A single nucleotide polymorphism in the matrix metalloproteinase 9 gene (–8202A/G) is associated with thoracic aortic aneurysms and thoracic aortic dissection

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Objective: Matrix metalloproteinase 9 plays an important role in the maintenance of the aortic extracellular matrix. Genetic variations that affect protease expression or activity might contribute to thoracic aortic disease. The purpose of this study was to determine whether 3 single nucleotide polymorphisms in the matrix metalloproteinase 9 gene are associated with thoracic aortic aneurysms and dissection.

Methods: Genomic DNA was isolated from blood or aortic tissue from 28 patients with degenerative thoracic aortic aneurysms, 60 patients with thoracic aortic dissection, and 111 control patients. The frequency distributions of 3 matrix metalloproteinase 9 single nucleotide polymorphisms (–8202A/G, IVS4+3G/T, and 2003A/G [Q668R]) were determined by using genotyping accomplished with a real-time detection system. Associations between polymorphisms and disease were estimated with odds ratios and their 95% confidence intervals.

Results: The frequency of the –8202G allele was significantly higher in patients with thoracic aortic aneurysms and aortic dissection (0.52 and 0.56, respectively) than in control subjects (0.36, $P < .001$). Patients with thoracic aortic aneurysms or dissection were nearly 5 times more likely than control subjects to have the G allele (adjusted odds ratio, 4.87; 95% confidence interval, 2.04–11.64). There were no significant associations between the IVS4+3G/T or 2003A/G polymorphisms and thoracic aortic disease.

Conclusions: The matrix metalloproteinase 9 –8202A/G polymorphism is associated with thoracic aortic aneurysms and dissection. Further studies are warranted to elucidate the functional role of the –8202A/G variant in matrix metalloproteinase 9 expression.

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Thoracic aortic dissection (TAD) and degenerative thoracic aortic aneurysms (TAAs) are major causes of mortality in the United States. Data from the National Center for Health Statistics at the Centers for Disease Control and Prevention reveal that aortic dissection and aneurysms rank among the 15 leading causes of death for all Americans (all races, both sexes) between the ages of 55 and 84 years.¹ Despite the lethality of these disease processes, their underlying mechanisms remain poorly understood.

The medial layer of the aorta is composed of vascular smooth muscle cells and extracellular matrix (ECM) proteins, primarily elastin and collagen. Maintaining a balanced composition of vascular smooth muscle cells and ECM proteins appears to be critical for preserving the important functional properties of the thoracic aorta, especially its mechanical compliance with pulsatile blood flow. Disturbances in the metabolic balance that result in excessive ECM degradation might lead to progressive aortic wall deterioration, expansion, and rupture.²

Abbreviations and Acronyms

ECM	= extracellular matrix
MMP	= matrix metalloproteinase
OR	= odds ratio
PCR	= polymerase chain reaction
SNP	= single nucleotide polymorphism
TAA	= thoracic aortic aneurysm
TAD	= thoracic aortic dissection

The matrix metalloproteinases (MMPs) are a family of more than 20 zinc-dependent proteolytic enzymes.³⁻⁵ These enzymes play vital roles in diseases related to ECM metabolism and aortic wall remodeling, which might be relevant to the development of aneurysms or dissection.^{6,7} Recent studies have shown that excessive activation of one MMP, MMP-9, occurs in abdominal aortic aneurysms and might contribute to rapid aortic expansion and rupture.^{8,9} Increased MMP-9 expression has also been observed in patients with thoracic aortic disease.¹⁰⁻¹²

The genetic aspects of TAA and TAD remain relatively unexplored, except in patients with connective tissue disorders, such as the Marfan and Ehlers-Danlos syndromes, and familial syndromes caused by rare genetic mutations.¹³⁻¹⁵ Because there is extensive literature about the role of MMP-9 in abdominal aortic aneurysms, the MMP-9 gene (*MMP9*) has emerged as a target for investigation in patients with thoracic aortic disease.

A single nucleotide polymorphism (SNP) is a common nucleotide variant in DNA at a single nucleotide site. Each individual has many SNPs that together create a unique DNA sequence. SNPs are highly conserved during evolution and within populations. They are thought to be the cause of most human diseases that have a genetic component or, at least, to be positional markers of disease genes. A number of *MMP9* SNPs have been identified and recorded in the National Center for Biotechnology Information dbSNP database. Some *MMP9* SNPs have been associated with specific phenotypic features of breast cancers, with outcomes in patients with breast cancer, with invasiveness of gastric cancer, and with risk for coronary artery disease.¹⁶⁻¹⁸ Although one SNP, found at -1562bp (ie, the nucleotide that is 1562 base pairs away from the start of transcription) in the promoter region of *MMP9*, has been associated with abdominal aortic aneurysms,¹⁹ the role of *MMP9* polymorphisms in thoracic aortic disease is unknown.

The purpose of this case-control study was to examine the association of 3 *MMP9* SNPs with TAA and TAD in a white population. The 3 SNPs examined were -8202A/G (dbSNP ID: rs11697325) in the 5' untranslated region, IVS4+3G/T (dbSNP ID: rs2274755) at the fourth intron, and 2003A/G (Q668R; dbSNP ID: rs2274756) at the twelfth exon.

Materials and Methods**Patients**

Three groups of patients were compared: 2 groups of patients with thoracic aortic disease and 1 group of control patients (Table 1). The 2 disease groups consisted of patients undergoing treatment at Baylor College of Medicine for TAD (n = 60) or degenerative TAA not caused by aortic dissection (n = 28). Because the frequencies of SNPs vary among ethnic groups, we restricted our study to white patients. Patients with Marfan syndrome, Ehlers-Danlos syndrome, traumatic aneurysms, or aortic coarctation were excluded from the study. The TAD group included 1 patient with a bicuspid aortic valve, 1 with giant cell arteritis, and 1 with aortic rupture. Fifty-nine patients had classic dissection, and 1 patient had an intramural hematoma. The TAA group included 1 patient with aortic rupture; there were no patients with pseudoaneurysms or mycotic aneurysms in this group.

Although TAD and degenerative TAAs represent distinct disease processes, occasionally patients present with both conditions. This occurs when either (1) a TAD is superimposed on a preexisting TAA or (2) a TAA exists isolated from a TAD. Three patients had both TAD and degenerative TAA and therefore were included in both of the disease groups for this analysis. One of these patients had a degenerative thoracoabdominal aortic aneurysm and had previously undergone ascending aortic repair for a DeBakey type II dissection. The other 2 patients had DeBakey type III dissection (1 classic dissection and 1 intramural hematoma) and unrepaired degenerative ascending aortic aneurysms.

The control group consisted of 111 white patients with chest pain who visited the Eastern Heart Clinic in Australia for angiographic examination. Control patients were enrolled only if angiography revealed no evidence of coronary artery disease, ascending aortic dissection, or abnormal ascending aortic diameter.

Informed consent was obtained from all patients. The genotype analysis of the control patients was approved by the Ethics Committee of the University of New South Wales, Sydney, Australia. The patients with TAA and the patients with TAD were recruited for this study under a protocol approved by the Institutional Review Board at Baylor College of Medicine.

Sample and Data Collection

Blood samples were obtained from 161 patients (24 patients with TAA, 26 patients with TAD, and 111 control subjects), and tissue samples were obtained from 35 patients (1 patient with TAA, 31 patients with TAD, and 3 patients with TAA+TAD). Blood was collected in 4-mL ethylenediamine tetraacetate tubes. Within 15 minutes of collection, blood was placed in storage at -80°C. Samples of aneurysmal or dissected aortic wall were collected during surgical repair; this tissue is routinely excised during the operation and would normally be discarded. All tissue specimens were rinsed with sterile saline. Blood clots and adipose tissue were removed from the aortic samples. The samples were placed in cryogenic vials and snap-frozen in liquid nitrogen for 1 minute. The frozen samples were stored at -80°C until batch analysis was performed.

For each patient, a complete data sheet containing detailed clinical information was filled out at the time of sample collection. Patients were considered to have a positive family history of TAA or TAD if they reported having any first-degree relative with these

TABLE 1. Demographic and clinical characteristics of the study groups

Variable	Group		
	TAA (n = 28)	TAD (n = 60)	Control (n = 111)
Male sex	21 (75%)	46 (77%)	63 (57%)
Mean age $\pm$ SD (y)	67.2 $\pm$ 10.0	59.9 $\pm$ 12.6	51.7 $\pm$ 11.0
Age range (y)	42-81	22-81	17-80
Smoking history			
None	5 (18%)	22 (37%)	*
Current	10 (36%)	11 (18%)	*
Past	13 (46%)	27 (45%)	*
Previous cardiac-aortic surgery	7 (25%)	29 (48%)	*
Coronary artery bypass	4	7	0
Aortic valve replacement	0	7	*
Ascending aorta	1	19	0
Aortic arch	1	6	*
Descending thoracic aorta	1	2	*
Abdominal aorta	2	1	*
Thoracoabdominal aorta	2	0	*
Taking lipid-lowering medication	19 (68%)	23 (38%)	*
Family history of aneurysm	4 (14%)	4 (7%)	*
Family history of dissection	0	3 (5%)	*
Bicuspid aortic valve	0	1 (2%)	*
Arteritis	0	1 (2%)	*
Rupture	1 (4%)	1 (2%)	NA
Location of degenerative aneurysm(s)			
Aortic root	3 (11%)	0	NA
Ascending aorta	10 (36%)	2 (3%)†	NA
Transverse aortic arch	6 (21%)	0	NA
Descending thoracic aorta	1 (4%)	0	NA
Thoracoabdominal aorta	17 (61%)	1 (2%)†	NA
Acuity of dissection			
Acute	2 (7%)†	13 (22%)	NA
Chronic	1 (4%)†	47 (78%)	NA
Extent of dissection			
DeBakey type I	0	25 (42%)	NA
DeBakey type II	1 (4%)†	11 (18%)	NA
DeBakey type III	2 (7%)†	24 (40%)	NA
Mean no. of segments involved $\pm$ SD‡	2.6 $\pm$ 1.4	2.9 $\pm$ 1.3	NA
Mean maximum aortic diameter $\pm$ SD (cm)	6.2 $\pm$ 1.3	6.1 $\pm$ 1.4	*
Range of maximum aortic diameter (cm)	4.0-9.0	3.6-10.0	*

TAA, Thoracic aortic aneurysm; TAD, thoracic aortic dissection; SD, standard deviation; NA, not applicable. *Not obtained. †Three patients had both thoracic aortic aneurysm and thoracic aortic dissection and are included in both groups. ‡Based on involvement of up to 5 aortic segments: ascending, transverse arch, descending thoracic, suprarenal abdominal, and infrarenal abdominal.

TABLE 2. Single nucleotide polymorphism genotyping assay information

SNP	Reporter		Context sequence‡
	VIC*	FAM†	
−8202A/G	A	G	5'GCCCAGCCCAGCCTGCAGCCCTGGG[A/G]CTCTGCAGCAGCACAGTCAGAAATG 3'
IVS4+3G/T	G	T	5'GTGGTCCCTGGGCAAGGGCGTCGGT[G/T]AGATTCTGAGTCCTCCTGGCCCCCTG 3'
2003A/G (Q668R)	A	G	5'GACACGCACGACGTCTTCCAGTACC[A/G]AGGTGAGGGCTGAGGAGGATCCCTT 3'

SNP, Single nucleotide polymorphism. *VIC dye used to label the TaqMan probe that detects the allele 1 sequence. †FAM dye used to label the TaqMan probe that detects the allele 2 sequence. ‡Twenty-five nucleotides on both sides of the SNP site, which is indicated in brackets (ie, NNN[A1/A2]NNN, where A1 = allele 1 and A2 = allele 2).

TABLE 3. —8202A/G genotypes, their association with the risk of thoracic aortic aneurysm and thoracic aortic dissection, and G allele frequencies in patients and control subjects

Genotype	Control subject (n = 111)		Patients with TAA (n = 28)			
	No.	%	No.	%	Crude OR (95% CI)	Adjusted OR (95% CI)
AA	46	41.4	1	3.6	1.00	1.00
AG	50	45.1	25	89.3	23.00 (3.00-176.62)	15.42 (1.77-134.18)
GG	15	13.5	2	7.1	6.13 (0.52-72.52)	6.81 (0.48-96.87)
AG + GG	65	58.6	27	96.4	19.11 (2.51-145.68)	13.80 (1.60-119.15)
G allele frequency	0.36		0.52			

Odds ratios and their 95% confidence intervals were calculated with logistic regression, with the *MMP9* variant genotype AA as the reference group, and adjusted for age and sex. TAA, Thoracic aortic aneurysm; TAD, thoracic aortic dissection; OR, odds ratio; CI, confidence interval.

conditions. The maximum aortic diameter of each patient with TAA and TAD was measured from the patient's most recent computed tomographic scan.

MMP9 Genotyping

DNAzol DNA extraction kits (Invitrogen, Carlsbad, Calif) were used to isolate genomic DNA from blood and aortic tissue. The Assays-on-Demand TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, Calif) of 3 SNPs (Table 2) were used to determine *MMP9* genotypes; each assay consisted of a 20× mix of unlabeled polymerase chain reaction (PCR) primers and a TaqMan minor groove binder probe. Primers were designed against a conserved region of the genome flanking the locus of interest. Two probes were designed across the locus of interest, one for each allele. Each probe was labeled with a different reporter dye, as well as a quencher molecule. Proximity to the quencher dye inhibited the fluorescence of the reporter molecule. During thermocycling, the probe annealed to the locus of interest in an allele-specific manner. As the Taq DNA polymerase extended the primers, it also degraded the annealed probe, allowing the fluorescent dye to come out of the sphere of influence of the quencher and thus become detectable. Genotyping was accomplished with the ICycler iQ Real-Time Detection System (Bio-Rad Laboratories, Hercules, Calif). Allelic discrimination real-time PCR was accomplished with a 25-μL reaction mixture containing 20 ng of DNA, 12.5 μL of TaqMan Universal PCR Master Mix (Applied Biosystems), and 1.25 μL of 20× TaqMan SNP Genotyping Assay Mix (Applied Biosystems). The PCR profile consisted of an initial melting step

of 10 minutes at 95°C, 40 cycles of 15 seconds at 92°C, and 1 minute at 60°C.

Statistical Analysis

The frequency distributions of the 3 SNPs were determined for each group. The associations between *MMP9* polymorphisms and TAA and TAD were estimated with odds ratios (ORs) and their 95% confidence intervals, which were calculated by using unconditional logistic regression models. The ORs were adjusted for age and sex. The Pearson χ^2 test was used to examine differences in distributions of genotypes between control subjects and an Applied Biosystems white sample set. All analyses were carried out with Statistical Product and Service Solutions software (Version 12.0; SPSS Inc, Chicago, Ill).

Results

The allelic frequency of *MMP9*–8202G was significantly higher among the patients with TAA and the patients with TAD than among the control subjects ($P < .001$, Table 3). Additionally, the proportion of –8202G carriers was significantly higher among the patients with TAA (27/28 [96%]) and patients with TAD (53/60 [88%]) than among the control subjects (65/111 [59%], $P < .001$). Patients with TAA were 14 times more likely to have the G allele than were control subjects. Patients with TAD were 4 times more likely to have the G allele than were control subjects. When

TABLE 4. IVS4+3G/T genotypes, their association with the risk of thoracic aortic aneurysm and thoracic aortic dissection, and T allele frequencies in patients and control subjects

Genotype	Control subjects (n = 111)		Patients with TAA (n = 28)			
	No.	%	No.	%	Crude OR (95% CI)	Adjusted OR (95% CI)
GG	83	74.8	21	75.9	1.00	1.00
TG	26	23.4	7	24.1		
TT	2	1.8	0	0	0.99 (0.38-2.57)	0.65 (0.19-2.28)
T allele frequency	0.14		0.13			

Odds ratios and their 95% confidence intervals were calculated with logistic regression, with the *MMP9* variant genotype (GG) as the reference group, and adjusted for age and sex. TAA, Thoracic aortic aneurysm; TAD, thoracic aortic dissection; OR, odds ratio; CI, confidence interval.

TABLE 3

Patients with TAD (n = 60)				Patients with TAA or TAD (n = 85)			
No.	%	Crude OR (95% CI)	Adjusted OR (95% CI)	No.	%	Crude OR (95% CI)	Adjusted OR (95% CI)
7	11.7	1.00	1.00	8	9.4	1.00	1.00
39	65.0	5.13 (2.09-12.59)	4.02 (1.57-10.32)	63	74.1	7.25 (3.14-16.74)	4.96 (2.04-12.07)
14	23.3	6.13 (2.09-18.03)	5.19 (1.61-16.74)	14	16.5	5.37 (1.89-15.28)	4.52 (1.42-14.37)
53	88.3	5.36 (2.24-12.84)	4.26 (1.70-10.66)	77	90.6	6.81 (3.00-15.47)	4.87 (2.04-11.64)
		0.56				0.54	

all patients with TAA and patients with TAD were combined into a single group, their −8202G allele frequency (0.535) was significantly higher than that of the control subjects (0.360, $P < .001$); the adjusted OR for the −8202G allele carriers was 4.87 (95% confidence interval, 2.04-11.64). No allele or genotype of IVS4+3G/T or 2003A/G (Q668R) was significantly associated with TAA or TAD (Tables 4 and 5).

The frequency distributions of all 3 *MMP9* SNPs in our control population were not significantly different from those previously published for white subjects (−8202A/G: $\chi^2 = 0.011$, $P = .92$; IVS4+3G/T: $\chi^2 = 0.19$, $P = .67$; and 2003A/G: $\chi^2 = 0.29$, $P = .56$) by Applied Biosystems. The minor allele frequencies for the SNPs among the 111 control subjects were 0.36 for −8202A/G, 0.14 for IVS4+3G/T, and 0.13 for 2003A/G. These frequencies were 0.37, 0.15, and 0.15, respectively, in a separate white population (from the Applied Biosystems sample set, $n = 45$; data from Applied Biosystems, <http://www.appliedbiosystems.com>).

Overall, 9 (82%) of the 11 patients with family histories of thoracic aortic disease had the −8202G allele. In the TAA group all 4 patients with family histories of aneurysms were −8202G allele carriers. In the patients with TAD, 3 of the 4 patients with family histories of aneurysms and 2 of 3 patients with family histories of dissection were −8202G allele carriers. The −8202G allele was present in the patient

with TAD with aortic rupture but not in the patient with the ruptured TAA.

Discussion

Both TAA and TAD involve progressive weakening of the aortic wall and are primarily associated with a characteristic histologic appearance of medial degeneration, historically described as “Erdheim cystic medial necrosis,” in which there is degeneration and fragmentation of elastic fibers, loss of smooth muscle cells, and an accumulation of basophilic ground substance.¹⁵ The medial degenerative process in TAA and TAD might be limited to one aortic segment or might involve the entire aorta. The role of MMP-9 in aortic degeneration has stimulated interest in its potential as a therapeutic target.²⁰⁻²⁴ The MMP-9 protein plays a major role in the maintenance of the aortic wall ECM and, as a degrading enzyme, is critical to arterial wall remodeling. When MMP-9 is overproduced, increased elastolytic activity can result in a weakened aortic wall that is prone to expansion. Additionally, MMP-9 can act synergistically with MMP-1 to degrade interstitial collagens. These mechanisms might explain the association between MMP-9 overexpression and abdominal aortic aneurysm formation.²⁵⁻³⁰ Upregulated MMP-9 synthesis has also been reported in patients with thoracic aortic pathology.¹⁰⁻¹²

TABLE 4

Patients with TAD (n = 60)				Patients with TAA or TAD (n = 85)			
No.	%	Crude OR (95% CI)	Adjusted OR (95% CI)	No.	%	Crude OR (95% CI)	Adjusted OR (95% CI)
45	75.4	1.00	1.00	64	75.6	1.00	1.00
12	19.7			18	20.9		
3	4.9	0.99 (0.48-2.04)	0.86 (0.38-1.92)	3	3.5	0.97 (0.51-1.87)	0.78 (0.36-1.67)
		0.15				0.14	

ACD

TABLE 5. 2003A/G (Q668R) genotypes, their association with the risk of thoracic aortic aneurysm and thoracic aortic dissection, and A allele frequencies in patients and control subjects

Genotype	Control subjects (n = 111)		Patients with TAA (n = 28)			
	No.	%	No.	%	Crude OR (95% CI)	Adjusted OR (95% CI)
GG	84	75.7	21	75.9	1.00	1.00
AG	25	22.5	7	24.1		
AA	2	1.8	0	0	1.04 (0.40-2.71)	0.68 (0.19-2.41)
A allele frequency	0.13				0.13	

Odds ratios and their 95% confidence intervals were calculated with logistic regression, with the *MMP9* variant genotype (GG) as the reference group, and adjusted for age and sex. TAA, Thoracic aortic aneurysm; TAD, thoracic aortic dissection; OR, odds ratio; CI, confidence interval.

Polymorphisms are DNA sequence variants that are present in at least 1% of the population when the variant is first observed. SNPs, locations on a DNA sequence where a single base varies among persons, are the most common sequence variations in the human genome and occur in about 1 in every 1000 bases. These SNPs can occur in both coding and noncoding regions of DNA, producing varying results. Because of the degeneracy of the genetic code, not all SNPs have deleterious effects; however, SNPs in coding regions might change the amino acids within proteins, thereby reducing DNA binding, catalytic activity, and receptor-ligand contact. Likewise, SNPs in noncoding regions (ie, 5' untranslated regions, 3' untranslated regions, and introns) can affect RNA processing, stability and translation; SNPs in the 5' untranslated region might also affect transcription.³¹

In the present study we examined the relative frequency of 3 SNPs in the *MMP9* gene among white patients with and without TAA or TAD. We chose these 3 SNPs because of their potential to affect MMP-9 production and function. The -8202A/G SNP, which was overrepresented among the patients with TAA and the patients with TAD, occurs in the 5' untranslated region of the *MMP9* gene that might have an enhancer element that increases *MMP9* transcription, resulting in high lifetime MMP-9 levels that can increase susceptibility to TAA and TAD. Alternatively, this SNP might result in loss of a repressor, thereby increasing *MMP9* transcription. It is also possible that the -8202A/G variant is in linkage disequilibrium with functional variants at other sites that might directly regulate MMP-9 expression. Enhancer-repressor elements are normally located upstream of the core promoter of a gene, although this needs to be shown experimentally for each individual SNP and gene. The IVS4+3G/T SNP occurs at the third base of the fourth intron and therefore has the potential to influence the RNA splicing process. The 2003A/G SNP is a nonsynonymous SNP (ie, an SNP whose 2 forms produce different amino acids) in the coding region that can cause a glutamine-to-arginine substitution at the 668th amino acid (Q668R) of the MMP-9 protein, potentially affecting the protein's function. Our results, however, sug-

gest that neither IVS4+3G/T nor 2003A/G is associated with TAA or TAD.

Like all genetic polymorphisms, SNPs can predetermine the susceptibility of the allele carrier to certain diseases. However, in most situations, the functional effect of an SNP depends on the presence of an environmental trigger. Because of the interdependence of genetic and environmental factors, a genetic defect might never manifest itself phenotypically if the carrier is never exposed to a specific trigger. For example, the 27th repeat polymorphism in the endothelial nitric oxide synthase gene contributes to the risk of myocardial infarction, although only in smokers.³² Additionally, different alleles might respond differently to the same environmental condition (eg, inflammation), so that each exposed individual's risk of a given disease phenotype is determined by the particular allele the individual carries. The relationship between *MMP9* polymorphisms and environmental factors in thoracic aortic disease will require investigation.

Traditionally, SNPs have been identified through the recognition of restriction enzymes that digest specific short DNA sequence spans of 4 to 6 bp. When one or more nucleotides are changed or mutated, either the existing restriction enzyme recognition site is abolished or a new one is created. Genomic regions are normally amplified with PCR and digested with a specific restriction enzyme, and the end product is resolved in electrophoresis. Different alleles are determined by different nucleotide lengths after digestion. However, of the increasing number of SNPs that have been identified with direct sequencing techniques, not all involve changes that can be detected by means of the restriction enzymes currently in use.

The probe-based direct genomic amplification approach circumvents this limitation. Taqman SNP analysis uses the 5' exonuclease activity of DNA Taq polymerase and the quenching effects of specific fluorescent dyes to determine the relative frequency of each allele within an individual genome.

An important limitation of our study was the nature of the control population, which consisted of patients initially

TABLE 5

Patients with TAD (n = 60)				Patients with TAA or TAD (n = 85)			
No.	%	Crude OR (95% CI)	Adjusted OR (95% CI)	No.	%	Crude OR (95% CI)	Adjusted OR (95% CI)
45	75.4	1.00	1.00	64	75.6	1.00	1.00
12	19.7			18	20.9		
3	4.9	1.04 (0.50-2.15)	0.92 (0.41-2.07)	3	3.5	1.02 (0.53-1.97)	0.84 (0.39-1.79)
		0.15				0.14	

believed to have heart disease. Although the control patients' angiograms were unremarkable and their ascending aortic diameters were within normal limits, we cannot rule out the possibility that these patients might have had isolated aneurysms at other segments of the aorta. Because individuals with asymptomatic aortic pathology are frequently regarded as healthy, it is almost impossible to obtain a group of volunteers or healthy donors who are all confirmed free of TAA and TAD unless complete thoracic aortic imaging is conducted. Another limitation of this study was that only basic demographic information was collected from the control patients, and thus we had no information about the control subjects' family histories of TAA or TAD.

Because our investigation is a genetic association study, our findings do not confirm a functional role for the -8202G allele in MMP-9 expression; functional investigation of this allele is warranted. Elucidating the molecular mechanisms of AG-allele-mediated MMP-9 regulation will require many experiments to identify transcription factors, characterize their transactivating effects with the promoter or other enhancer, generate recombinant vectors, and so forth. We have initiated experiments regarding the functional characterization of MMP9 polymorphisms.

In summary, our study found that the MMP9 -8202A/G polymorphism was associated with TAA and TAD in a white population. Although further studies are needed to prospectively analyze the association between the -8202A/G genotypes and the incidence of thoracic aortic disease and to evaluate the potential causal relationships between them, these results support the hypothesis that MMP9 plays a role in the pathogenesis of TAA and TAD. The discovery of disease-associated SNPs might ultimately refine the assessment of TAA and TAD risk in individual patients and provide prognostic information to guide patient care. Our ultimate goal is to create a genetic test that will allow physicians to screen individuals for their susceptibility to TAA and TAD by analyzing their DNA for specific SNP patterns.

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References

1. National Center for Health Statistics, Centers for Disease Control and Prevention, United States Department of Health and Human Services. Deaths, percent of total deaths and death rates for the 15 leading causes of death in 10-year age groups by race and sex: United States, 2001. Available at: http://www.cdc.gov/nchs/data/dvs/LCWK2_2001.pdf. Accessed May 3, 2005.
2. Powell J, Greenhalgh RM. Cellular, enzymatic, and genetic factors in the pathogenesis of abdominal aortic aneurysms. *J Vasc Surg.* 1989; 9:297-304.
3. Nagase H, Woessner JF Jr. Matrix metalloproteinases. *J Biol Chem.* 1999;274:21491-4.
4. Brinckerhoff CE, Matrisian LM. Matrix metalloproteinases: a tail of a frog that became a prince. *Nat Rev Mol Cell Biol.* 2002;3:207-14.
5. Parks WC, Mecham RP. Matrix metalloproteinases. San Diego: Academic Press; 1998.
6. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res.* 2002; 90:251-62.
7. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res.* 2003; 92:827-39.
8. Yamashita A, Noma T, Nakazawa A, Saito S, Fujioka K, Zempo N, et al. Enhanced expression of matrix metalloproteinase-9 in abdominal aortic aneurysms. *World J Surg.* 2001;25:259-65.
9. Petersen E, Wagberg F, Angquist KA. Proteolysis of the abdominal aortic aneurysm wall and the association with rupture. *Eur J Vasc Endovasc Surg.* 2002;23:153-7.
10. LeMaire SA, Wang X, Wilks JA, Carter SA, Wen S, Won T, et al. Matrix metalloproteinases in ascending aortic aneurysms: bicuspid versus trileaflet aortic valves. *J Surg Res.* 2005;123:40-8.
11. Ishii T, Asuwa N. Collagen and elastin degradation by matrix metalloproteinases and tissue inhibitors of matrix metalloproteinase in aortic dissection. *Hum Pathol.* 2000;31:640-6.
12. Lesauskaite V, Tanganelli P, Sassi C, Neri E, Diciolla F, Ivanoviene L, et al. Smooth muscle cells of the media in the dilatative pathology of ascending thoracic aorta: morphology, immunoreactivity for osteopontin, matrix metalloproteinases, and their inhibitors. *Hum Pathol.* 2001; 32:1003-11.
13. Guo D, Hasham S, Kuang SQ, Vaughan CJ, Boerwinkle E, Chen H, et al. Familial thoracic aortic aneurysms and dissections: genetic heterogeneity with a major locus mapping to 5q13-14. *Circulation.* 2001; 103:2461-8.
14. Vaughan CJ, Casey M, He J, Veugelers M, Henderson K, Guo D, et al. Identification of a chromosome 11q23.2-q24 locus for familial aortic aneurysm disease, a genetically heterogeneous disorder. *Circulation.* 2001;103:2469-75.
15. Hasham SN, Willing MC, Guo DC, Muilenburg A, He R, Tran VT, et al. Mapping a locus for familial thoracic aortic aneurysms and dissections (TAAD2) to 3p24-25. *Circulation.* 2003;107:3184-90.

16. Grieu F, Li WQ, Iacopetta B. Genetic polymorphisms in the MMP-2 and MMP-9 genes and breast cancer phenotype. *Breast Cancer Res Treat.* 2004;88:197-204.
17. Matsumura S, Oue N, Nakayama H, Kitadai Y, Yoshida K, Yamaguchi Y, et al. A single nucleotide polymorphism in the MMP-9 promoter affects tumor progression and invasive phenotype of gastric cancer. *J Cancer Res Clin Oncol.* 2005;131:19-25.
18. Morgan AR, Zhang B, Tapper W, Collins A, Ye S. Haplotypic analysis of the MMP-9 gene in relation to coronary artery disease. *J Mol Med.* 2003;81:321-6.
19. Jones GT, Phillips VL, Harris EL, Rossaak JJ, van Rij AM. Functional matrix metalloproteinase-9 polymorphism (C-1562T) associated with abdominal aortic aneurysm. *J Vasc Surg.* 2003;38:1363-7.
20. Thompson RW, Holmes DR, Mertens RA, Liao S, Botney MD, Mecham RP, et al. Production and localization of 92-kilodalton gelatinase in abdominal aortic aneurysms: an elastolytic metalloproteinase expressed by aneurysm-infiltrating macrophages. *J Clin Invest.* 1995;96:318-26.
21. Curci JA, Mao D, Bohner DG, Allen BT, Rubin BG, Reilly JM, et al. Preoperative treatment with doxycycline reduces aortic wall expression and activation of matrix metalloproteinases in patients with abdominal aortic aneurysms. *J Vasc Surg.* 2000;31:325-42.
22. Mosorin M, Juvonen J, Biancari F, Satta J, Surcel HM, Leinonen M, et al. Use of doxycycline to decrease the growth rate of abdominal aortic aneurysms: a randomized, double-blind, placebo-controlled pilot study. *J Vasc Surg.* 2001;34:606-10.
23. Baxter BT, Pearce WH, Waltke EA, Littooy FN, Hallett JW Jr, Kent KC, et al. Prolonged administration of doxycycline in patients with small asymptomatic abdominal aortic aneurysms: report of a prospective (phase II) multicenter study. *J Vasc Surg.* 2002;36:1-12.
24. Prall AK, Longo GM, Mayhan WG, Waltke EA, Fleckten B, Thompson RW, et al. Doxycycline in patients with abdominal aortic aneurysms and in mice: comparison of serum levels and effect on aneurysm growth in mice. *J Vasc Surg.* 2002;35:923-9.
25. Lindholt JS, Vammen S, Fasting H, Henneberg EW, Heickendorff L. The plasma level of matrix metalloproteinase 9 may predict the natural history of small abdominal aortic aneurysms: a preliminary study. *Eur J Vasc Endovasc Surg.* 2000;20:281-5.
26. Tamarina NA, McMillan WD, Shively VP, Pearce WH. Expression of matrix metalloproteinases and their inhibitors in aneurysms and normal aorta. *Surgery.* 1997;122:264-71.
27. Hovsepian DM, Ziporin SJ, Sakurai MK, Lee JK, Curci JA, Thompson RW. Elevated plasma levels of matrix metalloproteinase-9 in patients with abdominal aortic aneurysms: a circulating marker of degenerative aneurysm disease. *J Vasc Interv Radiol.* 2000;11:1345-52.
28. McMillan WD, Pearce WH. Increased plasma levels of metalloproteinase-9 are associated with abdominal aortic aneurysms. *J Vasc Surg.* 1999;29:122-7.
29. McMillan WD, Tamarina NA, Cipollone M, Johnson DA, Parker MA, Pearce WH. Size matters: the relationship between MMP-9 expression and aortic diameter. *Circulation.* 1997;96:2228-32.
30. Thompson RW, Baxter BT. MMP inhibition in abdominal aortic aneurysms: rationale for a prospective randomized clinical trial. *Ann N Y Acad Sci.* 1999;878:159-78.
31. Antonarakis SE, Cooper DN. Mutations in human genetic diseases. In: Cooper DN, editor. *Nature encyclopedia of the human genome.* London: Nature Publishing Group; 2003. p. 227-53.
32. Wang XL, Sim AS, Badenhop RF, McCredie RM, Wilcken DE. A smoking-dependent risk of coronary artery disease associated with a polymorphism of the endothelial nitric oxide synthase gene. *Nat Med.* 1996;2:41-5.