

## Cardiovascular Risk and Matrix Metalloproteinase Polymorphisms Not Just a Simple Substitution

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Cardiovascular remodeling is a process by which structural changes occur within the vascular compartment and the myocardium and are hallmark events in the development and progression of cardiovascular disease. This remodeling process is multifactorial entailing biological shifts in molecular, cellular, and extracellular matrix (ECM) structure and function. The ECM, for example, plays a critical role in maintaining normal vascular and myocardial architecture, and proteolytic turnover of the ECM, driven in large part by the induction and activation of matrix metalloproteinases (MMPs), is a major determinant of ECM structure and function. The MMPs are tightly regulated by transcriptional, post-transcriptional, and post-translational checkpoints. Transcriptional regulation of MMPs is primarily determined by upstream gene promoter activity, whereby several intracellular signaling factors bind to specific sequences within the MMP promoter sequence. As such, there has been considerable interest in nucleic acid substitutions (ie, polymorphisms) that occur within the MMP promoter regions and relation to overall MMP levels, and most importantly, relation to cardiovascular outcomes.<sup>1,2</sup>

### See Article by Salminen et al

There have been several MMP polymorphisms identified in key MMP types, which include the collagenases (MMP-1, -8), the gelatinases (MMP-2, MMP-9), and stromelysins (MMP-3). A brief synopsis of MMP polymorphisms with respect to cardiovascular remodeling processes and selected citations is provided in Table.<sup>2-19</sup> This summary table is by no means exhaustive but underscores the fact that several polymorphisms, primarily within the MMP promoter regions, have been identified and associated with subsets of patients at risk for cardiovascular events. Several of the MMP types identified in Table are associated with acute/chronic inflammation in that these proteases are expressed by inflammatory cells, as well as induced by mediators of inflammation, such as through

cytokine signaling. For example, MMP-8 and MMP-9 are highly expressed by neutrophils and macrophages, and rapid induction of these MMP types through inflammatory signaling pathways has been well established. Coronary artery disease, and more specifically vulnerable coronary plaques, has been associated with inflammation and local activation of MMP-9 and MMP-8. Precisely, increased local MMP-8 levels and polymorphisms within the MMP-8 promoter region have been identified with coronary plaque progression and acute events.<sup>17,18</sup> In this issue of *Circulation: Cardiovascular Genetics*, Salminen et al<sup>18</sup> performed a genome-wide association study to that of plasma MMP-8 levels, as well as overall cardiovascular events in a large cohort of patients.<sup>18</sup> These investigators identified that in certain subsets of patients, polymorphisms within the MMP-8 promoter region were associated with different plasma MMP-8 levels. Past studies have identified that polymorphisms within the MMP-9 promoter region would also result in higher plasma MMP-9 levels and in turn impart increased risk for cardiovascular events.<sup>7,8</sup>

More importantly, however, Salminen et al<sup>18</sup> identified several important polymorphisms distal to that of MMP-8 itself using a genome-wide association study approach: that of complement factor-H and in a specific member of the S100A family, both of which directly or indirectly may have influenced MMP-8 levels.<sup>18</sup> For example, the minor allele of S100A9 (rs1560833) was associated with differences in steady-state plasma MMP-8 levels. An association between plasma S100 protein levels, inflammatory signaling, and cardiovascular risk has been identified.<sup>20</sup> While an oversimplification, the findings by Salminen et al<sup>18</sup> add to the body of evidence about an interrelationship between inflammatory signaling pathways, MMP induction, and cardiovascular risk. With respect to complement factor-H polymorphisms, Salminen et al<sup>18</sup> demonstrated that a complement factor-H polymorphism (rs800292) was associated with reduced MMP-8 release in a neutrophil functional assay.<sup>18</sup> In this case, it is likely that the complement factor-H polymorphism interferes in the release of preformed MMP-8 from neutrophils. These findings underscore how gene mutations, in what initially would seem to be unrelated pathways, can hold biological relevance, and in this case, alter a downstream MMP-8 post-translational regulatory step.

In the study by Salminen et al,<sup>18</sup> the minor allele of S100A9 (rs1560833) was not only associated with changes in MMP-8 plasma levels but also associated with overall cardiovascular events.<sup>18</sup> Specifically, the S100A9 minor allele A of rs1560833 was associated with lower plasma MMP-8 levels and an inverse relative risk of cardiovascular events. Interestingly, the association with this S100A9 polymorphism and cardiovascular events was identified in men but not

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**Table. Examples of MMP Polymorphisms and Association With Risk of Cardiovascular Disease**

Polymorphism	Clinical Association	References
MMP-9	CVD, post-MI remodeling, DCM	2–4,6–8,19
	TAA/AAA	15
MMP-2	CAD, HF, CVD, HTN, TAA	12,14
MMP-3	Post-MI remodeling, DCM, CAD, HTN	5,9,10,15,16
MMP-1	Post-MI remodeling, HF, CVA	3,5,11,13
MMP-8	CVD	17,18
MMP-12	CVD	4

AAA indicates abdominal aortic aneurysm; CAD, coronary artery disease; CVA, cerebrovascular accident; CVD, cardiovascular disease; DCM, dilated cardiomyopathy; HTN, hypertension; MI, myocardial infarction; MMP, matrix metalloproteinases; and TAA, thoracic aortic aneurysm.

women. Past studies of MMP polymorphisms and cardiovascular risk have identified both sex and ethnicity as important independent confounding variables in predictive cardiovascular risk models.<sup>17,21–24</sup> Indeed, a past study reported that polymorphisms contained within the MMP-8 promoter region conferred increased risk for the progression of carotid artery disease, specifically in women.<sup>17</sup> In another study, the severity of carotid artery disease was most strongly associated with a common MMP-9 polymorphism (-1562T) in women.<sup>24</sup> With respect to ethnicity, specific MMP polymorphisms have been identified to confer increased risk of cardiovascular disease progression and cardiovascular events in Asians and in blacks of African descent.<sup>21–23</sup> In addition, specific MMP polymorphisms confer additive risk for cardiovascular events in subjects with other risk factors, such as diabetes mellitus and obesity.<sup>2,19</sup> Thus, assessment of polymorphisms associated with MMP induction in at risk subpopulations will be an important area for further research and refinement in terms of cardiovascular risk assessment.

One important observation from the study by Salminen et al<sup>18</sup> is the influence of sample collection conditions in terms of assessing MMP levels and potential biological interactions of other enzyme pathways, such as complement factors.<sup>18</sup> MMP levels will not yield equivalent results when measured from decanted plasma or that from serum, and the study by Salminen et al<sup>18</sup> amplifies the importance of the sampling procedure. Although this may at first seem to be a minor methodological consideration, it underscores a relative lack of uniformity and standardized process for blood sample preparation in the assessment of labile biological factors, such as MMPs and other proteases. This issue must be resolved through an appropriate consensus panel if biomarker measurements at the genome and post-transcriptional level are to be strategically integrated into predictive models and clinical risk assessment algorithms. Finally, it should be emphasized that the majority of studies that have examined the relationship of MMP polymorphisms to that of MMP levels have primarily been performed in mixed venous blood samples. However, the proteolytic activity of MMPs is a highly compartmentalized process, and therefore systemic levels of MMPs may not necessarily reflect ECM degradation and remodeling

occurring locally. Furthermore, systemic MMP levels, and for that matter polymorphisms affecting MMP transcription, do not provide an index of actual MMP activity. Future studies that examine the inter-relationship of MMP polymorphisms to true indices of MMP activity, such as through next-generation MMP imaging,<sup>25</sup> are warranted.

In conclusion, simple sequence substitutions within the MMP gene or promoter region provide only a partial picture into the complex regulation of MMPs within the cardiovascular system. Indeed, the study reported by Salminen et al<sup>18</sup> demonstrated no association between polymorphisms within the MMP-8 gene itself and relative MMP-8 plasma levels.<sup>18</sup> However, this study provides further evidence that changes in unexpected distal pathways can affect MMP induction and release. It is not surprising that there are multiple checkpoints and intersections of the MMP system given the critical role these proteases play in cardiovascular remodeling and ultimately determining cardiovascular risk.

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### Disclosures

None.

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