Review

Influence of matrix metalloproteinase genotype on cardiovascular disease susceptibility and outcome

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Abstract

Data have been accumulating that indicate that matrix metalloproteinase (MMP) gene polymorphisms contribute to inter-individual differences in susceptibility to and outcome of cardiovascular disease. This is currently best exemplified by the MMP3 gene 5A/6A polymorphism which has an effect on MMP3 expression and has been shown to be associated with coronary stenosis, myocardial infarction, coronary artery calcification, post-angioplasty coronary restenosis, carotid atherosclerosis, stroke, arterial stiffness, and blood pressure. Functional polymorphisms in the MMP1, MMP2, MMP7, MMP9, MMP12, and MMP13 genes have also been related to coronary artery disease, arterial stiffness, and/or abdominal aortic aneurysm. These genetic findings support the notion that MMPs play important roles in the pathogenesis of these conditions. There is also some evidence suggesting that MMP genotyping could aid in identifying patients who are likely to have unfavourable prognosis and/or adverse response to treatment.

Keywords: Matrix metalloproteinase; Genetics; Polymorphisms; Cardiovascular disease

1. Introduction

The matrix metalloproteinase (MMP) family consists of over 20 secreted or cell surface enzymes that are capable of degrading extracellular matrix proteins as well as clotting factors, lipoproteins, latent growth factors, and chemotactic and cell adhesion molecules [1–3]. The expression of MMPs is primarily regulated at the transcriptional level, whilst the activity of MMPs is also dependent on activation of MMP zymogens and influenced by TIMPs (tissue inhibitors of MMPs) which inhibit active MMPs [1–3]. Changes in the expression of MMPs in vascular and cardiac tissues have been implicated in the pathogenesis of several cardiovascular conditions including atherosclerosis, aneurysms, post-angioplasty restenosis, and heart failure (reviewed in Refs. [4,5]).

Naturally occurring DNA sequence variations have been identified in several MMP genes, and there is accumulating evidence indicating that individuals of certain MMP genotypes are predisposed to the development of the cardiovascular conditions mentioned above. This article will review some of these findings, focusing on polymorphisms in MMP3 and MMP9 and then touching upon polymorphisms in several other MMPs.

2. MMP3 (stromelysin-1)

2.1. Polymorphisms identified in the MMP3 gene

Polymorphisms that have been identified in the MMP3 gene are depicted in Fig. 1. The 5A/6A polymorphism in the promoter has been shown to have an effect on MMP3 expression and is associated with a number of cardiovascular conditions.
Expression of MMP3 is subject to tight transcriptional regulation, which involves a number of cis-elements in the promoter of the gene [6]. The transcription factor AP-1 binding site located at position −70 to −64 relative to the transcriptional start site plays an essential role in regulating MMP3 transcription in response to various cytokines and growth factors [7–11]. Other cis-elements identified in the MMP3 promoter include two transcription factor Ets binding sites located between positions −217 to −200, an interleukin-1 responsive element at position −1614 to −1595, a platelet-derived growth factor responsive element at position −1659 to −1643, and several glucocorticoid responsive elements (Fig. 1) [8–12].

The 5A/6A polymorphism is located within the interleukin-1 responsive element (Fig. 1). In in vitro experiments using the reporter gene assay technique, the 5A allelic promoter had greater activity in driving gene expression than the 6A allelic promoter [13,14]. This difference has been observed in several cell types including macrophages, smooth muscle cells, and fibroblasts [13,14]. In agreement, studies of the levels of MMP3 mRNA and protein in ex vivo tissues including vascular tissues from individuals of different genotypes for the 5A/6A polymorphism showed that the levels were highest in 5A homozygotes, intermediate in heterozygotes and lowest in 6A homozygotes [15,16]. This suggests that the 5A/6A polymorphism has an effect on MMP3 transcription in vivo, resulting in different MMP3 levels among individuals of different MMP3 genotypes.

DNA-protein interaction assays have demonstrated that at least two nuclear proteins can bind to DNA elements encompassing the run of 5As (or 6As), and one of these proteins interacts with the 6A allele more effectively than with the 5A allele [13]. One of the nuclear proteins capable of binding to this region of the MMP3 promoter is transcription factor ZBP89 (also named ZNF148) which has a similar affinity with the 5A and 6A alleles and acts as a transcriptional enhancer [17]. Another nuclear protein capable of binding to this region is transcription factor NFκB composed of various dimers of subunits, including p65/p50 heterodimers, p65/p65 homodimers and p50/p50 homodimers [18]. The p65/p50 dimer has similar affinity for the 5A and 6A alleles, so does the p65/65 dimer [18]. However, the p50/p50 dimer binds to the 6A allele more effectively than to the 5A allele [18].

The NFκB p50 homodimer has been shown to function as a transcriptional repressor for a number of genes, by inhibiting transactivation of the p50/p65 heterodimer [19–25]. Since the optimal binding sequences for p65 and p50 are similar but not identical, [19,26] it is likely that NFκB mediated transcriptional regulation can be fine-tuned by DNA sequence variations that alter the relative binding of p65/p50, p50/50 and p65/65 to gene promoters [23].

The findings described above suggest a functional model in which the higher promoter activity of the 5A allele is a result of reduced binding of the transcriptional repressor p50/p50 to the 5A allele compared with the 6A allele. Direct experimentation to test this hypothesis is warranted.

Interestingly, a tumour necrosis factor-α gene promoter polymorphism has been shown to have an allele-specific effect on the binding of p50 homodimers, but does not affect the binding of p65/p50 heterodimers, to the tumour necrosis factor-α gene promoter [23].

2.3. Cardiovascular diseases that have been shown to be associated with the MMP3 gene 5A/6A polymorphism

MMP3 has proteolytic activity on a number of extracellular matrix proteins, including types II, IV, and IX collagen, proteoglycans, laminin, fibronectin, gelatins and

Fig. 1. MMP3 gene polymorphisms. Open boxes denote cis-elements: SPRE, stromelysin platelet derived growth factor responsive element; SIRE, stromelysin interleukin-1 responsive element; Ets, transcription factor Ets binding site; API, transcription factor API binding site; TATA, TATA box. Closed boxes denote exons.
elastin [27,28]. It can also break down cross-linked fibrin [29]. In addition, it can activate several other MMPs [27,28]. This versatile enzyme is believed to play important roles in vascular and cardiac matrix remodelling.

There have been a number of genetic epidemiological studies on the MMP3 5A/6A polymorphism, which provide evidence indicating that the polymorphism is associated with various cardiovascular conditions. In general, the 6A/6A genotype is associated with phenotypes involving increased matrix protein deposition, whereas the 5A/5A and 5A/6A genotypes are associated with phenotypes involving increased matrix protein degradation.

2.3.1. Coronary artery disease

2.3.1.1. Coronary stenosis. Several studies have shown that compared with individuals of the 5A/5A or 5A/6A genotype, individuals of the 6A/6A genotype have a higher rate of coronary atherosclerotic lesion growth defined by the change in coronary artery luminal diameter over 2–3 years, measured by angiographic examinations [30–32]. In agreement, several cross-sectional studies of patients with coronary atherosclerosis documented by coronary angiography show that patients of the 6A/6A genotype have more coronary arteries with significant stenosis than those of the 5A/5A or 5A/6A genotype [14,33,34].

2.3.1.2. Myocardial infarction. Coronary atherosclerotic plaque rupture or erosion is the most common cause of myocardial infarction [35,36]. Rupture or erosion of the plaque results in exposure of its thrombogenic contents (e.g. tissue factor, collagens and lipids) to the blood, leading to thrombus formation and consequently myocardial infarction [35,36]. Pathological studies have indicated an association between increased MMP3 expression and plaque rupture [37,38]. The notion that increased MMP3 expression may promote plaque rupture has prompted genetic epidemiological studies of the MMP3 gene 5A/6A polymorphism in relation to risk of myocardial infarction. Several such studies have shown that the frequencies of the genotypes bearing the 5A allele (i.e. 5A/5A and 5A/6A) are higher in patients with myocardial infarction than in controls [14,39–42]. These studies indicate that individuals carrying the 5A allele have about 1.5–2 fold higher risk of myocardial infarction than non-carriers. A synergistic effect of the 5A allele bearing genotypes and smoking or risk of myocardial infarction has also been suggested [41,43]. Two other studies, however, have not detected the effect of the 5A allele on risk of myocardial infarction [34,44]. The discrepancy could be due to differences in study design, genetic background of the subjects, and environmental exposures.

2.3.1.3. Possible explanation of the associations of the 5A/6A polymorphism with coronary stenosis and risk of myocardial infarction. The major constituents of athero-
2.3.2. Restenosis after coronary angioplasty

Restenosis occurs in over 30% of patients after percutaneous transluminal coronary angioplasty without stent implantation, and in 20% to 30% in patient with stenting [53]. There is evidence suggesting that arterial remodelling is the primary cause of restenosis after balloon angioplasty, whereas neointimal hyperplasia appears to be an important mechanism for in stent restenosis [53].

Humphries et al. studied the 5A/6A polymorphism in relation to restenosis in patients who had undergone balloon coronary angioplasty and patients who had successful implantation of an endovascular stent [54]. In the former group, patients of the 6A/6A genotype showed about 50% more reduction in luminal diameter 6 month after the angioplasty than patients of the 5A/5A or 5A/6A genotype [54]. This genetic effect, however, was not detected in the stenting group [54]. The different findings in the two different patient groups might reflect the different mechanisms underlying restenosis after balloon angioplasty and in stent restenosis.

Hoppmann et al. has also examined the 5A/6A polymorphism in patients who had undergone coronary angioplasty without stenting and patients with stenting [55]. In the non-stenting group, there was more reduction in luminal diameter 6 month after the procedure in patients of the 6A/6A genotype than in patients of the 5A/5A genotype or 5A/6A genotype, although the difference was not statistically significant [55].

Increased incidence of restenosis in individuals of the 6A/6A genotype was also observed in a study of de Maat et al. which showed that patients of the 6A/6A genotype were more likely to require a repeat angioplasty due to restenosis-related symptoms [32].

2.3.3. Carotid intima-media thickness, carotid atherosclerosis, and stroke

Increased carotid artery intima-media thickness can be used as a measure of early atherosclerosis. Three independent studies have shown that the 5A/6A polymorphism is associated with carotid intima-media thickness, such that individuals of the 6A/6A genotype have greater intima-media values than individuals of the 5A/5A or 5A/6A genotype [56–58].

Furthermore, individuals of the 6A/6A genotype are more like to have advanced carotid atherosclerosis resulting in significant carotid stenosis. In a study of patients with carotid atherosclerosis and controls with no evidence of the disease, Ghilardi et al. showed that the frequency of the 6A/6A genotype was higher in the case group than in the control group, and that among the cases, carriers of the 6A/6A genotype have a higher degree of carotid stenosis [59].

The 5A/5A genotype, on the other hand, has been associated with increased risk of stroke. In a study of individuals with a history of ischemic stroke and a group of age- and gender-matched controls, Flex et al. found that the frequency of the 5A/5A genotype was higher in the case group, and that the association of the 5A/5A genotype with increased risk of stroke was independent of classic risk factors [60]. Although the mechanisms leading to the stroke incidences are likely to be complex, some of the incidences might have been triggered by atherosclerotic plaque rupture where increased MMP3 expression may play a role.

Thus, there are similarities between the findings regarding coronary disease and the findings regarding cerebrovascular disease, such that the 6A/6A genotype is associated with increased stenosis in both coronary arteries and carotid arteries, whilst the 5A allele bearing genotypes are associated with higher risk of both myocardial infarction and stroke. In most, but not all, of these studies, the results are consistent with a genetic model with the 5A allele having a dominant effect and the 6A allele having a recessive effect, i.e. 5A allele homozygotes and heterozygotes having a similar phenotype whilst 6A homozygotes having a different phenotype. This suggests that the intermediate level of MMP3 expression in heterozygotes is sufficient to cause the phenotype associated with the high MMP3 expression 5A/5A genotype, or sufficient to prevent the phenotype associated with the low MMP3 expression 6A/6A genotype.

2.3.4. Arterial stiffness and blood pressure

Large artery stiffness, which increases with increasing age, is a major determinant of pulse pressure and the principal mechanism underlying systolic hypertension [61]. Large artery stiffness is influenced by the relative amounts of structural proteins (particularly elastin and collagens) and by smooth muscle tone [61]. Medley et al. reported that in elderly people (age ≥61 years), aortic stiffness measured by aortic impedance was greater in 5A allele homozygotes and 6A allele homozygotes, compared with heterozygotes [62]. This association was not detected in younger individuals (age 30–60) [62].

A cross-sectional study of 1111 randomly selected community subjects, aged 27–77 years, showed that the 5A/6A polymorphism is associated with blood pressure [63]. Subjects of the 5A/5A genotype were found to have higher systolic and diastolic blood pressure than subjects of the 5A/6A or 6A/6A genotype, and this association remained significant after adjusting for classic cardiovascular risk factors [63]. It is speculated that increased blood pressure in individuals of the 5A/5A genotype may be related to increased degradation of...
elastin in the blood vessel wall, leading to increased blood vessel stiffening [63].

2.3.5. Prognostic impact of MMP3 genotype in patients with cardiovascular disease

As discussed above, follow-up studies of coronary artery disease patients have shown that the rate of coronary atherosclerotic lesion growth is higher in patients of the 6A/6A genotype and that post-angioplasty restenosis is more likely to occur in patients of this genotype [30–32,54,55]. On the other hand, a prospective study of 2743 middle-aged men from the UK showed that the relative risk of acute coronary ischaemic events was higher in smokers who had the 5A/5A genotype [43].

Furthermore, a follow-up study of patients with heart failure suggested that the 5A/5A genotype was an independent predictor of cardiac mortality in patients with non-ischaemic cardiomyopathy [64]. There was, however, no evidence of an effect of MMP3 genotype on cardiac events in patients with ischaemic cardiomyopathy [64]. The different findings in these two patient groups may be related to different patterns of MMP3 expression in myocardial tissues, as it has been shown that MMP3 expression is increased in myocardial tissues in patients with non-ischaemic cardiomyopathy but not in patients with ischaemic cardiomyopathy [65,66].

3. MMP9 (type IV collagenase 92kDa, gelatinase B)

3.1. Polymorphisms identified in the MMP9 gene

A number of polymorphisms in the MMP9 gene have been identified (Fig. 2) [67]. Most functional analyses and genetic epidemiological studies of this gene have focused on the −1562 C>T polymorphism and the (CA)n polymorphism, both in the promoter region.

3.2. Functional studies of MMP9 gene polymorphisms

MMP9 expression is under strict transcriptional regulation which involves a number of cis-elements depicted in Fig. 2 [68,69]. Functional studies indicate that the −1562 C>T polymorphism has an allele-specific effect on MMP9 transcription. DNA-protein interaction assays have revealed that the sequence between nucleotide position −1567 and −1559 relative to the transcription start site of the MMP9 gene, which encompasses the −1562 polymorphic site, can interact with a nuclear protein whose entity is still unknown [70]. This nuclear protein has considerably higher affinity with the T-1562 allele than the C-1562 allele [70]. In vitro experiments using the reporter assay technique have showed that the T-1562 allele has higher promoter activity in driving gene expression than the C-1562 allele [70].

In agreement, a study of aortic tissues by Medley et al. showed that MMP9 mRNA levels, MMP9 protein levels, and MMP9 activity were higher in T-1562 allele carriers than in non-carriers [71]. In addition, Blankenberg et al. showed that plasma MMP9 levels were also higher in T-1562 allele carriers than in non-carriers [72]. These findings suggest that the −1562 C>T polymorphism not only has an effect on MMP9 promoter activity in in vitro experiments but also has an influence on MMP9 transcription in vivo, and that this effect is translated into differences in MMP9 protein level and activity between individuals of different MMP9 genotypes.

There is evidence suggesting that the (CA)n polymorphism also has an effect on MMP9 expression [73,74]. Reporter assays have demonstrated that the alleles containing 21, 22, or 23 CA repeats have higher promoter activity than the alleles containing 14 or 18 CA repeats [73,74]. DNA-protein interaction assays have shown that the CA repeats sequences interact with a nuclear protein that binds more effectively to the alleles that have higher promoter activity [74].
3.3. Cardiovascular diseases that have been shown to be associated with MMP9 polymorphisms

MMP9 possesses proteolytic activity on type IV collagen, a major constituent of the basement membrane that surrounds every vascular smooth muscle cell and underlies the endothelium in the blood vessel wall [4]. Studies have shown that MMP9 plays an important role in vascular smooth muscle cell migration and macrophage infiltration in atherogenesis, both of which require degradation of the basement membrane [75–77]. MMP9 can also degrade elastin, and this elastinolytic activity is implicated in arterial stiffening and the development of aneurysms [78–81].

3.3.1. Coronary artery disease

Evidence from genetic epidemiological studies indicates that T-1562 allele carriers are predisposed to the development of coronary atherosclerosis that results in significant coronary stenosis [70,82]. In accordance, an autopsy study by Pollanen et al. shows that carriers of the T-1562 allele have larger atheromas than non-carriers, and this difference is more pronounced in older people [83].

MMP9 knockout studies in mice have also demonstrated a role of MMP9 in the development of atherosclerosis [75–77]. Compared with MMP9 wild-type mice, MMP9 deficient mice have fewer and smaller atherosclerotic lesions [77]. Smooth muscle cell migration into the intima is reduced in MMP9 deficient mice [75,76]. In addition, atherosclerotic lesions in MMP9 deficient mice contain fewer macrophages [77]. Thus, increased vascular smooth muscle migration and macrophage infiltration are likely a mechanism that, at least partly, explains the finding of increased coronary atherosclerosis in carriers of the MMP9 high expression T-1562 allele in humans.

3.3.2. Arterial stiffness and blood pressure

As MMP3, MMP9 can degrade elastin. There is a correlation between aortic stiffness and MMP9 levels [78]. As the MMP3 5A/6A polymorphism, the MMP9 C>T polymorphism has been associated with arterial stiffness. Medley et al. showed that coronary artery disease patients who carried the T-1562 allele had significantly greater aortic stiffness assessed by input impedance and characteristic impedance, and these relationships remained significant after adjusting for age, gender, mean arterial pressure, total cholesterol, low-density lipoprotein cholesterol, and triglycerides [71]. In addition, T-1562 allele carriers had higher brachial systolic and pulse pressure as well as carotid systolic and pulse pressure [71].

3.3.3. Aneurysms

The development and rupture of aneurysms involve degradation of vascular structural proteins including collagens and elastin. Elastinolysis is particularly pertinent to vessel dilatation, whilst collagenolysis plays a more important role in aneurysmal rupture [84]. It has been shown that MMP9 expression is increased in abdominal aortic aneurysmal tissues compared with normal blood vessels and that development of experimental abdominal aortic aneurysms is suppressed in MMP9 knockout animals [79–81]. Jones et al. studied the MMP9 C>T polymorphism in patients with abdominal aortic aneurysm and healthy controls, and found that the frequency of the T-1562 allele bearing genotypes was significantly higher in the case group than in the control group [85].

In addition, a study of the MMP9 gene (CA)n polymorphism showed that the frequency of the (CA)23 allele which has higher MMP9 promoter activity was higher in patients with intracranial aneurysm than in age- and gender-matched controls [73]. This difference, however, was not detected in another study [86]. Further investigations with larger sample sizes would be required to test this putative association.

3.3.4. Prognostic influence of MMP9 genotype in patients with cardiovascular disease

Blankenberg et al. investigated whether plasma MMP9 levels and MMP9 genotype were determinants of cardiovascular mortality in patients with coronary artery disease, in a prospective study of 1127 cases with a follow-up period of approximately 4 years [72]. The study showed that plasma MMP9 concentration was a predictor of cardiovascular mortality, which remained significant after adjusting for clinical variables and treatment. Plasma MMP9 levels were found to be higher in patients carrying the MMP9 T-1562 allele than in non-carriers. Although there was no significant association between cardiovascular death and two MMP9 gene polymorphisms tested (C>T and Arg279Gln), overall cardiovascular events including cardiovascular death as well as non-fatal myocardial infarction occurred significantly more frequently in patients carrying the 279 Gln allele.

In a study of patients suffering from heart failure and followed up for approximately 2 years, Mizon-Gérard et al. found that patients carrying the MMP9 gene T-1562 allele had higher cardiac mortality rate than non-carriers [64]. This was observed in patients with non-ischaemic cardiomyopathy as well as in patients with ischaemic cardiomyopathy [64]. In this study, the authors also examined the effect of the MMP3 gene 5A/6A polymorphism and found that the MMP3 gene variation was associated with cardiac mortality in patients with non-ischaemic cardiomyopathy but not in those with ischaemic cardiomyopathy. The different findings for the MMP9 and MMP3 genes could be explained by different patterns of expression of these two MMPs in different types of cardiomyopathy. Spinale et al. had previously showed that myocardial MMP9 levels are elevated in both ischaemic and non-ischaemic cardiomyopathy, whereas myocardial MMP3 levels are increased in...
non-ischaemic but not in ischaemic cardiomyopathy [65,66].

4. Polymorphisms in other MMP genes

Functional polymorphisms in the MMP1, MMP2, MMP7, MMP12 and MMP13 genes have also been identified. These polymorphisms are summarised in Table 1. Studies have shown that the MMP1 gene −1607 G/GG polymorphism has an effect on binding of an Ets transcription factor, [87,88] the MMP2 gene −1575 G>A and −1306 C>T polymorphisms affect binding of estrogen receptor and the SP1 transcription factor respectively, [89,90] and the MMP12 gene −82 A>G polymorphism influences binding of the AP1 transcription factor, to the respective gene promoters [91]. These polymorphisms have been shown to have allele-specific effects on the activity of the respective promoters in driving gene expression [87–91]. The MMP7 gene −181 A>G and −153 C>T polymorphisms and the MMP13 gene −77 A>G polymorphism have also been shown to exert allele-specific effects on the activity of the respective promoters, although it is unknown whether these polymorphisms affect the binding of transcription factors [92,93]. These MMP1, MMP2, MMP7, MMP12 and MMP13 gene polymorphisms have all been reported to be associated with coronary artery disease or aorta atherosclerosis [91–95].

5. Concluding remarks

The data regarding influences of MMP genotypes on susceptibility to and progression of the cardiovascular diseases discussed above are pertinent to the understanding of the genetic basis and biological mechanisms underlying the pathogenesis of these complex disorders. These genetic data support the notion that MMPs play important roles in the development of these diseases which all involve extracellular matrix remodelling. The data also suggest that the MMP gene variations are strong candidate genetic factors for these disorders which all have a multifactorial, polygenic aetiology. Additional genetic epidemiological studies with large sample size to further test this hypothesis are warranted.

Recent studies of genetic variations in relation to outcome of treatment have indicated the possibility of devising genetic tests for identifying individuals who will have a favourable or adverse response to certain treatment. Some of the studies discussed in this article provide evidence suggesting that some MMP polymorphisms might have utility in this respect, e.g. potentially increased susceptibility to developing post-angioplasty restenosis in patients with the 6A/6A genotype. Further studies in this important area are indicated.

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