Published in final edited form as:
J Vasc Surg. 2005 June ; 41(6): 1036–1042.

Genetic analysis of polymorphisms in biologically relevant candidate genes in patients with abdominal aortic aneurysms

Toru Ogata, MD[a], **Hidenori Shibamura, MD, PhD**[a], **Gerard Tromp, PhD**[a], **Moumita Sinha, MStat**[b], **Katrina A. B. Goddard, PhD**[b], **Natzi Sakalihasan, MD, PhD**[c], **Raymond Limet, MD, PhD**[c], **Gerald L. MacKean, MD**[d], **Claudette Arthur, BN, MBA**[d], **Taijiro Sueda, MD, PhD**[e], **Susan Land, PhD**[a,f], and **Helena Kuivaniemi, MD, PhD**[a,g]

[a]*From the Center for Molecular Medicine and* [f]*Genetics, Applied Genomics Technology Center, and* [g]*Department of Surgery, Wayne State University School of Medicine;* [b]*Department of Epidemiology and Biostatistics, Case Western Reserve University;* [c]*Department of Cardiovascular Surgery, University Hospital of Liège;* [d]*Department of Surgery, Dalhousie University; and* [e]*Department of Surgery, Hiroshima University.*

Abstract

Background—Abdominal aortic aneurysms (AAAs) are characterized by histologic signs of chronic inflammation, destructive remodeling of extracellular matrix, and depletion of vascular smooth muscle cells. We investigated the process of extracellular matrix remodeling by performing a genetic association study with polymorphisms in the genes for matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and structural extracellular matrix molecules in AAA. Our hypothesis was that genetic variations in one or more of these genes contribute to greater or lesser activity of these gene products, and thereby contribute to susceptibility for developing AAAs.

Methods—DNA samples from 812 unrelated white subject (AAA, n = 387; controls, n = 425) were genotyped for 14 polymorphisms in 13 different candidate genes: MMP1(nt−1607), MMP2(nt−955), MMP3(nt−1612), MMP9(nt−1562), MMP10(nt+180), MMP12(nt−82), MMP13(nt−77), TIMP1(nt +434), TIMP1(rs2070584), TIMP2(rs2009196), TIMP3(nt−1296), TGFB1(nt−509), ELN(nt+422), and COL3A1(nt+581). Odds ratios and *P* values adjusted for gender and country of origin using logistic regression and stratified by family history of AAA were calculated to test for association between genotype and disease status. Haplotype analysis was carried out for the two TIMP1 polymorphisms in male subjects.

Results—Analyses with one polymorphism per test without interactions showed an association with the two TIMP1 gene polymorphisms (nt+434, $P = .0047$; rs2070584, $P = .015$) in male subjects without a family history of AAA. The association remained significant when analyzing TIMP1 haplotypes (χ^2 $P = .014$ and empirical $P = .009$). In addition, we found a significant interaction between the polymorphism and gender for MMP10 ($P = .037$) in cases without a family history of AAA, as well as between the polymorphism and country of origin for ELN ($P = .0169$) and TIMP3 ($P = .0023$) in cases with a family history of AAA.

Reprint requests: Helena Kuivaniemi, MD, PhD, Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, 3106 Gordon H. Scott Hall of Basic Medical Sciences, 540 East Canfield Avenue, Detroit, MI 48201 (e-mail: kuivan@sanger.med.wayne.edu) 0741-5214/$30.00

Supported in part by grants from the National Heart, Lung, and Blood Institute (HL064310 to H.K.), the National Human Genome Research Institute (HG01577 to K.G.), and the National Center for Research Resources (RR03655).

Detroit, Mich; Cleveland, Ohio; Liège, Belgium; Halifax, Canada; and Hiroshima, Japan

Competition of interest: none.

Additional material for this article may be found online at www.mosby.com/jvs.

Conclusions—These findings suggest that genetic variations in TIMP1, TIMP3, MMP10, and ELN genes may contribute to the pathogenesis of AAAs. Further work is needed to confirm the findings in an independent set of samples and to study the functional role of these variants in AAA. It is noteworthy that contrary to a previous study, we did not find an association between the MMP9 (nt−1562) polymorphism and AAA, suggesting genetic heterogeneity of the disease.

Clinical Relevance—Abdominal aortic aneurysms (AAAs) are an important cardiovascular disease, but the genetic and environmental risk factors, which contribute to individual's risk to develop an aneurysm, are poorly understood. Histologically, AAAs are characterized by signs of chronic inflammation, destructive remodeling of the extracellular matrix, and depletion of vascular smooth muscle cells. We hypothesized that genes involved in these events could harbor changes that make individuals more susceptible to developing aneurysms. This study identified significant genetic associations between DNA sequence changes in tissue inhibitor of metalloproteinase 1 (TIMP1), TIMP3, matrix metalloproteinase 10 (MMP10) and elastin (ELN) genes, and AAA. The results will require confirmation using an independent set of samples. After replication it is possible that these sequence changes in combination with other risk factors could be used in the future to identify individuals who are at increased risk for developing an AAA.

About 15,000 individuals die every year because of the rupture of abdominal aortic aneurysms (AAAs) in the United States.[1,2] An estimated 1% to 6% of the population in the industrialized countries harbor aneurysms.[1] Despite the major advances in surgical treatment, the survival rate after a ruptured AAA is low.[1] Early diagnosis of AAA is therefore important for improving outcome. However, diagnosing AAAs is difficult because most AAAs are asymptomatic before rupture and ultrasonography screening can only tell if the person currently has an AAA but is not able to estimate the risk of developing an AAA later. If it were possible to predict who is at risk for developing an AAA, many lives and health care dollars would be saved. Finding a susceptibility gene for AAA could lead to a simple DNA test to identify individuals who are at risk for developing an AAA. Those individuals would then be screened routinely to detect an AAA before it reaches a critical size and ruptures.

It has been suggested that AAAs are a complex disease with both genetic and environmental risk factors.[3–6] Two formal statistical analyses, so-called segregation studies, favored a genetic model in explaining the familial aggregation of AAA and suggested the presence of a major gene effect.[3,4] Recently, we reported on a collection of 233 families with at least two individuals affected with AAA,[7] and identified two genetic susceptibility loci for AAA on chromosomes 19q13 and 4q31.[8]

Several distinct processes contribute to the pathologic changes observed in AAAs. The most apparent of these are chronic inflammation, destructive remodeling of the extra-cellular matrix, and depletion of vascular smooth muscle cells.[9] Our hypothesis was that genes involved in these events could be considered candidate genes for AAA.

The matrix metalloproteinases (MMPs) are a family of endopeptidases that degrade extracellular matrix proteins.[10] The MMPs have been studied extensively and implicated in the pathogenesis of AAA.[9,11–20] Many studies have measured mRNA and protein levels of various MMPs in the aneurysmal wall and found them to be elevated.[11–14,21,see 15] We hypothesized that such elevated levels could be caused by genetic differences in the promoter sequences of these genes influencing transcription. Indeed, functional studies have shown that many of the promoter variants in MMP genes show differential binding of transcription factors.[22–27] In a previously published preliminary study, we described a suggestive genetic association between a 5A/6A polymorphism in the MMP3 gene and AAA, and found that the transcriptionally more active 5A allele was more common in AAA cases than in controls.[15,27] Other investigators found an association between a polymorphism in the MMP9 gene and AAA.[16]

Tissue inhibitors of metalloproteinases (TIMPs) are major inhibitors of MMPs.[10] Downregulation of these inhibitors could lead to an increase in the activity of extra-cellular matrix degrading enzymes such as MMPs, and therefore could contribute to the pathogenesis of AAAs. In fact, two studies have shown decreased mRNA levels of TIMPs in AAA.[21,28] Furthermore, the ratio of MMP mRNA to TIMP mRNA was higher in AAA than in normal aortas when assayed using competitive reverse-transcriptase polymerase chain reaction (RT-PCR).[17] We analyzed previously the coding sequences of TIMP1 and TIMP2 genes in patients with AAA and observed a significant difference in the frequency of the nt+573 TIMP2 polymorphism between AAA patients and controls.[29]

We have now extended our genetic studies to the polymorphisms in genes for MMP1,[23] MMP2,[26] MMP3,[15] MMP9,[25] MMP10, MMP12,[24] MMP13,[22] TIMP1,[29] TIMP2, TIMP3,[30] transforming growth factor β-1 (TGFB1),[31] elastin (ELN),[32] and type III procollagen (COL3A1),[33] and genotyped 387 AAA patients and 425 controls. Nine of the 14 polymorphisms under study were known to be functional based on previous studies.

METHODS

Study population

AAA was defined as an infrarenal aortic diameter of 3.0 cm or greater.[34] Altogether, 387 unrelated AAA cases (male subjects: n = 316, 81.7%), 180 Belgian admitted to the University Hospital of Liège in Liège and 207 Canadian admitted to Dalhousie University Hospital in Halifax, were included in the study. Seventeen patients were admitted for emergency repair of ruptured AAA, and 335 patients were admitted for elective surgery. Thirty-five patients were diagnosed with AAA using ultrasonography and did not undergo surgery because of old age or because the size of the aneurysm was relatively small. Altogether, 152 cases (39.3%) had a family history of AAA. All patients were white.

Control samples were obtained from 425 white subjects (male subjects: n = 217, 51.1%), 269 Belgian and 156 Canadian, and included spouses of AAA patients (n = 113) and individuals admitted to the same hospitals as the AAA patients for reasons other than AAA (n = 312).

The study was approved by the Institutional Review Boards of Wayne State University School of Medicine and of each patient recruiting center. All subjects gave informed written consent to participate in the study.

Genotyping

We isolated genomic DNA from peripheral blood using a Puregene kit (Gentra Systems, Minneapolis, Minn). Before performing genotyping using PCR-based methods, a whole-genome amplification using primer extension preamplification (PEP) was carried out to increase the amount of template DNA available for genotyping and to ensure that limited resources were used cost effectively.[35] The PEP products were diluted 100-fold and used for genotyping.

The PCR conditions and methods used to assay the 14 polymorphisms (Table I) are summarized in Table II, online only. Five microliters of 100-fold diluted PEP products were used for each genotyping reaction. The genotyping assays for MMP1,[23] MMP3,[15] MMP9,[25] MMP12,[24] MMP13,[22] TIMP1(+434),[29] TIMP3,[30] TGFB1,[31] ELN,[32] and COL3A1[33] were carried out as described previously. Allele-specific PCR was used to genotype MMP2[26] polymorphism (Table II, online only). Three polymorphisms, dbSNP rs486055 in MMP10 (MMP10 nt+180), rs2070584 in TIMP1, and rs2009196 in TIMP2, were identified from the National Center for Biotechnology Information LocusLink database (www.ncbi.nih.gov/LocusLink). Two polymorphisms, rs2070584 and rs2009196, were genotyped by 5′-nuclease assay (TaqMan

Assay; Applied Biosystems, Foster City, Calif). Allele-specific TaqMan minor groove binder (MGB) probes and PCR primers were designed by using Primer Express version 1.5 software (Applied Biosystems). Reactions were carried out in 5-μL volumes in an ABI PRISM Sequence Detection System 7900 (SDS; Applied Biosystems). The results were analyzed using SDS software version 1.7 (Applied Biosystems).

Power calculations

Power calculations were performed using the Genetic Power Calculator (http://statgen.iop.kcl.ac.uk/gpc/cc2.html).[36] To compute the power, we assumed that the polymorphism and the disease locus were in complete linkage disequilibrium with the same allele frequencies, ie, the polymorphism was the disease locus. The population prevalence of AAA was taken as 5%.[1] Power was computed for different values of several different model parameters including mode of inheritance, allele frequency, and genotype relative risk.[36]

Statistical analysis

The two populations (Belgians and Canadians) were tested separately to determine whether the genotypes were in Hardy-Weinberg equilibrium by comparing the observed genotype frequencies in AAA cases and controls with their expected frequencies at equilibrium based on the χ^2 test. Odds ratios (ORs) and *P* values adjusted for gender and country of origin using logistic regression and stratified by family history of AAA were calculated to test the association between genotype and AAA. Next, possible interactions between the polymorphism, country of origin, and gender were included in the model. We modified the input files for the HAPFREQS program[37] to estimate haplotype frequencies via the expectation-maximization algorithm for two X-linked polymorphisms (TIMP1 polymorphisms [nt+434 and rs2070584]). In this case, female subjects who are heterozygous and have two different alleles at both polymorphisms have ambiguous phase, whereas all homozygous female subjects and all male subjects (who have only one X chromosome and therefore only one TIMP1 allele) have known haplotypes. Haplotype frequencies were estimated separately for cases (stratified by family history of AAA) and controls, and then compared using the χ^2 test. Empirical *P* values were also obtained using a permutation test, as implemented in the CLUMP program.[38] Linkage disequilibrium (which means nonrandom segregation of polymorphisms in a population) between the two TIMP1 gene polymorphisms used in the study was estimated by computing the squared correlation coefficient (r^2).[39]

RESULTS

The observed genotype counts and their frequencies are shown in Table III, online only. All results, except TIMP3 in the Belgian cases, were in Hardy-Weinberg equilibrium. The minor allele frequencies varied from 0.12 to 0.46 in the study population (Table III, online only). The allele frequencies of both Belgian and Canadian controls in our study were remarkably similar to those reported in previous studies or public databases, suggesting that the control groups used in our study were representative of the general population (Table IV, online only).

Our power calculations indicated that in general, the power was high (>90%) for common alleles (frequency >0.2). For alleles with lower frequency, a higher genetic relative risk was required to maintain high power. The dominant model had the greatest power of the modes of inheritance that were tested (not shown).

ORs and *P* values adjusted for gender and country of origin using logistic regression were calculated (Table III, online only). This analysis was performed for all polymorphisms except TIMP1 (nt+434) and TIMP1 (rs2070584), which are X-linked, and which genotypes were

analyzed separately for each gender. None of the polymorphisms were associated significantly with risk of AAA (Table III, online only).

We stratified the AAA cases based on family history of AAA and repeated the analyses. Interestingly, the two polymorphisms located in TIMP1 were significantly associated with AAA in male cases without family history (n = 235) of AAA (nt+434, $P = .0047$; rs2070584, $P = .015$; Table V).

We then proceeded to carry out a haplotype analysis using the results of the two polymorphisms in the TIMP1 gene (Table VI). The TT haplotype was more common in the AAA cases without family history than in controls, whereas the CG haplotype was more common in controls than AAA cases without family history (TT: AAA 60% vs control, 47%; CG: AAA 37% vs control, 51%). There was a significant difference between the AAA cases without family history and controls (χ^2 $P = .014$, and empirical $P = .009$). CT and TG haplotypes were rare in both AAA cases and controls. The two polymorphisms in the TIMP1 gene were linked together tightly using r^2 as a measure of linkage disequilibrium (Belgians, $r^2 = .892$; Canadians, $r^2 = .778$; total, $r^2 = .827$).

The final analyses examined possible interactions between the polymorphisms, country of origin, and gender. We found a significant interaction between the polymorphism and gender for MMP10 ($P = .037$) in cases without a family history of AAA. For male subjects, the adjusted ORs were 1.97 and 1.40 for the GG and AG genotypes compared with the AA genotype, respectively, and for female subjects, the adjusted ORs were .377 and .614 for the GG and AG genotypes compared with the AA genotype, respectively. We also found a significant interaction between the polymorphism and country of origin for ELN ($P = .0169$) in cases with a family history of AAA. For Belgians, the adjusted ORs were .933 and .966 for the GG and AG genotypes compared with the AA genotype, respectively, and for Canadians, the adjusted ORs were 4.15 and 2.04 for the GG and AG genotypes compared with the AA genotype, respectively. Finally, there was a significant interaction between the polymorphism and country of origin for TIMP3 ($P = .0023$) in cases with a family history. For the Belgians, the adjusted ORs were 2.19 and 1.48 for the CC and CT genotypes compared with the TT genotype, respectively, and for the Canadians, the adjusted ORs were .31 and .56 for the CC and CT genotypes compared with the TT genotype, respectively.

DISCUSSION

We selected polymorphisms from genes encoding for proteins important as structural molecules of the aortic wall or involved in the process of extracellular matrix remodeling. Many of these proteins had been implicated in the pathogenesis of AAA previously based on protein and mRNA expression.[9,11–20]

Few genetic association studies between polymorphisms in MMPs and AAA have been reported.[15,16,19] Jones et al[16] found an association between AAA and MMP9 (nt−1562) polymorphism in the population of New Zealand, and we previously reported a borderline association between AAA and MMP3 (nt−1612) polymorphism.[15] Our current study was designed to replicate these previous observations, and it was therefore somewhat surprising that we did not find an association between these polymorphisms and AAA. There was no significant difference between the allele frequencies of the MMP9 polymorphism in controls in the study by Jones et al[16] and our study, and both studies had about the same number of cases and controls. One possible explanation of the differences in the results may be ethnic variations, although as pointed out in a recent review article by Colhoun et al,[40] other explanations also exist.

Our results showed a significant difference in the frequencies of both two TIMP1 polymorphisms and haplo-types between AAA cases without a family history and controls in male subjects. This observation supports the hypothesis that genetic variations responsible for down-regulation of TIMPs contribute to the pathogenesis of AAAs.[5,6,14,17] These two TIMP1 polymorphisms, which have haplotypes that were found to be associated with AAA, are unlikely to be the causative changes because one of them is at the third position of a codon and does not change the amino acid and the other lies within an intron. It is therefore likely that this haplotype is in linkage disequilibrium with other functional sequence changes that contribute to the disease.

Intriguing findings were the significant interactions between the MMP10 (nt+180) polymorphism, gender, and AAA; between the ELN (nt+422) polymorphism, country of origin, and AAA; and between the TIMP3 (nt−1296) polymorphism, country of origin, and AAA. The biological significance of these statistical interactions has yet to be defined.

Because the power of detecting an association was high, we can exclude the polymorphisms, which did not show an association with AAA in the study, as important for AAA. A limitation of our study was that we analyzed only one polymorphism in most of the genes and could have missed an association that was to a specific polymorphism not studied here. It should also be emphasized that the results obtained here require confirmation in an independent set of samples before the information can be considered definitive and useful for estimating an individual's risk for developing an AAA.

In conclusion, we investigated 14 polymorphisms in 13 biologically relevant candidate genes for AAA and found evidence for an association between TIMP1 polymorphisms and AAA in male subjects without a family history of AAA. In addition, we identified significant interactions between MMP10 (nt+180) polymorphism and gender as well as between TIMP3 (nt−1296) polymorphism or ELN (nt+422) polymorphism and country of origin and AAA. If the results are confirmed in another study, further work will be needed to explain the functional role of these variants in the pathogenesis of AAA.

Acknowledgements

We thank Dr. Jane M. Olson for her invaluable input in the design of the study before she died.

References

1. Ernst CB. Abdominal aortic aneurysm. N Engl J Med 1993;328:1167–72. [PubMed: 8455684]
2. Kochanek KD, Smith BL. Deaths: preliminary data for 2002. Natl Vital Stat Rep 2004;52:1–47. [PubMed: 14998175]
3. Majumder PP, St Jean PL, Ferrell RE, Webster MW, Steed DL. On the inheritance of abdominal aortic aneurysm. Am J Hum Genet 1991;48:164–70. [PubMed: 1985458]
4. Verloes A, Sakalihasan N, Koulischer L, Limet R. Aneurysms of the abdominal aorta: familial and genetic aspects in three hundred thirteen pedigrees. J Vasc Surg 1995;21:646–55. [PubMed: 7707569]
5. van Vlijmen-van Keulen CJ, Pals G, Rauwerda JA. Familial abdominal aortic aneurysm: a systematic review of a genetic background. Eur J Vasc Endovasc Surg 2002;24:105–16. [PubMed: 12389231]
6. Kuivaniemi H, Shibamura H. Candidate genes for abdominal aortic aneurysms. In: Liotta D, Del Rio M, Cooley D, editors. Diseases of the aorta. Buenos Aires, Argentina: Liotta Foundation Medical; 2003. p. 89–100.
7. Kuivaniemi H, Shibamura H, Arthur C, Berguer R, Cole CW, Juvonen T, et al. Familial abdominal aortic aneurysms: collection of 233 multiplex families. J Vasc Surg 2003;37:340–5. [PubMed: 12563204]
8. Shibamura H, Olson JM, van Vlijmen-van Keulen C, Buxbaum SG, Dudek DM, Tromp G, et al. Genome scan for familial abdominal aortic aneurysm using sex and family history as covariates

suggests genetic heterogeneity and identifies linkage to chromosome 19q13. Circulation 2004;109:2103–8. [PubMed: 15096456]

9. Steinmetz EF, Buckley C, Thompson RW. Prospects for the medical management of abdominal aortic aneurysms. Vasc Endovasc Surg 2003;37:151–63.
10. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 2001;17:463–516. [PubMed: 11687497]
11. Goodall S, Crowther M, Hemingway DM, Bell PR, Thompson MM. Ubiquitous elevation of matrix metalloproteinase-2 expression in the vasculature of patients with abdominal aneurysms. Circulation 2001;104:304–9. [PubMed: 11457749]
12. 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. [PubMed: 11099248]
13. Newman KM, Jean-Claude J, Li H, Scholes JV, Ogata Y, Nagase H, et al. Cellular localization of matrix metalloproteinases in the abdominal aortic aneurysm wall. J Vasc Surg 1994;20:814–20. [PubMed: 7526009]
14. Knox JB, Sukhova GK, Whittemore AD, Libby P. Evidence for altered balance between matrix metalloproteinases and their inhibitors in human aortic diseases. Circulation 1997;95:205–12. [PubMed: 8994438]
15. Yoon S, Tromp G, Vongpunsawad S, Ronkainen A, Juvonen T, Kuivaniemi H. Genetic analysis of MMP3, MMP9, and PAI-1 in Finnish patients with abdominal aortic or intracranial aneurysms. Biochem Biophys Res Commun 1999;265:563–8. [PubMed: 10558909]
16. Jones GT, Phillips VL, Harris EL, Rossaak JI, van Rij AM. Functional matrix metalloproteinase-9 polymorphism (C-1562T) associated with abdominal aortic aneurysm. J Vasc Surg 2003;38:1363–7. [PubMed: 14681642]
17. 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. [PubMed: 9288131]
18. Petersen E, Gineitis A, Wagberg F, Angquist KA. Activity of matrix metalloproteinase-2 and -9 in abdominal aortic aneurysms. Relation to size and rupture. Eur J Vasc Endovasc Surg 2000;20:457–61. [PubMed: 11112465]
19. St Jean PL, Zhang XC, Hart BK, Lamlum H, Webster MW, Steed DL, et al. Characterization of a dinucleotide repeat in the 92 kDa type IV collagenase gene (CLG4B), localization of CLG4B to chromosome 20 and the role of CLG4B in aortic aneurysmal disease. Ann Hum Genet 1995;59:17–24. [PubMed: 7762981]
20. Pyo R, Lee JK, Shipley JM, Curci JA, Mao D, Ziporin SJ, et al. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. J Clin Invest 2000;105:1641–9. [PubMed: 10841523]
21. Defawe OD, Colige A, Lambert CA, Munaut C, Delvenne P, Lapiere CM, et al. TIMP-2 and PAI-1 mRNA levels are lower in aneurysmal as compared to athero-occlusive abdominal aortas. Cardiovasc Res 2003;60:205–13. [PubMed: 14522424]
22. Yoon S, Kuivaniemi H, Gatalica Z, Olson JM, Buttice G, Ye S, et al. MMP13 promoter polymorphism is associated with atherosclerosis in the abdominal aorta of young black male subjects. Matrix Biol 2002;21:487–98. [PubMed: 12392760]
23. Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ, et al. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res 1998;58:5321–5. [PubMed: 9850057]
24. Jormsjo S, Ye S, Moritz J, Walter DH, Dimmeler S, Zeiher AM, et al. Allele-specific regulation of matrix metalloproteinase-12 gene activity is associated with coronary artery luminal dimensions in diabetic patients with manifest coronary artery disease. Circ Res 2000;86:998–1003. [PubMed: 10807873]
25. Zhang B, Ye S, Herrmann SM, Eriksson P, de Maat M, Evans A, et al. Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. Circulation 1999;99:1788–94. [PubMed: 10199873]

26. Price SJ, Greaves DR, Watkins H. Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. J Biol Chem 2001;276:7549–58. [PubMed: 11114309]
27. Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE, Henney AM. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. J Biol Chem 1996;271:13055–60. [PubMed: 8662692]
28. Brophy CM, Marks WH, Reilly JM, Tilson MD. Decreased tissue inhibitor of metalloproteinases (TIMP) in abdominal aortic aneurysm tissue: a preliminary report. J Surg Res 1991;50:653–7. [PubMed: 2051779]
29. Wang X, Tromp G, Cole CW, Verloes A, Sakalihasan N, Yoon S, et al. Analysis of coding sequences for tissue inhibitor of metalloproteinases 1 (TIMP1) and 2 (TIMP2) in patients with aneurysms. Matrix Biol 1999;18:121–4. [PubMed: 10372551]
30. Beranek M, Kankova K, Muzik J. Identification of novel common polymorphisms in the promoter region of the TIMP-3 gene in Czech population. Mol Cell Probes 2000;14:265–8. [PubMed: 10970732]
31. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, et al. Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet 1999;8:93–7. [PubMed: 9887336]
32. Tromp G, Christiano A, Goldstein N, Indik Z, Boyd C, Rosenbloom J, et al. A to G polymorphism in ELN gene. Nucleic Acids Res 1991;19:4314. [PubMed: 1871001]
33. Tromp G, Kleinert C, Kuivaniemi H, Prockop DJ. C to T polymorphism in exon 33 of the COL3A1 gene. Nucleic Acids Res 1991;19:681. [PubMed: 2011540]
34. Lederle FA, Johnson GR, Wilson SE. Abdominal aortic aneurysm in women. J Vasc Surg 2001;34:122–6. [PubMed: 11436084]
35. Kuivaniemi H, Yoon S, Shibamura H, Skunca M, Vongpunsawad S, Tromp G. Primer-extension preamplified DNA is a reliable template for genotyping. Clin Chem 2002;48:1601–4. [PubMed: 12194946]
36. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003;19:149–50. [PubMed: 12499305]
37. Goddard KA, Yu CE, Oshima J, Miki T, Nakura J, Piussan C, et al. Toward localization of the Werner syndrome gene by linkage disequilibrium and ancestral haplotyping: lessons learned from analysis of 35 chromosome 8p11.1–21.1 markers. Am J Hum Genet 1996;58:1286–302. [PubMed: 8651307]
38. Sham PC, Curtis D. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. Ann Hum Genet 1995;59:97–105. [PubMed: 7762987]
39. Devlin B, Risch N. A comparison of linkage disequilibrium measures for fine-scale mapping. Genomics 1995;29:311–22. [PubMed: 8666377]
40. Colhoun HM, McKeigue PM, Davey Smith G. Problems of reporting genetic associations with complex outcomes. Lancet 2003;361:865–72. [PubMed: 12642066]

Table I

Polymorphisms used in this study

Gene[*]	*Locus ID*[†]	*Chromosomal localization*	*Polymorphism*	*Nucleotide position*[‡]	*GenBank accession number*	*Function based on previous studies*	*Reference*
MMP1	4312	11q22.3	G/GG	nt−1607	AF023338	Different transcriptional activity	Rutter et al[23]
MMP2	4313	16q21	A/C	nt−955	U96098	Different transcriptional activity	Price et al[26]
MMP3	4314	11q22.3	5A/6A	nt−1612	J04732	Different transcriptional activity	Ye et al[27]
MMP9	4318	20q11.2–3	C/T	nt−1562	J05070	Different transcriptional activity	Zhang et al[25]
MMP10	4319	11q22.3	A/G	nt+180	X07820	Lysine to arginine	www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs = 486055
MMP12	4321	11q22.3	A/G	nt−82	U25346	Different transcriptional activity	Jormsjo et al[24]
MMP13	4322	11q22.3	A/G	nt−77	X81640	Different transcriptional activity	Yoon et al[22]
TIMP1	7076	Xp11.3–1.23	C/T	nt+434	D11139	No amino acid change	Wang et al[29]
TIMP1	7076	Xp11.3–1.23	T/C	rs2070584[§]	NT_011568	Not known	www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs = 2070584
TIMP2	7077	17q25	G/C	rs2009196[§]	NT_010641	Not known	www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs = 2009196
TIMP3	7078	22q12.1–q13.2	T/C	nt−1296	AL023282	Not known	Beranek et al[30]
TGFB1	7040	19q13.2	T/C	nt−509	X02812	Different transcriptional activity	Grainger et al[31]
ELN	2006	7q11.23	G/A	nt+422	M16983	Glycine to serine	Tromp et al[32]
COL3A1	1281	2q31	T/C	nt+581	X14420	No amino acid change	Tromp et al[33]

[*] Gene symbols used are HGNC-approved symbols obtained from www.gene.

[†] LocusID was obtained from www.ncbi.nih.gov/LocusLink.

[‡] Minus indicates promoter region; plus indicates coding region.

[§] Polymorphisms located within introns.

Table II, online only

Genotyping assays used in the study

Gene	*Polymorphism*	*Primers*	*Primer sequence*	*Annealing temperature (° C)*	*Product size (base pair)*	*Method of detection*	*Restriction enzyme*	*Fragment sizes after digestion (base pair)*
MMP1	nt−1607	MMP1–1	5-GTTATGCCACTTAGATGAGG-3	56	148/149	PAGE	N/A	N/A
		MMP1–2	5-TTCCTCCCCTTATGGATTCC-3					
MMP2*	nt−955	MMP2–7	5-TTTAGGGGCTGAAGTCAGG-3	57 (first PCR)	399	ASP	N/A	N/A
		MMP2–8	5-AAGAAGCCAGCCAAAACC-3					
		MMP2- PROM3	5-AGGAAAGGATTCAAGAGTGAGT-3					
		MMP2–31	5-ACCAGTGCCATGGCAGTT-3	60 (second A-specific PCR)	120			
		MMP2–32	5-ACCAGTGCCATGGCAGTG-3	63 (second C-specific PCR)	120			
MMP3	nt−1612	MMP3-3N	5-ACTAGTATTCTATGGTTCTCC-3	57	124/125	PAGE	N/A	N/A
		MMP3–5N	5-GCCACCACTCTGTTCTCC-3					
MMP9	nt−1562	MMP9–1562F	5-GCCTGGCACATAGTAGGCCC-3	68	435	RE	Nla III	C: 435, T: 244, 191
		MMP9–1562R	5-CTTCCTAGCCAGCCGGCATC-3					
MMP10	nt+180†	MMP10-3	5-CAACCTCGAAAAGGATGTG-3	56	170	RE	Mbo II	A: 170, G137,: 33
		MMP10-4	5-AGTGACCAACGTCAGGAAC-3					
MMP12	nt−82	MMP12-F82	5-GTCAAGGGATGATATCAGCT-3	50	137	RE	Pvu I	A: 137, G: 116, 21
		MMP12-RC82	5-CTTCTAAACGGATCAATTCAG-3					
MMP13	nt−77	MMP13-1N	5-GATACGTTCTTACAGAAGGC-3	56	445	RE	Bsr I	A: 445, G: 248, 197
		MMP13-2	5-GACAAATCATCTTCATCACC-3					
TIMP1	nt+434‡	TIMP1-01	5-TGGGGACACCAGAAGTCAAC-3	55 (first PCR)	653			
		TIMP1-02	5-TAAGCTCAGGCTGTTCCAGG-3					
		TIMP1-03	5-AGGCTGTTCCAGGGAGTCGC-3	55 (second PCR)	339	RE	Nru I	T:3 39, C: 320, 19
		TIMP1-SP5	5-CCGCCATGGAGAGTGTCTGC-3					
	rs2070584	Forward primer	5-CTATTTGCCCAGGGCTTTCTAGTTA-3	60	91	TaqMan	N/A	N/A
		Reserve primer	5-GCTGGCAAGATGTGTGAATGG-3					
		FAM probe	5-FAM-AATCACTGCCTTACTGGAA-MGB-3					
		VIC probe	5-VIC-AATCACTGCCTTACTGGAA-MGB-3					
TIMP2	rs2009196	Forward primer	5-GGCCTATTGGAAACAAGCTTTTCTG-3	60	142	TaqMan	N/A	N/A
		Reserve primer	5-TCAGGAAAGATGAGAAGAGCTGGAT-3					
		FAM probe	5-FAM-CCCCCAAACCTAAATA-MGB-3					
		VIC probe	5-VIC-CCCCCAAAGCTAAATA-MGB-3					
TIMP3	nt−1296	TIMP3–11A	5-CAAAGCAGAATCAAGATGTCAAT-3	58	488	RE	Alu I	T: 204, 128, 69, 55, 32, C: 204, 160, 69, 55
		TIMP3–11B	5-CTGGGTTAAGCAACACAAAGC-3					
TGFB1	nt−509	TGFB1.31	5-CAGACTCTAGAGACTGTCAG-3	60	265	RE	Bsu36 I	T: 265, C: 196, 69
		TGFB1.32	5-GTCACCAGAGAAAGAGGAC-3					
ELN	nt+422	ELN-29	5-GCTTTCCCGGCTTTGGTGTCG-3	59	183	RE	Bfa I	G: 183, A: 125, 58
		ELN-30	5-CCTGCAGAGCCGAGCAGACAA-3					

Gene	*Polymorphism*	*Primers*	*Primer sequence*	*Annealing temperature (° C)*	*Product size (base pair)*	*Method of detection*	*Restriction enzyme*	*Fragment sizes after digestion (base pair)*
COL3A1	nt+581	IVS32F	5-CAACACTCCTGGAAAGTAATCG-3	56	326	RE	Hae III	T: 257, 69, C: 224, 69, 33
		IVS32R	5-AGTGCAGGACTGTCCCATATG-3					

FAM, 6-Carboxyfluorescein;*VIC*, •••; *MGB*, minor groove binder; *PAGE*, polyacrylamide gel electrophoresis; *ASP*, allele-specific PCR; *N/A*, not applicable; *RE*, restriction endonuclease digestion of PCR products.

* MMP2: The first PCR was performed with primers MMP2–7 and MMP2–8. The second A-specific PCR was performed with primers MMP2-PROM3 and MMP2–31, and the second C-specific PCR was performed with primers MMP2-PROM3 and MMP2–32.

† dbSNP rs486055.

‡ TIMP1(nt+434): The first PCR was performed with primers TIMP1-01 and TIMP1-02. The second PCR was performed with primers TIMP1-03 and TIMP1-SP5.

Table III, online only

Genotype and allele counts and frequencies in cases and controls with odds ratios and *P* values for tests of association between genotype and AAA adjusted for gender and country of origin using logistic regression

		AAA				*Control*					
		Belgian		*Canadian*		*Belgian*		*Canadian*			
Gene and position of polymorphism	***Genotype/ allele*†**	**n**	***%***	**n**	***%***	**n**	***%***	**n**	***%***	**OR‡**	***P***
MMP1	GG/GG	34	19	33	16	51	19	21	14	1.11	.64
nt−1607	GG/G	87	49	113	55	140	52	85	56	1.06	
	G/G	58	32	60	29	77	29	46	30		
	Allele GG	155	43	179	43	242	45	127	42		
MMP2	A/A	13	7	38	19	37	14	31	20		.14
nt−955	A/C	78	44	91	44	115	44	67	43	1.18	
	C/C	86	49	76	37	112	42	58	37	1.40	
	Allele A	104	29	167	41	189	36	129	41		
MMP3	5A/5A	56	31	53	26	71	28	38	24		.087
nt−1612	5A/6A	87	48	109	54	132	51	74	47	0.82	
	6A/6A	37	21	41	20	54	21	44	28	0.68	
	Allele 6A	161	45	191	47	240	47	162	52		
MMP9	C/C	131	74	140	72	204	76	107	74		.57
nt−1562	C/T	43	24	49	25	61	23	35	24	1.10	
	T/T	3	2	5	3	4	1	3	2	1.20	
	Allele T	49	14	59	15	69	13	41	14		
MMP10	A/A	3	2	3	1	11	4	2	1		.81
nt+180	A/G	49	27	59	29	74	28	38	24	0.96	
	G/G	128	71	143	70	184	68	117	75	0.93	
	Allele A	55	15	65	16	96	18	42	13		
MMP12	A/A	136	78	161	79	204	77	121	78		.77
nt−82	A/G	35	20	38	19	60	23	33	21	0.95	
	G/G	3	2	5	2	1	0	2	1	0.91	
	Allele G	41	12	48	12	62	12	37	12		
MMP13	A/A	92	52	94	47	136	51	80	52		.99
nt−77	A/G	69	39	81	41	103	39	58	37	0.99	
	G/G	16	9	25	13	26	10	17	11	0.99	
	Allele G	101	29	131	33	155	29	92	30		
TIMP1 male	T	83	53	91	60	86	48	12	38		.063
nt+434*	C	74	47	61	40	92	52	20	63	0.70	
TIMP1 female	T/T	6	33	18	36	29	34	42	34	0.96	.91
nt+434*	C/T	9	50	21	42	38	45	60	49	0.98	
	C/C	3	17	11	22	18	21	21	17		
	Allele T	21	58	57	57	96	56	144	59		
TIMP1 male	T	84	54	92	61	89	49	14	44		.116
rs2070584*	G	72	46	59	39	91	51	18	56	0.97	
TIMP1 female	T/T	7	37	20	39	30	35	42	34	1.03	.93
rs2070584*	G/T	9	47	19	37	39	42	60	49	1.02	
	G/G	3	16	12	24	17	20	21	17		
	Allele T	23	61	59	58	99	58	144	59		
TIMP2	C/C	6	3	16	8	13	5	18	12	1.18	.53
rs2009196	C/G	62	35	86	43	73	27	61	39	1.08	
	G/G	108	61	100	50	183	68	77	49		
	Allele C	74	21	118	29	99	18	97	31		
TIMP3	T/T	87	49	108	53	140	53	66	42		.95
nt−1296	C/T	61	34	74	36	102	38	67	43	0.99	
	C/C	29	16	21	10	24	9	23	15	0.99	
	Allele C	119	34	116	29	150	28	112	36		
TGFB1	T/T	16	9	19	9	26	10	16	10		.66
nt−509	C/T	84	47	93	45	115	43	70	45	0.95	
	C/C	79	44	93	45	127	47	71	45	0.90	
	Allele T	116	32	131	32	167	31	102	32		
ELN	A/A	40	23	35	17	41	15	31	20		.62
nt+422	A/G	77	44	87	42	118	44	85	54	1.05	
	G/G	60	34	83	40	107	40	40	26	1.11	
	Allele A	157	44	157	38	200	38	147	47		
COL3A1	T/T	83	46	105	51	125	47	84	54		.22
nt+581	C/T	80	45	88	43	122	46	63	40	1.17	
	C/C	16	9	12	6	19	7	9	6	1.36	
	Allele C	112	31	112	27	160	30	81	26		

AAA, Abdominal aortic aneurysm; *OR*, odds ratio.

[*] Because the TIMP1 gene is located on the X chromosome, genotyping results were analyzed separately for male and female subjects and were adjusted for country of origin only.

[†] The genotype counts and frequencies of the minor alleles are shown.

[‡] The reference group is the homozygous genotype for which OR is not listed.

Table IV, online only

Comparison of minor allele frequencies between controls in the current study and other studies

Gene	*Minor allele*	*Belgian controls (n = 269)*	*Canadian controls (n = 156)*	*Other studies*			
				Frequency	*N*	*CI*	*Reference*
MMP1	GG	0.45	0.42	0.50	100	0.43–0.57	Rutter et al [23]
MMP2	A	0.36	0.41	0.40	32	0.26–0.54	Price et al [26]
MMP3	6A	0.47	0.52	0.49	266	0.45–0.53	Ye et al [28]
MMP9	T	0.13	0.14	0.18	192	0.14–0.22	Zhang et al [25]
MMP10	A	0.18	0.13	0.04	36	0.01–0.12	www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs = 486055
MMP12	G	0.12	0.12	0.16	367	0.13–0.19	Jormsjo et al [24]
MMP13	G	0.29	0.30	0.30	987	0.28–0.32	Yoon et al [22]
TIMP1 male	T	0.49	0.31	0.48	29	0.35–0.62	Wang et al [29]
nt +434 female	T	0.45	0.43	0.27	22	0.15–0.43	Wang et al [29]
TIMP1 male	T	0.50	0.45	0.48*	1186*	0.46–0.50*	www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs = 2070584
rs2070584 female	T	0.45	0.43				
TIMP2	C	0.18	0.31	0.27	304	0.24–0.31	www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs = 2009196
TIMP3	C	0.28	0.36	0.39	95	0.32–0.46	Beranek et al [30]
TGFB1	T	0.31	0.32	0.33	246	0.29–0.37	Grainger et al [31]
ELN	A	0.38	0.47	0.42	64	0.34–0.51	Tromp et al [32]
COL3A1	C	0.30	0.26	0.29	50	0.20–0.39	Tromp et al [33]

CI, 95% confidence interval.

*For TIMP1 rs 2070284 polymorphism, the minor allele frequency, number of individuals studied, and 95% CI included both male and female subjects.

Table V

Odds ratios and *P* values for tests of association between genotype and AAA adjusted for gender and country of origin using logistic regression, and stratified by family history of AAA

Gene and position of polymorphism	*Genotype*	*With family history*(n = *152*)		*Without family history*(n = *235*)	
		OR[†]	P	*OR*[†]	P
MMP1	GG/GG	1.06	.85	0.82	.48
nt−1607	GG/G	1.03		0.91	
MMP2	A/C	1.14	.37	1.15	.29
nt−955	C/C	1.30		1.32	
MMP3	5A/6A	0.71	.025	0.90	.43
nt−1612	6A/6A	0.50		0.82	
MMP9	C/T	1.27	.25	0.97	.89
nt−1562	T/T	1.61		0.94	
MMP10	A/G	0.79	.22	1.15	.43
nt+180	G/G	0.62		1.32	
MMP12	A/G	0.89	.61	0.95	.82
nt−82	G/G	0.79		0.91	
MMP13	A/G	0.89	.48	1.06	.64
nt−77	G/G	0.80		1.13	
TIMP1 male nt+434[*]	C	1.00	.99	0.55	.0047
TIMP1 female nt+434[*]	C/T	1.26	.37	0.73	.25
	T/T	1.59		0.54	
TIMP1 male rs2070584[*]	G	1.03	.90	0.59	.015
TIMP1 female rs2070584[*]	G/T	1.30	.31	0.77	.32
	T/T	1.69		0.59	
TIMP2	C/G	1.03	.86	1.14	.36
rs2009196	C/C	1.06		1.31	
TIMP3	C/T	0.94	.68	1.01	.97
nt−1296	C/C	0.88		1.01	
TGFB1	C/T	0.92	.60	0.95	.68
nt−509	C/C	0.84		0.89	
ELN	A/G	1.38	.030	0.89	.34
nt+422	G/G	1.91		0.79	
COL3A1	C/T	1.35	.066	1.07	.63
nt+581	C/C	1.83		1.15	

AAA, Abdominal aortic aneurysm; *OR*, odds ratio.

[*] Because the TIMP1 gene is located on the X chromosome, genotyping results were analyzed separately for male and female subjects and were adjusted for country of origin only. There were 115 male subjects with a family history of AAA, 201 male subjects without a family history of AAA, 37 female subjects with a family history of AAA, and 34 female subjects without a family history of AAA.

[†] Reference group is the homozygous genotype for which OR is not listed.

Table VI

Haplotypes for the TIMP1 gene in male subjects

Haplotype		AAA						Control	
		With family history*		Without family history†		Total‡			
nt+434	rs2070584	n	%	n	%	n	%	n	%
T	T	48	44	118	60	166	54	97	47
C	T	5	5	4	2	9	3	4	2
T	G	3	3	2	1	5	2	0	0
C	G	53	49	72	37	125	41	107	51

AAA, Abdominal aortic aneurysm.

*χ^2 P = .016 and empirical P = .053 for comparison between AAA cases with family history and controls.

†χ^2 P = .014 and empirical P = .0089 for comparison between AAA cases without family history and controls.

‡χ^2 P = .039 and empirical P = .036 for comparison between all AAA cases and controls.