

MMP-3 polymorphism: Genetic marker in pathological processes (Review)

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Abstract. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are collectively capable of cleaving virtually all extracellular matrix (ECM) substrates and play an important role in diverse physiological and pathological processes. The activity of MMPs is controlled at multiple levels, and the transcriptional regulation of MMPs appears to represent a necessary step in its regulation. MMP-3 is a key member of the MMP family with broad substrate specificity, and is crucial to the connective tissue remodeling process. It is also involved in the turnover of the numerous ECM components. A common functional promoter polymorphism of MMP-3, 5A/6A, affects its activity and has been associated with various diseases. This polymorphism may be used as a genetic marker for certain pathologies to identify individual susceptibility. This review discusses various topics related to MMP-3 in pathological processes, with a focus on the 5A/6A polymorphism.

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Abbreviations: ECM, extracellular matrix; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of MMP; 5A, 5 consecutive adenines; 6A, 6 consecutive adenines

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1. Introduction

The evidence that individual characteristics play an important role in physiological and pathological processes has resulted in the targeted study of genetic polymorphisms in the clinical setting. In this context, mediators that degrade the extracellular matrix (ECM), such as matrix metalloproteinases (MMPs), are highlighted in studies involving inflammatory and degenerative diseases, and principally the prognosis and metastasis of cancer.

Degradation of the ECM is essential in many physiological processes, such as during development, growth and repair of tissue, and the microenvironment plays a central role in controlling both normal and transformed cell functions, as well as normal tissue integrity (1).

MMPs are a pivotal family of zinc enzymes responsible for the degradation of ECM components, including basement membrane collagen, interstitial collagen, fibronectin and various proteoglycans, during normal remodeling and repair processes in development and inflammation. MMPs are also peptidase enzymes responsible for clotting factors, lipoproteins, latent growth factors and chemotactic and cell adhesion molecules (2,3). In this way, MMPs play a key role in the physiologic remodeling of tissues, including embryogenesis and tissue morphogenesis, angiogenesis, cell migration, proliferation, apoptosis, alteration of cell motility, effects on the immune system, wound repair and the inflammatory response (4).

MMPs are a family of more than 25 enzymes. The expression of most MMPs is normally low in tissues and is induced when remodeling of the ECM is required. MMP gene expression is regulated primarily at the transcriptional level, but there is also evidence of the modulation of mRNA stability in response to growth factors and cytokines (5). The promoter region of inducible MMP genes (i.e., MMP-3) shows remarkable conservation of regulatory elements, and their expression is induced by growth factors, cytokines and other environmental factors, such as contact with the ECM (6). The potent proteolytic activities of MMPs are also regulated by conversion of the pro-enzyme to the activated form and the balance with specific tissue inhibitors of metalloproteinases (TIMPs) (7).

MMPs may alter the cell cycle checkpoint controls, promote genomic instability conceivably by affecting cell adhesion, and contribute to tumor initiation and development

by altering the cellular microenvironment that facilitates tumor formation (8). In fact, excessive or inappropriate expression of MMPs may contribute to the pathogenesis of tissue destructive processes in a wide variety of diseases, including various types of cancer, invasion and metastasis of tumor cells, inflammation and degenerative diseases (9-14).

Many studies have investigated the association between polymorphic variants in MMP candidate genes and several diseases. The aim of our review is to discuss various topics related to MMP-3 in pathological processes, with a focus on the 5A/6A polymorphism.

2. Matrix metalloproteinase-3

Matrix metalloproteinase-3 (also called stromelysin-1, STR1 and STMY1) is an important member of the MMP family and degrades collagen types II, IV, V, IX, X and XI, proteoglycans, laminin, fibronectin, gelatins and elastin and other ECM proteins. It also activates other metalloproteinases, such as MMP-1, -2 and -9 (15,16), as well as its own pro-enzyme, pro-MMP-3 (17).

MMP-3 is a key member of the MMP family, with broad substrate specificity. In this way, MMP-3 is crucial to the connective tissue remodeling process; it is involved in the turnover of numerous ECM components (18).

Stromelysin-1 is produced by various types of cells, such as fibroblasts, smooth muscle cells, macrophages, synovial cells and chondrocytes (19). The expression of MMP-3 is primarily regulated at the level of transcription, where the promoter of the gene responds to various stimuli, including growth factors, cytokines, tumor promoters and oncogene products (20). MMP-3 expression is also induced in response to local conditions, such as mechanical loading (21) and inflammation (22).

MMP-3 may be particularly significant to arterial wall remodeling, as it potentially contributes to the development of structural alterations in the vessel wall by degradation of ECM proteins (23).

MMP-3 is known to lyse basal membrane collagen, and may play a role in both local invasiveness and metastatic spread, the latter of which involves the ability of neoplastic cells to cross the basal membrane of both the epithelium and the vascular endothelium. MMP-3 overexpression by some tumor types is implicated in tumor angiogenesis, invasion and metastasis (16).

3. 5A/6A polymorphism of MMP-3

Polymorphisms represent natural sequence variants (alleles). They may occur in more than one form that is present in at least 1% of a population and is considered biologically normal (24). A polymorphism exerts allele-specific effects on the regulation of gene expression or function of the coded protein, thus underlying individual differences in various biological traits and in susceptibility to disease (23).

A common functional promoter polymorphism of MMP-3 has been associated with various diseases. The MMP-3 gene is located adjacent to the telomere side of the MMP-1 gene on 11q22 (23). A naturally occurring and common polymorphism (rs3025058) affects the MMP-3 promoter at a position -1171, where either 5 or 6 consecutive adenines (5A/6A) alter

transcription factor binding and affect MMP-3 promoter activity (23).

The 5A allele was found to have greater promoter activity in various experiments with cell cultures, such as fibroblasts and vascular smooth muscle cells (23); however, this has not yet been confirmed in human tissue.

In vitro data suggest that the 5A/6A polymorphism in the MMP-3 gene promoter is potentially functionally relevant, where the 5A allele is associated with higher and the 6A allele with lower transcriptional activity (25,26). DNA-protein interaction assays showed that nuclear protein binds more strongly to the 6A sequence than to the 5A sequence, suggesting that it may be a transcriptional repressor (23).

The control of MMP-3 expression *in vivo* is complex and not well understood, and could be subject to modulation by other transcription or post-transcription factors, such as cytokines (27,28); one of its polymorphisms is located within the interleukin-1 responsive element for several transcriptional regulators (29).

4. Pathological processes and the MMP-3 5A/6A polymorphism

MMP-3 plays an important role in connective tissue remodeling during tissue repair, cell migration, angiogenesis, tissue morphogenesis and growth. These physiological processes require accurate control; however, the disruption of this balance may lead to several pathological states.

The inflammatory and degenerative reaction is orchestrated by several molecules belonging to different families of inflammatory mediators, such as cytokines, chemokines, adhesion molecules and proteolytic enzymes (30,31). Importantly, plasma levels and/or functional activity of these inflammation determinants may be strongly influenced by functional single nucleotide polymorphisms of the corresponding genes, with important clinical implications. This is the case of the polymorphism of MMP-3, which has important roles at different stages in inflammatory and degenerative processes.

The 5A/6A polymorphism in the MMP-3 gene promoter was previously investigated in patients with cardiovascular disease, suggesting an association with more severe coronary atherosclerosis (23,32,33). The 5A allele has been associated with acute coronary events (34,35), abdominal aortic and intracranial aneurysms (36) and peripheral arterial occlusive disease (37), all of which may reflect increased matrix degradation. By contrast, the 6A allele has been associated with carotid intima-media thickening (38) and the progression of coronary artery disease (32), restenosis after balloon angioplasty (39), angiographic coronary atherosclerotic lesion growth (32,40) and stenosis (41,42), suggesting that a lower expression may result in matrix accumulation, faster arterial wall thickening and plaque progression (43).

Atherosclerosis is characterized by a complex multi-factorial pathophysiology, and extensive expression of the MMP-3 gene was found to be particularly localized in plaque regions prone to rupture, such as the fibrous cap and its adjacent tissues (44). Other MMPs also influence arterial wall pathologies, but while some studies have identified local expression of MMP-3 in coronary plaque (45), other MMPs, such as MMP-1 and -9, were found mainly in carotid plaque (46).

Table I. Association of the 5A/6A polymorphism in the MMP-3 gene with various diseases.

	Disease	Reference
Allele		
5A	Rheumatoid arthritis	Scherer <i>et al</i> , 2010 (75)
5A	Lumbar disc degeneration	Yuan <i>et al</i> , 2010 (10)
5A	Myopia	Hall <i>et al</i> , 2009 (76)
6A	Ankylosing spondylitis	Wei <i>et al</i> , 2009 (77)
6A	Angiographic coronary plaque	Chen <i>et al</i> , 2009 (52)
5A	Coronary artery disease	Ozkök <i>et al</i> , 2008 (78)
6A	Susceptibility to carotid atherosclerosis	Djurić <i>et al</i> , 2008 (79)
5A	Peripheral arterial occlusive disease	Flex <i>et al</i> , 2007 (37)
5A	Migraine	Kara <i>et al</i> , 2007 (80)
5A	Oral cancer	Vairaktaris <i>et al</i> , 2007 (60)
5A	Breast cancer progression	Holliday <i>et al</i> , 2007 (59)
5A	Vascular dementia	Flex <i>et al</i> , 2006 (85)
5A	Acute coronary syndrome	Liu <i>et al</i> , 2006 (81)
5A	Hepatocellular carcinoma	Okamoto <i>et al</i> , 2005 (61)
5A	Acute myocardial infarction	Liu <i>et al</i> , 2003 (82)
6A	Severity of rheumatoid arthritis	Constantin <i>et al</i> , 2002 (19)
5A	Breast cancer	Ghilardi <i>et al</i> , 2002 (83)
6A	Carotid stenosis	Ghiraldi <i>et al</i> , 2002 (38)
Haplotype		
2G/1G - 6A/5A (MMP-1 - MMP-3)	Coronary artery disease	Horne <i>et al</i> , 2008 (12)
2G/2G - 6A/6A (MMP-1 - MMP-3)	Colorectal cancer	Lièvre <i>et al</i> , 2006 (13)
1G-6A-82A-1082G (MMP-1 - MMP-3 - MMP-12)	Lung cancer	Su <i>et al</i> , 2006 (14)
2G-6A (MMP-1 - MMP-3)	Colorectal cancer susceptibility	Hinoda <i>et al</i> , 2002 (84)

Although these studies suggest a profound influence of the MMP-3 genotype on matrix composition, the potential influence of this polymorphism on the elastic properties of the large arteries has not been clarified (47). Given the known associations between large artery stiffness and cardiovascular risk (48), particularly myocardial ischemic risk (49), it would be useful from a risk stratification perspective to understand how the MMP-3 genotype affects large artery stiffness.

In Kawasaki disease, a multi-systemic type of vasculitis including coronary involvement, the MMP-3 6A/6A genotype may be an independent risk factor for coronary artery lesions (50).

In diabetes patients, the MMP-TIMP system in vessel walls is substantially dysregulated (51), demonstrating that polymorphisms of MMP-3 affect angiographic coronary plaque progression in non-diabetic and type 2 diabetic patients (52).

A previous study found that the MMP-3 promoter genotype was associated with intervertebral disc degeneration in an elderly group (>64 years of age), but not in subjects less than 28 years of age. In fact, the accumulative effects resulting from differences in MMP-3 expression when the genotypes are compared may be significant in ageing (53). Kauppila (54) suggested that intervertebral disc degeneration may be due to disease of the lumbar artery. This indicates that the association of the MMP-3 promoter polymorphism and intervertebral disc degeneration may be related to arterial disease (53).

As summarized in Table I, this polymorphism has been associated with susceptibility to a variety of diseases, including cancer. Tumor invasion, metastasis and angiogenesis require controlled degradation of the ECM; therefore, it is clear that the expression of MMPs is associated with the invasion and metastasis of various malignancies.

The relationship between the MMP-3 5A/6A polymorphism and susceptibility to cancer remains ambiguous (55). For example, its association with breast cancer is controversial. While certain studies (38,56) have correlated the 5A allele with breast cancer susceptibility and have demonstrated that 5A homozygosity confers a worse prognostic, this has not been confirmed in other studies (57,58).

Holliday *et al* (59) suggested that, in women with breast cancer, a highly expressed MMP-3 genotype may promote tumor progression to a greater extent. Vairaktaris *et al* (60) demonstrated the association between the MMP-3 5A/6A polymorphism and oral cancer. This polymorphism was also found to influence hepatocellular carcinoma (61), while negative findings have been reported for endometrial cancer (62), brain astrocytoma (63), colorectal cancer (64), renal cell carcinoma (55) and ovarian cancer (65).

Although this polymorphism alone does not influence certain diseases, it has been demonstrated that the MMP haplotype, including the 5A/6A polymorphism in MMP-3, is strongly associated with several pathological processes, including cancer. These findings may explain why the asso-

ciation of MMP-3 5A/6A with cancer was less unambiguous in previous case-control studies. In fact, carcinogenesis, like most diseases, is a multicellular and multistage process, and different genes that metabolize various types of proteins may be involved in its stages.

5. Haplotype influence

Haplotypes are a combination of alleles at multiple loci that are transmitted together on the same chromosome. Haplotype effects may provide more complete and reliable information than single polymorphism analysis, which may contribute only partially to the MMP pathway.

A previous study suggested that genetic variations in the MMP family, including MMP-1, -3, -8, -12 and -9, are associated with bladder cancer risk. Heavy carcinogen exposure may overwhelm some of the genetic effects of MMP polymorphisms. This confirms the importance of favoring a multigenic pathway-based approach to risk assessment (66).

It appears that there are at least two explanations for why a phenotype is associated with a haplotype, but not with the individual polymorphisms that make up the haplotype. First, a functional effect on gene expression is dependent on the interaction between two or more polymorphisms (67); second, haplotypes generally have a higher probability than individual polymorphisms of showing useful linkage disequilibrium with an unknown causal variant (68). However, for a complete explanation, analysis characterizing the nuclear proteins involved and their interactions is required.

In fact, the MMP-3 5A/6A polymorphism is in linkage disequilibrium with the MMP-1 1G/2G polymorphism (63). Fang *et al* (69) showed that the 1G/6A haplotype may play a protective role in the development of adult astrocytoma, whereas the MMP-3 5A/6A polymorphism is not necessarily an independent factor influencing susceptibility to astrocytoma in individuals from northern China.

The MMP 2G/6A haplotype was associated with lower risk of lymphatic metastasis of lung cancer when compared to the 1G/5A haplotype in a Chinese population (69). However, in a US population, another haplotype (1G/6A/82A/1082G), including MMP-1 (1G/2G), MMP-3 (5A/6A) and MMP-12 (-82AG, 1082A/G), showed an association with a higher risk of lung cancer among never smokers (14). These conflicting results are explained by ethnic variations in the polymorphisms.

In fact, the association between different diseases and the frequencies of diverse polymorphisms has been shown to vary with race/ethnicity (70,71). In the 5A/6A polymorphism in MMP-3, a high frequency of the 6A allele is found in approximately 50% of Caucasian populations (40). This allelic frequency is in agreement with that reported in Australian (47), British (72) and Swedish (73) populations. However, Lanfear *et al* (74) showed that the 6A allele is more common in African American than in European American subjects, suggesting a potential genetic contribution to the observed racial differences in genotype distribution. A differential impact of this polymorphism, if present, could suggest either that they are in linkage with a causative variant or that their influence requires other genetic modifiers that may differ between races.

Environmental influences, such as diet and smoking (39), appear to have an important interaction with the MMP-3 genotype, and may be particularly important in explaining ethnic differences in the relationship between the MMP-3 genotype and coronary artery disease (40).

Therefore, in order to clarify the contribution of genetic polymorphisms to the development and progression of disease, it is important to analyze such genotype distributions and allele frequencies between diverse races. This may help confirm the positive correlation reported in different populations.

The 5A/6A polymorphism in MMP-3 may contribute to the pathogenesis of tissue destructive processes in a wide variety of diseases. However, analysis is required to determine whether its effect is buffered or intensified in a haplotype with other MMP polymorphisms, which participate in a complex network of interactions in such diseases in a particular manner in each ethnic population. Thus, the discovery of genetic markers related to pathologies becomes clinically invaluable for identifying susceptible individuals. Further study of polymorphisms in the MMP-3 gene will provide additional insight into the biology of diverse diseases, and will aid in a better understanding of the molecular influence of polymorphisms.

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