
<td>2.04</td>
<td>1.2 to 3.3</td>
</tr>
<tr>
<td>Yamada et al(^{48}), 2002</td>
<td>173/590</td>
<td>260/704</td>
<td>0.71</td>
<td>0.6 to 0.9</td>
</tr>
<tr>
<td>Naijiri et al(^{47}), 2003 (sample 1)</td>
<td>54/164</td>
<td>78/335</td>
<td>1.62</td>
<td>1.1 to 2.4</td>
</tr>
<tr>
<td>Naijiri et al(^{47}), 2003 (sample 2)</td>
<td>94/302</td>
<td>78/335</td>
<td>1.49</td>
<td>1.0 to 2.1</td>
</tr>
<tr>
<td>Zhou et al(^{45}), 2004</td>
<td>179/509</td>
<td>133/518</td>
<td>1.57</td>
<td>1.2 to 2.1</td>
</tr>
<tr>
<td>Samnegard et al(^{49}), 2005</td>
<td>263/374</td>
<td>284/385</td>
<td>0.84</td>
<td>0.6 to 1.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1103/2552</strong></td>
<td><strong>1568/3567</strong></td>
<td><strong>1.26</strong></td>
<td><strong>1.1 to 1.4</strong></td>
</tr>
</tbody>
</table>

**Figure 1** Odds, for patients with myocardial infarction (MI) versus controls, of carrying the 5A allele (matrix metalloproteinase 3 5A/6A polymorphism). x axis: odds ratio (OR) and 95% CI; y axis: studies included in the meta-analysis.

**Figure 2** Odds of carrying the T allele (matrix metalloproteinase MMP9 –1562C/T polymorphism) for angiographically documented patients with coronary artery disease (CAD). x axis: odds ratio (OR) and 95% CI; y axis: studies included in the meta-analysis.
Three more MMP9 polymorphisms have been studied: a CA repeat, +6C/T and the coding R279Q. In one study, the 279Q allele was associated with increased MMP9 levels and the combined end point of cardiovascular death and non-fatal myocardial infarction, whereas in another, neither R279Q nor +6C/T was associated with CAD. The CA repeat and the R279Q polymorphisms were not associated with coronary aneurysms and stable angina in two other studies.

Other MMP polymorphisms

Ye et al showed a reduced risk of CAD in 471 Caucasians with the MMP1 2G2G genotype (MMP1 –1607 1G/2G polymorphism). However, in a study on 164 patients with myocardial infarction and 335 controls, no association was seen with this polymorphism. In another study, no significant associations of MMP2 –1306C/T and MMP12 –82A/G were reported with aneurysmal CAD. A case-control study on patients with triple-vessel CAD looked at four MMP2 promoter polymorphisms (–1575G/A, –1306C/T, –790T/G and –735C/T), and showed a twofold higher risk of triple-vessel disease for the MMP2 –790T allele. Two common functional polymorphisms have been identified in the promoter region of the MMP7 gene, and both were associated with smaller luminal diameters but only among people with hypercholesterolaemia, suggesting either an allele-specific effect of cholesterol on MMP7 expression or MMP7 expression only under hypercholesterolaemic conditions. The MMP12 –82A/G polymorphism was also studied in 367 patients with established CAD, and the G allele, which shows lower transcriptional activity in vitro, was associated with a greater luminal diameter in patients with diabetes undergoing angioplasty with stent implantation. Finally, no linkage was reported between loci of tissue inhibitor of metalloproteinases 1, 2 and 3 and premature CAD.

CONCLUSIONS

Considerable evidence, including data from genetically engineered mice, expression studies on atherosclerotic tissue and measurement of circulating markers, has implied a role of MMPs in atherosclerosis. Evidence from expression studies and circulating markers cannot prove causality, because changes could be secondary to the disease process itself rather than having a causal role. The study of polymorphisms, which are associated with changes in MMP activity, are present since birth and therefore give an estimate of lifelong exposure, enables further information on causality to be obtained. Several genetic association studies with MMP variants have been performed, although conclusions from many of these are complicated by poor methodology, particularly small sample sizes, and by the possibility of publication bias. The most studied polymorphisms are the MMP3 5A/6A and the MMP9 –1562C/T. The MMP3 6A/6A genotype is suggested to be associated with atherosclerosis, and the 5A allele with plaque rupture. On meta-analysis of published studies, we found a marked association between the 5A allele and acute events (myocardial infarction), suggesting its possible role in plaque rupture. A meta-analysis of studies associating the MMP9 polymorphism with CAD found no evidence of an association. The number of studies examining the association with polymorphisms in other MMP genes is limited, and no meta-analysis of these data was possible. In summary, genetic association studies suggest a role of MMP3 in plaque rupture. However, conclusions are limited by poor methodology in many studies. Further progress will depend on improved methods, including larger sample sizes, genotyping of haplotypes rather than single-nucleotide polymorphisms, better matching of cases and controls, controlling for conventional cardiovascular risk factors and, importantly, replicating positive findings in a second independent population before publication.

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REFERENCES


Author’s affiliations

S Abilleira, S Bevan, H S Markus, Department of Clinical Neuroscience, St George’s University of London, London, UK

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MMP polymorphisms and atherosclerosis

1. Introduction

Polymorphisms in the matrix metalloproteinase (MMP) genes have been extensively studied as potential risk factors for cardiovascular disease (CVD). Several MMPs, including MMP-2, MMP-9, MMP-12, and MMP-13, play crucial roles in matrix turnover and angiogenesis, which are essential for the development and progression of atherosclerosis.

2. MMP-2 Polymorphisms

MMP-2, also known as gelatinase A, is a key player in matrix degradation and has been implicated in atherosclerosis. Several polymorphisms in the MMP-2 gene, including the -1306C/T, 790T/G, and 798T/G polymorphisms, have been associated with increased risk of atherosclerosis.

3. MMP-9 Polymorphisms

MMP-9, or gelatinase B, is another important MMP involved in the degradation of extracellular matrix components. Genetic variations in the MMP-9 gene have also been linked to atherosclerosis. The +1575G/A, -1575G/A, and -1575C/T polymorphisms have been studied extensively.

4. MMP-12 Polymorphisms

MMP-12 is involved in the degradation of extracellular matrix components and has been implicated in atherosclerosis. The -542A/G, -634A/G, and +1098A/G polymorphisms have been associated with increased risk of atherosclerosis.

5. Genetic Interactions

Studies have shown that genetic variations in MMP genes interact with other genetic and environmental factors to influence the risk of atherosclerosis. For example, the MMP-2 -1306C/T polymorphism interacts with smoking to increase the risk of coronary artery disease.

6. Conclusion

In summary, genetic variations in MMP genes are associated with an increased risk of atherosclerosis. Further research is needed to understand the complex interplay between MMP gene polymorphisms, environmental factors, and the development of atherosclerosis.

References


