Variation in the matrix metalloproteinase-1 gene and risk of coronary heart disease

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Aims Matrix metalloproteinase-1 (MMP-1), a proteolytic enzyme able to degrade types I and III collagens, is present in atherosclerotic lesions but absent from the normal blood vessel wall. The recent observation that, in a transgenic mouse model, MMP-1 gene expression slows the development and progression of atherosclerotic plaques suggests that it may play a role in human atherogenesis. We investigated whether coronary heart disease was associated with a functional polymorphism in the human MMP-1 gene. In addition, we examined a polymorphism in the human MMP-3 gene that was previously reported to be associated with progression of coronary atherosclerosis.

Methods and results We genotyped 471 Caucasian men and women, aged 66–75 years, from Sheffield, UK, for the 1G/2G polymorphism in the MMP-1 gene and the 5A/6A polymorphism in the MMP-3 gene and ascertained the prevalence of coronary heart disease. People homozygous for the more transcriptionally active 2G allele of the MMP-1 gene had a reduced risk of coronary heart disease (OR 0.5, 95% CI 0.3 to 0.9) compared to people homozygous for the less transcriptionally active 1G allele. Heterozygotes had an intermediate risk (OR 0.7, 95% CI, 0.5 to 1.1). We found no association between the 5A/6A polymorphism in the MMP-3 gene and risk of coronary heart disease.

Conclusion Sequence variants at the MMP-1 genomic locus may influence risk of coronary heart disease in humans.

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KEYWORDS
Coronary heart disease; Matrix metalloproteinase-1; Matrix metalloproteinase-3; Gene expression; Polymorphism; Epidemiology

Introduction

Atherosclerotic plaques typically contain a lipid-rich core surrounded by a fibrous capsule consisting predominantly of smooth muscle cells and extracellular matrix proteins. The principal matrix proteins in plaques are types I and III collagens, proteoglycans and elastin, with collagens accounting for up to 60% of the total protein content. The cellular constituents of atherosclerotic lesions, particularly macrophages, also express a number of proteolytic enzymes, matrix metalloproteinases (MMPs), that are thought to influence rates of atherogenesis and the stability of atherosclerotic plaques. Among the best characterized is MMP-1 (collagenase-1) which plays an important part in the degradation of collagen types I, II and III.

Recent data from animal experiments suggest that MMP-1 can prevent or delay the progression of atherosclerotic plaques. The apoE knockout mouse rapidly develops atherosclerotic lesions similar to those found in humans when fed a high cholesterol, Western-type diet. Insertion of a human MMP-1 transgene into such mice (wild-type mice do not possess a gene homologous to human MMP-1) reduces both the severity and extent of aortic atheromatous lesions.
A functional polymorphism is present in the promoter region of the human MMP-1 gene. The two alleles have either one (1G) or two (2G) guanine nucleotides at position—1607 relative to the transcriptional start site of the MMP-1 gene. It has been shown that the 2G allelic promoter of the MMP-1 gene has over 20 fold higher transcriptional activity than the 1G allelic promoter and is associated with elevated MMP-1 mRNA levels in ovarian carcinomas.

We examined the MMP-1 gene polymorphism in a group of British Caucasian subjects and investigated whether they were related to the prevalence of coronary heart disease. In addition, we investigated whether the risk of coronary heart disease was associated with a MMP-3 gene variant, referred to as the 5A/6A polymorphism which had previously been reported to be associated with progression of coronary atherosclerosis.

**Methods**

We studied 471 men and women, aged 66–75 years, who had been born in the Jessop Hospital for Women, Sheffield, UK and who had previously taken part in research into the processes by which environment in early life influences adult cardiovascular disease. The participants had been traced using the National Health Service Central Register and were still living in Sheffield. All participants were Caucasians. The study was approved by the North Sheffield Research Ethics Committee and all participants gave written informed consent. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Participants were interviewed by a fieldworker who administered the Rose/WHO Cardiovascular Questionnaire and enquired about history of cardiovascular disease, smoking habits and current medication. She also measured height, weight and blood pressure, recorded a 12-lead electrocardiogram and took a fasting venous blood sample for measurement of total cholesterol and MMP-1 and MMP-3 genotyping. Samples were stored at –80 °C for later analysis. The methods used to determine genotypes for the MMP-1 1G/2G polymorphism and the MMP-3 5A/6A polymorphism have been described. In brief, using subjects’ genomic DNA as template, PCR reactions were carried out to amplify the DNA sequence containing the polymorphism. The PCR products were then subjected to cleavage by restriction endonuclease Xmn I which cuts the 1G allele of the MMP-1 gene and the 5A allele of the MMP-3 gene. The digests were fractionated by non-denaturing polyacrylamide gel electrophoresis and subsequently the DNA bands were visualized by Vistra Green (Amersham) staining.

We tested that the allele frequencies conformed to Hardy-Weinberg equilibrium proportions by use of the χ² test. We used ANOVA and the χ² test to examine the relation between polymorphism of the MMP-1 gene and cardiovascular risk factors. Logistic regression was used to examine the relation between MMP-1 and MMP-3 genotype and the presence of coronary heart disease, defined as the presence of one or more of the following: angina according to the Rose/WHO Cardiovascular Questionnaire, Minnesota codes 1-1, 1-2 (Q and QS codes) on an electrocardiogram or a history of coronary-artery bypass grafting or coronary angioplasty. In the multivariate logistic regression analyses we adjusted for age, gender, body mass index, smoking habit, total cholesterol concentration, pulse pressure, and use of medication to treat hypertension, diabetes or hyperlipidaemia. We also examined whether there were any interactions between MMP-1 and MMP-3 polymorphisms and smoking habit, body mass index, total cholesterol concentration or pulse pressure.

**Results**

Two hundred and four (43%) of the 471 participants had evidence of coronary heart disease. Table 1 shows the characteristics of the participants according to the presence or absence of coronary heart disease.

Information on MMP-1 genotype was available to 465 participants. In total, 128 (27.5%) participants were homozygous for the 1G allele, 215 (46.2%) were heterozygous and 122 (26.2%) were homozygous for the 2G allele. Information on MMP-3 genotype was available for 471 participants. Of these 125 (26.5%) were homozygous for the 5A allele, 242 (51.4%) were homozygous for the 6A allele. For both polymorphisms, the genotype distribution was in agreement with the Hardy–Weinberg equilibrium. There were no statistically significant associations between MMP-1 or MMP-3 polymorphisms and age, gender, blood pressure, body mass index, total cholesterol concentration or smoking habit.

Risk of coronary heart disease was reduced in men and women who were homozygous for the 2G allele of the

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<tr>
<th>Characteristic</th>
<th>CHD (n=204)</th>
<th>No CHD (n=267)</th>
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<tr>
<td>Age, yrs (mean SD)</td>
<td>70.3±2.0</td>
<td>70.1±2.0</td>
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<tr>
<td>Female (no, %)</td>
<td>84 (41.2%)</td>
<td>1132 (42.3%)</td>
</tr>
<tr>
<td>Body mass index, kg/m² (mean SD)</td>
<td>27.8±4.9</td>
<td>26.5±4.1</td>
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<tr>
<td>Total cholesterol, mmol/l (mean SD)</td>
<td>6.1±1.3</td>
<td>6.1±1.2</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg (mean SD)</td>
<td>146.0±21.9</td>
<td>141.9±20.8</td>
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<tr>
<td>Diastolic blood pressure, mmHg (mean SD)</td>
<td>80.4±10.5</td>
<td>80.0±9.9</td>
</tr>
<tr>
<td>Pulse pressure, mmHg (mean SD)</td>
<td>65.3±15.9</td>
<td>61.9±15.5</td>
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<tr>
<th>Smoking habit (no, %)</th>
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<tr>
<td>Never</td>
<td>63 (30.9%)</td>
<td>80 (30.0%)</td>
</tr>
<tr>
<td>Ex</td>
<td>105 (52.9%)</td>
<td>137 (51.9%)</td>
</tr>
<tr>
<td>Current</td>
<td>33 (16.2%)</td>
<td>48 (18.08%)</td>
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*aP<0.001.  bP=0.05.
MMP-1 gene compared to those who were homozygous for the 1G allele (OR 0.6, 95% CI 0.3 to 0.9 (Table 2). Heterozygotes had an intermediate risk (OR 0.7, 95% CI 0.5 to 1.1). This relation remained statistically significant after adjustment for age, gender and other cardiovascular risk factors. We examined whether the relation between coronary heart disease and MMP-1 polymorphisms varied according to smoking habit, blood pressure, body mass index or total cholesterol concentration, but there were no statistically significant interactions. We repeated the analysis using a more rigorous definition of coronary heart disease, i.e. Minnesota codes 1-1, 1-2 (Q and QS codes) on an electrocardiogram or a history of coronary-artery bypass grafting or coronary angioplasty. Risk estimates changed little: the odds ratio for coronary heart disease in men and women who were homozygous for the 2G allele of the MMP-1 gene compared to those who were homozygous for the 1G allele was 0.5, 95% CI 0.3 to 0.9, after multivariate adjustment.

We found no statistically significant association between the MMP-3 polymorphism and risk of coronary heart disease, either in univariate analysis or after adjustment for other risk factors (Table 2). Since a previous study showed an additive effect of the MMP-1 gene 2G allele and the MMP-3 6A allele on carotid atherosclerosis, we tested whether there was an additive effect on risk of coronary heart disease. No such effect was detected in this study. In addition, as in vitro data show that the 5A allele of the MMP-3 gene and the 2G allele of the MMP-1 gene have higher promoter activity, we examined whether homozygosity for both these alleles was associated with a lower risk of coronary heart disease. We found no evidence of a reduction in risk (OR 0.9, 95% CI 0.2 to 3.1), but only 10 participants had this combination of alleles.

**Discussion**

These findings suggest that the protective effect of MMP-1 expression on the development of atherosclerotic lesions seen in transgenic mice also operates, at least to a modest extent, in humans. In vitro studies have shown that the 2G allelic promoter has a higher transcriptional activity than the 1G allelic promoter. Thus it is possible that MMP-1 expression in the arterial wall is increased in individuals carrying the 2G allele, which would retard collagen accumulation. This hypothesis is consistent with the findings in transgenic mice that MMP-1 expression results in smaller, less advanced atherosclerotic lesions with diminished collagen content. In addition, an increase in MMP-1 expression may have indirect effects on lipid deposition and vascular smooth muscle cell migration, since there is evidence indicating that type I collagen enhances differentiation of monocytes into lipid-laden macrophages and that collagen is necessary for migration of vascular smooth muscle cells.

Some studies have shown an association between progression of atherosclerosis and a functional polymorphism in the promoter of the gene encoding MMP-3, another important matrix-degrading enzyme, such that lesion progression is more rapid in individuals who are homozygous for the transcriptionally less active 6A allele than those who carry the more active 5A allele. Genetic factors can be classed into disease susceptibility genes and disease modifying genes. The former may contribute to the initiation of the disease process, whereas the latter may influence the progression and outcome of the disease after it has been initiated. The finding of an association between the MMP-3 gene 5A/6A polymorphism and progression of atherosclerosis in previous studies suggest that this genetic variant can exert a disease modifying effect. Whether this genetic variant can also influence disease susceptibility is not known and this was examined in the present study. In the sample studied, we did not find an association between this genetic variant and risk of coronary heart disease.

There is accumulating evidence that MMPs play diverse roles in atherosclerosis. Although imbalanced MMP-1 activity in advanced atheroma may be one of the factors that contribute to plaque instability, we speculate that increased MMP-1 expression at the earlier stages could be protective.
of atherogenesis may be a beneficial response to collagen accumulation and help deter lesion progression.

Acknowledgements

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References