Polymorphisms of genes involved in extracellular matrix remodeling and abdominal aortic aneurysm

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Background: **Abdominal aortic aneurysm (AAA) has a multifactorial etiology and the relevance of genetic factors is getting increasing interest, in particular those related to the destructive remodeling of extracellular matrix.**

Methods: **We performed a candidate gene association study of polymorphisms in genes coding matrix metalloproteinases (MMPs), tissue inhibitors of MMPs (TIMPs), and elastin (ELN) in AAA. DNA samples from 423 AAA patients and 423 controls were genotyped for 12 polymorphisms in 10 genes: MMP1 (−1607G/GG), MMP2 (−735C/T; −1306C/T; −1575 G/A), MMP3 (5A/6A), MMP9 (−1562C/T), MMP10 (A180G), MMP-12 (−82A/G), MMP-13 (−77A/G), TIMP1 (C434T), TIMP3 (−1296T/C), and ELN (G1355A).**

Results: **Genotype distribution was significantly different between patients and controls for the following polymorphisms: −1306C/T MMP2; 5A/6A MMP3; −77A/G MMP-13; G1355A ELN; and C434T TIMP1. In a multivariable logistic regression analysis adjusted for traditional cardiovascular risk factors and chronic obstructive pulmonary disease, −1306C/T MMP2 (odds ratios [OR] = 0.55 [95% confidence interval, CI .34-.85], P < .007) and G1355A ELN (OR = 0.64 ([95% CI .41-.99], *P* = .046) polymorphisms resulted in independent protective factors for abdominal aortic aneurysm (AAA), whereas 5A/6A MMP3 (OR = 1.82 [95% CI 1.04-3.12], *P* = .034) and −77 A/G MMP-13 (OR = 2.14 [95% CI 1.18-3.86], *P* = .012) polymorphisms resulted in independent risk factors for AAA. In a multivariable logistic regression analysis adjusted for traditional cardiovascular factors and chronic obstructive pulmonary disease, the prevalence of the contemporary presence of three or four genetic risk conditions was a strong and independent determinant of AAA disease (OR = 2.96, 95% CI 1.67-5.24, *P* < .0001). For those polymorphisms independently associated with AAA in this study (−1306C/T MMP2, 5A/6A MMP3, −77A/G MMP-13, and G1355A ELN polymorphisms), we performed a meta-analysis of the available data (this paper and literature data). We found a significant association with an increased risk of AAA for MMP3 (AAA patients n = 1258, controls n = 1406: OR = 1.48 [95% CI = 1.23-1.78], I^2 = 0%) and MMP-13 (AAA patients n = 800, controls n = 843: OR = 1.37 [95% CI = 1.04-1.82], I^2 = 25%) polymorphisms and a trend that did not reach the statistical significance, toward a decreased risk of AAA for MMP2 (AAA patients n = 1090, controls n = 1077: OR = 0.83 [95% CI = .60-1.15], I^2 =7 1%) and ELN (AAA patients n = 904, controls n = 1069: OR = 0.79 [95% CI = .53-1.18], I^2 = 72%) polymorphisms.**

Conclusions: **These findings suggest that polymorphisms in MMP2, MMP3, MMP-13, and ELN genes may independently contribute to the pathogenesis of AAA. (J Vasc Surg 2012;55:171-9.)**

Clinical Relevance: **This study identifies polymorphisms in MMP2, MMP3, MMP-13, TIMP1, and ELN genes as genetic markers of abdominal aortic aneurysm (AAA) and underline the need to concentrate our efforts in studying the role of these markers in the aneurysmal disease to improve the understanding of its pathophysiology and pathogenesis. This study is part of the task for the identification of AAA genetic susceptibility factors, fundamental to design and develop gene-based clinical studies in the future to validate diagnostic or prognostic scores based on clinical, biochemical, genetic, and proteomic information to be applied in the everyday clinical practice.**

Abdominal aortic aneurysm (AAA) represents a severe chronic degenerative condition associated with atherosclerosis and characterized by segmental weakening and dilation of the aortic wall. AAA occurs in up to 9% of humans >65-years-old. It is estimated to be the tenth commonest cause of mortality and is responsible for >2% of all death.[1]

The pathogenesis of this complex disorder is the result of the interactions among multiple genes end environmental factors, but it has not been completely clarified yet.

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A key mechanism in the pathogenesis and progression of AAA is the proteolytic degradation of the aortic wall; both increased elastase and collagenase activity have been found in aortic aneurysms, and both have been positively correlated with diffuse alterations of extracellular matrix (ECM) composition and aneurysm size.[2,3] Several studies reported an increased expression and activity of matrix metalloproteinases (MMPs) in aortic aneurysm tissues and an imbalance between MMPs and their inhibitors (TIMPs) that may alter the equilibrium towards aortic wall matrix degradation.[4]

In knockout mice models of AAA, the absence of MMP2 and MMP9 is associated with a lower incidence of AAA.[5] Previous studies have found that different MMPs (MMP1, MMP2, MMP3, MMP9, and MMP-12) are expressed at elevated levels in aortic aneurysm compared with normal vessel wall.[3] On the other hand, decreased levels of TIMPs have been reported in aortic aneurysms.[6] In experimental models, inhibitors of MMPs, MMPs gene disruption,[7] or the overexpression of TIMPs may block aneurysm development.[8] Moreover, an ECM composition perturbation, due to structural protein alteration, could weaken the aortic vessel wall, and, consequently, determine a major tendency to aneurysmal disease.

Family history is a well-known risk factor for AAA. However, it remains unclear which genetic factors influence the formation of AAA in individuals who do not have genetic syndromes or a familial AAA.[9,10]

Altered levels of MMPs and TIMPs may be due to differences in the genetic sequences coding these enzymes. Genetic variations can influence the transcription of these genes or the function of the coded proteins. Indeed, functional studies have shown that many of the promoter variants in MMP genes determine differential binding of transcriptional factors.[11]

Several candidate genes (MMP3, MMP9, TIMP1, and TIMP2) have been investigated in different ethnic groups to identify a possible association between genetic variants and the sporadic AAA phenotype often in small cohorts of patients and with discordant results.[10-13]

As previous studies carried out in AAA cohorts of patients provided no definitive results on the association of different polymorphisms in different genes involved in ECM remodeling with AAA, the aim of our study was to evaluate the genetic susceptibility to AAA conferred by 12 polymorphisms in 10 genes coding enzymes involved in the ECM remodeling (MMP1, MMP2, MMP3, MMP9, MMP10, MMP-12, MMP-13, TIMP1, TIMP3, and elastin [ELN]).

METHODS

Study population. We enrolled 423 consecutive patients with AAA referred to the Vascular Surgery Unit of the University of Florence. Familial and inflammatory AAAs were excluded from the study. Familial genetic patterns might influence the predictive value of the gene polymorphisms that are included in this study. In several studies, some differences were reported between AAA patients with or without a first degree relative with a history of AAA, ie, higher prevalence of male and higher mean age in nonfamilial.[14] All controls had a negative personal and familial history of AAA. Patients and control subjects included in this study were previously investigated for polymorphisms involved in the methionine metabolism.[15] Definitions of the studied populations were detailed in a previous article.[15] At the duplex scanning examination, few controls (10/423) showed aortic diameters between 3 and 4.70 mm. We did not exclude these subjects as this situation could be more representative of the general control population, even if it could mitigate the significance of the associations. Therefore, we compared patients with AAA referred to the Vascular Surgery Unit of the University of Florence for surgery with the control subjects.

Patients and controls gave informed consent and the study was approved by the Ethical Committee.

DNA extraction. Genomic DNA was isolated from venous peripheral blood by using FlexiGene kit (Qiagen, Hilden, Germany).

Genotyping. We studied 12 polymorphisms in 10 candidate genes involved in ECM remodeling according to their demonstrated or putative function based on literature data and localization in functional gene regions, extracted from Single Nucleotide Polymorphism database (dbSNP) NCBI (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp&cmd=search&term=). Selection of the polymorphisms was also performed to better clarify contrasting data available in literature on the role of polymorphisms in MMPs, TIMPs, and ELN in AAA onset and progression.

Polymorphisms information were assessed in dbSNP NCBI and ENSEMBL (http://www.ensembl.org/index.html) databases. In Table I, information on the 12 selected polymorphisms are reported.

MMP1 (rs1799750), MMP2 (rs2285053, rs243865, rs243866), MMP10 (rs486055), MMP-12 (rs2276109), MMP-13 (rs2252070), TIMP1 (rs4898), TIMP3 (rs9619311), and ELN (rs2071307) polymorphisms were analyzed by using GenomeLab SNPstream genotyping platform and software (Beckman Coulter, Fullerton, Calif).[16] In Table A (online only; supplementary data), the sequences of polymerase chain reaction (PCR) and extension oligonucleotides are reported.

MMP3 (rs3025058) and MMP9 (rs3918242) polymorphisms were genotyped by Nanogene electronic microchip technology.[17] In Table B (online only; supplementary data), the sequences for PCR and hybridization oligonucleotides are reported.

Data analysis. Statistical analysis was performed using the SPSS package v11.5 (SPSS, Inc, Chicago, Ill). Hardy–Weinberg equilibrium (HWE) was evaluated by χ^2 test. HWE is commonly used for quality control of genotyping: it indicates inbreeding, population stratification, and systematic genotyping errors in unrelated individuals. Genotype distributions were compared between AAA and control groups or between patients with and without other atherosclerotic localizations by χ^2 analysis. We assessed the association between each polymorphism and AAA using different genetic models. The dominant model compares indi-

Table I. Polymorphisms investigated

Gene symbol and chromosome position	*Ensemble gene ID*	*dbSNP ID*	*Common polymorphism name*	*Position in gene region*
MMP1 11q22-q23	ENSG00000196611	rs1799750	−1607G/GG	5′ near gene
MMP2 16q13-q21	ENSG00000087245	rs2285053 rs243865 rs243866	−735C/T −1306C/T −1575G/A	5′ near gene 5′ near gene 5′ near gene
MMP3 11q22.3	ENSG00000149968	rs3025058	5A/6A	5′ near gene
MMP9 20q12-q13	ENSG00000100985	rs3918242	−1562C/T	5′ near gene
MMP10 11q22.3	ENSG00000166670	rs486055	A180G Lys53Arg	exon 2
MMP12 11q22.2-11q22.3	ENSG00000110347	rs2276109	−82A/G	5′ near gene
MMP13 11q22.3	ENSG00000137745	rs2252070	−77A/G	5′ near gene
TIMP1 Xp11.3-p11.23	ENSG00000102265	rs4898	C434T Phe124Phe	exon 5
TIMP3 22q12.1-q13.2	ENSG00000100234	rs9619311	−1296T/C	5′ near gene
ELN 7q11.1-q21.1	ENSG00000049540	rs2071307	G1355A Ser422Gly	exon 20

ID, Identification number.

viduals with one or two rare alleles (heterozygotes+ homozygotes) with the group of homozygous subjects. The recessive model compares individuals with two rare alleles (homozygotes) with the combined group of heterozygous+ wild-type homozygous subjects. The additive model assumes that there is a linear gradient in risk between the three genotypes. Categorical variables are expressed as frequencies and percentages. Unless otherwise indicated, data are given as median and range. Post-hoc sample size calculations indicated that a number of 423 AAA patients and 423 controls have a statistical power (β) to detect a significant difference in percentages of genotypes of 6% for MMP1 (−1607G/GG), 21%, 73%, and 36% for −735C/T, −1306C/T, −1575 G/A MMP2, respectively, 84% for MMP3 (5A/6A), 7% for MMP9 (−1562C/T), 5% for MMP10 (A180G), 12% for MMP-12 (−82A/G), 78% for MMP-13 (−77A/G), 96% for TIMP1 (C434T), 42% for TIMP3 (−1296T/C), and 74% for ELN (G1355A), with an $\alpha = .05$. Comparisons of continuous variables (age, aortic diameter) between patients and controls or among genotypes were performed by the nonparametric Mann–Whitney or Kruskal–Wallis test. Multivariable logistic regression analysis was used to estimate OR and 95% confidence intervals (CI) for the risk of AAA. To evaluate if polymorphisms were independently associated with AAA, multivariable analyses were adjusted for traditional risk factors for AAA: age, gender, hypertension, diabetes mellitus, dyslipidemia, smoking habit, and chronic obstructive pulmonary disease (COPD). The association between polymorphisms, which were significantly associated with AAA and aortic diameter, was estimated by general linear model. To reduce type I error, we applied the false discovery rate (FDR) multiple testing correction in all statistical analyses. A value of $P < .05$ was chosen as the cut-off level for statistical significance.

For meta-analyses, we pooled results from the individual studies by using Review Manager (RevMan) software for Macintosh (v5.0) by the Cochrane Collaboration and Statistical Package for Social Sciences (SPSS) software for Windows (v13.0). The results of each study were reported as dichotomous frequency data. We used a random-effects model that accounts for interstudy variation and provides a more conservative effect than the fixed model. Thus, we calculated random-summary OR with 95% CI, by using inverse-variance method. The potential sources of heterogeneity were assessed by using the Cochrane's Q test to assess between-study differences and the I^2 statistic to quantify the proportion of inconsistency across the study results.

RESULTS

Subjects. Demographic and clinical characteristics of investigated subjects are reported in Table II. As expected, based on selection criteria, no differences in gender and age between AAA patients and controls were observed; statistically significant differences were found in cardiovascular risk factors such as smoking habit, hypertension, dyslipidemia, and COPD, as well as in the prevalence of coronary artery, and cerebrovascular and peripheral obstructive artery disease (Table II).

Single nucleotide polymorphism (SNP) analysis. In Table III genotype distributions of the 12 polymorphisms and χ^2 analysis by different genetic models are reported.

Table II. Demographic and clinical characteristics of AAA patients and controls[15]

	Controls (n = 423)	*AAA patients (n = 423)*	P
Age	72.0 (41-94)	73.5 (40-94)	.651
Sex (male) N (%)	366 (86.5)	376 (88.9)	.295
Smoking N (%)	267 (63.1)	366 (86.5)	<.0001
Diabetes N (%)	49 (11.6)	41 (9.7)	.372
Hypertension N (%)	179 (42.3)	302 (71.4)	<.0001
Dyslipidemia N (%)	50 (11.8)	195 (46.1)	<.0001
COPD N (%)	66 (15.6)	311 (73.5)	<.0001
CAD N (%)	107 (25.3)	163 (38.5)	<.0001
CVD N (%)	38 (9.0)	111 (26.2)	<.0001
POAD N (%)	67 (15.8)	118 (27.9)	<.0001
Aortic diameter (mm)	19 (12-47) SEM 0.48	50 (31-98) SEM 0.58	<.0001

AAA, Abdominal aortic aneurysm; *CAD,* coronary artery disease; *COPD,* chronic obstructive pulmonary disease; *CVD,* cerebrovascular disease; *POAD,* peripheral obstructive artery disease; *SEM,* standard error mean.

The polymorphisms' genotype distributions were in HWE in AAA patients and controls.

Genotype distribution was significantly different between patients and controls for the following polymorphisms: rs243865 MMP2, rs3025058 MMP3, rs2252070 MMP-13, rs4898 TIMP1, and rs2071307 ELN.

Carriers of the T allele of the −1306C/T (rs243865) MMP2, of the A allele of the Ser422Gly (rs2071307) ELN, and of the C allele of the T434C (rs4898) TIMP1 polymorphism had a decreased risk of AAA (Table III). The status of carriers of the 5A allele of the 5A/6A (rs3025058) MMP3 polymorphism, as well as the presence of the G allele at the homozygous status for the −77A/G (rs2252070) MMP-13 polymorphism represented risk factors for AAA (Table III).

In a multivariable logistic regression analysis with AAA as dependent variable and age, gender, hypertension, diabetes mellitus, dyslipidemia, smoking habit, COPD and the single polymorphisms as independent variables (Table IV), rs243865 *MMP2* (OR = 0.55 [95% CI .34-.85], $P < .007$) and rs2071307 *ELN* (OR = 0.64 [95% CI .41-.99], $P = .046$) gene polymorphisms were independent protective factors for AAA, whereas rs3025058 *MMP3* (OR = 1.82 [95% CI 1.04-3.12], $P = .034$) and rs2252070 *MMP-13* (OR = 2.14 [95% CI 1.18-3.86], $P = .012$) gene polymorphisms were independent risk factors for AAA. Similar results were also observed in the comparison of AAA patients and controls, both free from other localization of atherosclerosis (coronary artery disease [CAD], cerebrovascular disease [CVD], and peripheral obstructive artery disease [POAD]) (data not shown).

No statistical differences in the genotype distribution of rs243865 MMP2, rs3025058 MMP3, rs2252070 MMP-13, rs4898 TIMP1, and rs2071307 ELN gene polymorphisms were observed between AAA patients with or without other manifestations of atherosclerosis by χ^2 (Table V). We obtained similar results also in a multivariable logistic regression analysis adjusted for the traditional cardiovascular risk factors and COPD.

Concerning the effect of the polymorphisms on aortic diameters, none of the polymorphisms influenced the aortic diameters in patients and controls (data not shown).

Meta-analysis of polymorphisms independently associated with AAA in this study. For those polymorphisms independently associated with AAA in this study (−1306C/T MMP2, 5A/6A MMP3, −77A/G MMP-13, and Ser422Gly ELN polymorphisms), we performed a meta-analysis of the available data (Table VI). We found a significant association with an increased risk of AAA for MMP3 (AAA patients n = 1258, controls n = 1406: OR = 1.48 [95% CI = 1.23-1.78], $I^2 = 0\%$) and MMP-13 (AAA patients n = 800, controls n = 843: OR = 1.37 [95% CI = 1.04-1.82], $I^2 = 25\%$) polymorphisms and a trend that did not reach the statistical significance toward a decreased risk of AAA for MMP2 (AAA patients n = 1090, controls n = 1077: OR = 0.83 [95% CI = .60-1.15], $I^2 = 71\%$) and ELN (AAA patients n = 904, controls n = 1069: OR = 0.79 [95% CI = .53-1.18], $I^2 = 72\%$) polymorphisms.

Combination of genetic risk conditions. To evaluate the effect of the combination of the different genetic risk conditions, we evaluated the association with AAA of the number of genetic conditions present for each subject. Genetic risk conditions were considered: CC genotype for −1306C/T MMP2 polymorphism, 5A allele for the 5A/6A MMP3 polymorphism, GG genotypes for the −77A/G MMP-13 polymorphism, and GG genotypes for the Ser422Gly ELN polymorphism. The prevalence of carriers of three or four genetic risk conditions was significantly higher in AAA patients (125/423, 29.6%) than in controls (83/423, 19.6%), $P < .0001$. In a logistic regression analysis adjusted for traditional cardiovascular risk factors and COPD, the prevalence of the contemporary presence of three or four genetic risk conditions was a strong and independent determinant of AAA disease (OR = 2.96, 95% CI 1.67-5.24, $P < .0001$).

DISCUSSION

The results of the present study suggest that polymorphisms in genes coding for important extracellular matrix components, MMP2, MMP3, MMP-13, and ELN are independently associated with AAA, extending and highlighting the role of these polymorphisms per se and, for the first time, in combination in the pathogenesis of the aneurysmal disease.

To favor the comparison with previous studies, we summarized results obtained in this article and literature data in Table VI.

In our study, the presence of the T allele of the polymorphism −1306C/T in the promoter of the MMP2 gene was associated to a significant and independent protective role for AAA. MMP2, also known as type IV collagenase or gelatinase (72-kDa), is an enzyme that specifically cleaves type IV collagen, the major structural component of basement membranes. Animal models and human aortic wall studies have suggested a role for MMP2 in the pathogenesis

Table III. Genotype distribution and allele frequency of the 12 investigated polymorphisms in AAA patients and control subjects

SNP	*Genotypes (%)* *Patients (n = 423)*			*Minor allele frequency*	*Genotypes (%)* *Control subjects (n = 423)*			*Minor allele frequency*	P
MMP1 rs1799750	AA(26.0)	AG(49.1)	GG(24.9)	*G* = .495	AA(24.8)	AG(51.3)	GG(23.8)	*G* = .495	1
MMP2 rs2285053	CC(71.2)	CT(27.2)	TT(1.6)	*T* = .152	CC(74.6)	CT(23.1)	TT(2.2)	*T* = .138	.524[c]
rs243865	CC(64.9)	CT(31.0)	TT(4.1)	*T* = .196	CC(56.3)	CT(37.6)	TT(6.1)	*T* = .249	.023[c]
rs243866	GG(67.2)	GA(29.5)	AA(3.3)	A = 0.180	GG(61.8)	GA(33.4)	AA(4.7)	A = 0.215	.296[c]
MMP3 rs3025058	5A/5A (26.5)	5A/6A (54.7)	6A/6A (18.8)	6A = 0.462	5A/5A (21.9)	5A/6A (50.8)	6A/6A (27.3)	6A = 0.572	.013[b]
MMP9 rs3918242	GG(73.9)	GA(23.1)	AA(3.0)	A = 0.146	GG(72.5)	GA(23.9)	AA(3.6)	A = 0.156	.159[a]
MMP10 rs486055	CC(95.4)	CT(4.3)	TT(0.3)	*T* = .024	CC(94.9)	CT(4.8)	TT(0.3)	*T* = .027	1
MMP-12 rs2276109	AA(78.1)	AG(19.1)	GG(2.9)	*G* = .124	AA(76.9)	AG(21.1)	GG(1.9)	*G* = .125	.719[b]
MMP-13 rs2252070	AA(34.8)	AG(43.9)	GG(21.4)	*G* = .433	AA(40.0)	AG(45.9)	GG(14.1)	*G* = .371	.023[b]
TIMP1 rs4898	TT(100.0)	CT(0.0)	CC(0.0)	*C* = .000	TT(96.8)	CT(3.2)	CC(0.0)	*C* = .016	<.0001[a]
TIMP3 rs9619311	TT(41.9)	CT(44.4)	CC(13.7)	*C* = .359	TT(46.1)	CT(44.1)	CC(9.8)	*C* = .318	.264[b]
ELN rs2071307	GG(39.9)	GA(43.8)	AA(16.3)	A = 0.382	GG(31.3)	GA(51.8)	AA(16.9)	A = 0.428	.022[c]

AAA, Abdominal aortic aneurysm; *SNP,* single nucleotide polymorphism.
P values were adjusted by using the false discovery rate (FDR) multiple-testing correction.
[a]*P* = according to the additive model.
[b]*P* = according to the recessive model.
[c]*P* = according to the dominant model

Table IV. Odds ratios for the occurrence of AAA according to rs243865 MMP2, rs3025058 MMP3, rs2252070 MMP13, and rs2071307 ELN polymorphisms

Variables	*Univariate analysis*	P	*Multivariate analysis*[a]	P
MMP2 rs243865 T allele	0.7 (0.52-0.93)	.013	0.55 (0.34-0.85)	.007
MMP3 rs3025058 5A allele	1.61 (1.13-2.33)	.007	1.82 (1.04-3.12)	.034
MMP13 rs2252070 GG genotype	1.65 (1.11-2.46)	.013	2.14 (1.18-3.86)	.012
ELN rs2071307 A allele	0.69 (0.51-0.92)	.012	0.64 (0.41-0.99)	.046

AAA, Abdominal aortic aneurysm; *COPD,* chronic obstructive pulmonary disease.
[a]Adjusted for age, gender, hypertension, diabetes mellitus, dyslipidemia, smoking habit, and COPD.

of AAAs.[18] The −1306 C/T polymorphism of the MMP2 affects the binding of estrogen receptor and the SP1 transcription factor, and it has an allele-specific effect on the activity of the promoter in driving gene expression.[19,20] A previous article investigated the role of this polymorphism in AAA incidence, but no significant association between this genetic variant and AAA was observed.[21] The discordant result might be due, at least in part, to the deviation from the HWE in the control subjects and the lower incidence of previous CAD and CVD in a previous article.[21] Moreover, in our study, patients had markedly larger AAA diameters with respect to the previously published papers[21] (52.4 ± 10.5 mm vs 35.7 ± 6.8 mm).

MMP3 or stromelysin-1 is capable of degrading proteoglycan, fibronectin, laminin, and type IV collagen. A large number of polymorphisms have been reported in this

Table V. Genotype distribution of the five polymorphisms associated with AAA in patients with and without other atherosclerotic localizations

SNP	*Genotypes (%)* *AAA patients without other atherosclerotic localizations (n = 118)*		*Genotypes (%)* *AAA patients with other atherosclerotic localizations (n = 305)*		P
MMP2 rs243865	CC (64.4)	CT + TT (35.6)	CC (65.7)	CT + TT (34.3)	.821[c]
MMP3 rs3025058	5A/5A+5A/6A (79.6)	6A/6A (20.4)	5A/5A+5A/6A (81.6)	6A/6A (18.4)	.641[b]
MMP-13 rs2252070	AA (82.9)	AG + GG (17.1)	AA (76.3)	AG + GG (23.7)	.137[b]
TIMP1 rs4898	TT (100.0)	CT + CC (0.0)	TT (100.0)	CT + CC (0.0)	—
ELN rs2071307	GG (38.3)	GA + AA (61.7)	GG (41.3)	GA + AA (58.7)	.550[c]

AAA, Abdominal aortic aneurysm; *SNP,* single nucleotide polymorphism.
[a]*P* = according to the additive model.
[b]*P* = according to the recessive model.
[c]*P* = according to the dominant model.

gene, but the 5A/6A polymorphism in the promoter region has been extensively investigated. The 5A/6A polymorphism is located within the interleukin-1 responsive element. This sequence variant arises from the insertion of an adenine nucleotide at position −1612 relative to the start of transcription, resulting in the wild-type allele having a run of five adenine nucleotide (5A) and the less common allele having six adenine nucleotides (6A). In in vitro experiments, the 5A allelic promoter had a greater activity in driving gene expression than the 6A allelic promoter.[22] Studies of the levels of MMP3 mRNA and protein, in ex vivo tissues, including vascular tissues from individuals of different genotypes for the 5A/6A polymorphism, showed that the levels were highest in 5A homozygotes, intermediate in heterozygotes and lowest in 6A homozygotes.[23] Our study shows the importance of the risk factor of the 5A/6A polymorphism in the pathogenesis of AAA, probably due to its effect on the gene expression level.[22] Previous studies showed that the 5A allele occurred more frequently in AAA patients,[24] or in AAA patients with family history of AAA.[11] A higher frequency of the 5A allele in AAA patients was also found in one small Finnish study although it failed to achieve statistical significance after correction for multiple testing.[25]

In the literature, only one study has investigated the role of the polymorphism −77A/G in the promoter region of MMP-13 in conferring genetic susceptibility to AAA, but failed to demonstrate any association.[11] Our data, on the contrary, demonstrated that this polymorphisms is significantly and independently associated with AAA. A possible explanation for this discrepancy might be due to the inclusion in the previous study[11] of AAA patients with family history of AAA or ethnic variations. On the other hand, previous studies demonstrated that MMP-13 is expressed at elevated levels in human aortic tissue.[26] MMP13 or collagenase 3 has a limited tissue distribution and a highly regulated pattern of expression. MMP-13 is active against types I and III collagens as MMP1 and MMP-8, but it is also capable of degrading the type-II collagen predominating in cartilage. MMP-13 is further distinguished from other interstitial collagenases by its activity against gelatin, proteoglycans, tenascin, fibronectin, and type IV collagen, as well as its capacity to activate latent pro-MMPs. This broad substrate specificity makes MMP-13 one of the most potent of the matrix-degrading metalloenzymes characterized to date. Our data are in keeping with previous studies demonstrating that MMP-13 is consistently expressed in affected human aortic tissue and that MMP-13 mRNA is expressed to a greater degree in AAA compared with atherosclerotic aorta.[26] The −77G/A polymorphism is associated with an altered transcriptional activity: the constructs with A nucleotide has approximately twice as much transcriptional activity as the constructs with G nucleotide in the same position.[27] The observed association could be attributed not only to the presence of reduced level of MMP-13 per se, determined by the GG genotype, but to a possible alteration of the balance between different MMPs.

Our data have not shown a role in AAA pathogenesis of the −1562 C/T promoter polymorphism in the MMP9. Our result is consistent with several literature data.[11,25,28]

TIMP1 regulates the activity of MMPs, preferentially inhibiting their activity. Our data demonstrated that the C434T polymorphism in TIMP1 is protective for AAA. The C434T is a same-sense polymorphism (Phe124Phe); the significant association with AAA might be due to the presence of the causal polymorphism in linkage disequilibrium. Two studies evaluated the role of polymorphisms in this gene in the AAA[11,13]; one small study did not find

Table VI. Polymorphisms investigated in relation to AAA incidence

SNP ID	*AAA vs control subjects (N)*	*Association with AAA*	*Reference*
MMP1	387/425	No	Ogata et al[11]
rs1799750			
(−1607G/GG)	423/423	No	Saracini et al[a]
MMP2	678/659	No	Smallwood et al[21]
rs243865			
(−1306C/T)	423/423	Yes	Saracini et al[a]
rs2285053			
(−735C/T)	423/423	No	Saracini et al[a]
rs243866			
(−1575G/A)	423/423	No	Saracini et al[a]
MMP3	405/405	Yes	Deguara et al[24]
rs3025058			
(−1612 5A/6A)	387/425	No Yes in subgroup analysis	Ogata et al[11]
	47/174	Yes (trend)	Yoon et al[25]
	423/423	Yes	Saracini et al[a]
MMP9	387/425	No	Ogata et al[11]
rs3918242			
(−1562C/T)	414/203	Yes	Jones et al[35]
	47/174	No	Yoon et al[25]
	676/649	No	Smallwood et al[28]
	423/423	No	Saracini et al[a]
MMP10	387/425	No	Ogata et al[11]
rs486055			
(A180G-Lys53Arg)	423/423	No	Saracini et al[a]
MMP12	387/425	No	Ogata et al[11]
rs2276109			
(−82A/G)	423/423	No	Saracini et al[a]
MMP13	387/425	No	Ogata et al[11]
rs2252070			
(−77A/G)	423/423	Yes	Saracini et al[a]
TIMP1	84/51	No	Wang et al[13]
rs4898			
(C434T-Phe124Phe)	387/425	No Yes in subgroup analysis	Ogata et al[11]
	423/423	Yes	Saracini et al[a]
TIMP3	387/425	No	Ogata et al[11]
rs9619311			
(−1296T/C)	423/423	No	Saracini et al[a]
ELN	387/425	No	Ogata et al[11]
rs2071307		Yes in subgroup analysis	
(G1355A-Ser422Gly)	99/225	No	Massart et al[36]
	423/423	Yes	Saracini et al[a]

AAA, Abdominal aortic aneurysm; *SNP*, single nucleotide polymorphism.
[a]Refers to this article.

association,[13] the other study found a significant association in the subgroup of patients without family history of AAA.[11]

ELN is one of the major determinants of arterial distensibility of large blood vessels and one of the principal components of elastic fibers of the media of arteries. Several polymorphisms of the ELN gene have been described. Tromp and coworkers identified an A-to-G polymorphism in exon 16 resulting in an amino acid substitution (Ser422Gly).[29] Ogata and coworkers observed that this polymorphism in ELN was associated with AAA, in particular in subjects with family history of disease.[11] Our data extend this result showing that the A allele is a significant and independent protector factor for AAA.

For polymorphisms independently associated with AAA in this study, we performed a meta-analysis. Even with limitations due to the absence or not complete information of some published studies (eg, confounding variables), we found a significant association with an increased risk of AAA for MMP3 and MMP-13 polymorphisms and a trend towards a decreased risk of AAA for −1306 C/T MMP2 and ELN polymorphisms.

Our study did not show any association of the polymorphisms with aortic diameters. Our data are consistent with

previous data investigating the association of −1306 C/T MMP-2, −1171 5A/6A MMP3, −1562 C/T MMP-9, and −82 A/G MMP-12 polymorphisms with AAA expansion.[21,30] Nevertheless, one of the limitations of our study is its inadequacy to evaluate the influence of the investigated polymorphisms on the progression of the disease, as our study was conducted on patients admitted to the observation of Vascular Surgery Unit for repair of the AAA.

Due to the presence of high prevalence of traditional cardiovascular risk factors and other clinical manifestations of atherosclerosis in AAA patients (CAD, CVD, and POAD), our data, at present, cannot definitely indicate whether the identified polymorphisms represent genetic markers of atherosclerosis or AAA development. On the other hand, AAA is strongly associated with atherosclerosis.[31] Even if our study was not aimed to investigate subgroup differences among AAA patients, in our population, no differences in the distribution of polymorphisms associated with AAA were observed between patients with or without other atherosclerotic localizations. Interestingly, patients with AAA plus other clinical manifestations of atherosclerosis were older and showed higher prevalence of different cardiovascular risk factors, in particular hypertension and COPD. Contrasting data and not for all the polymorphisms investigated in this study are available in the literature concerning association with other atherosclerotic diseases. Concerning 5A/6A MMP3, although data from some individual studies suggested a relationship with CAD, the result of a recent meta-analysis argued against an association of this polymorphism with atherosclerotic coronary diseases.[32] To better comprehend whether these polymorphisms represent markers of atherosclerosis or AAA, further studies in "pure" CAD or CVD patients comparable for gender and age to AAA patients are warranted.

In conclusion, present data have identified important polymorphisms in MMP2, MMP3, MMP-13, and ELN genes as genetic markers of AAA and underline the need to concentrate our efforts in studying the role of these markers in the aneurysmal disease to improve the understanding of its pathophysiology and pathogenesis.

For each gene, there are further SNPs that could be considered to better identify the susceptibility alleles. Due to the fundamental clinical implications, further studies on larger populations are needed (1) to confirm the role of the polymorphisms emerged associated with AAA in this study, (2) to evaluate the role of other polymorphisms in the same genes allowing a finer identification of the susceptibility alleles, and (3) to study other candidate genes involved in ECM remodeling as predictor of AAA disease. The polymorphisms that resulted susceptibility factor for AAA in this study could be part of a future panel, involving other important genetic variants, such as those identified in recent genome wide association studies for AAA in chromosome 9p21 and 9q33.[33,34] The identification of genetic susceptibility factors and the evaluation of their role per se or in combination is fundamental to design gene-based clinical studies in the future to validate diagnostic or prognostic scores based on clinical, biochemical, genetic, and proteomic information to be applied in the everyday clinical practice.

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AUTHOR CONTRIBUTIONS

Conception and design: BG, RA, GFG, CP
Analysis and interpretation: CS, RA, RP, BG
Data collection: GP, RP, PB, ES
Writing the article: CS, BG
Critical revision of the article: RA, RP, BG
Final approval of the article: CS, PB, ES, GP, RP, FS, CP, GG, RA, BG
Statistical analysis: BG, CS, FS
Obtained funding: RA
Overall responsibility: BG

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Table A, online only. Oligonucleotide designs for PCR and primer extension reactions[a]

SNP ID	*Panel*	*Oligo type*	*Sequence*
MMP1			
rs1799750	GA	PCRU	ATTCTTCTTACCCTCTTGAACTCA
		PCRL	TGATATCTTACTCATAAACAATACTTCAGTA
		SNPU	CGTGCCGCTCGTGATAGAATAAATTGTAGTTAAATAATTAGAAAG
MMP2			
rs2285053	CT	PCRU	TTTGTGACCTCTATCKTATTAAACCA
		PCRL	TGGGTAAAATGAGGCTGAGA
		SNPU	GGCTATGATTCGCAATGCTTTCATCCTGTGACCGAGAATGCGGAC
rs243865	CT	PCRU	CCATTGTCAATGTTCCCTAAA
		PCRL	TGACTTCAGCCCCTAAACTAGTAA
		SNPU	AGGGTCTCTACGCTGACGATTCCCCATATTCCCCACCCAGCACTC
rs243866	GA	PCRU	ACAAGCCTGAACTTGTCTGAA
		PCRL	AACAGTTTGAGAAGTAAGGTCAGG
		SNPU	AGAGCGAGTGACGCATACTATAGCTGTGATGATCAAGACATAATC
MMP10			
rs486055	GA	PCRU	ATTAGCAATACCTAGAAAAGTACTACAACC
		PCRL	CAACCCAAGGAACTTCTGC
		SNPU	GACCTGGGTGTCGATACCTAGAAAAGGATGTGAAACAGTTTAGAA
MMP-12			
rs2276109	GA	PCRU	TAATTGATCCATTGTCGTCTGA
		PCRL	TAAGTTCCTGAACTGTTCCTCTTTAT
		SNPU	AGCGATCTGCGAGACCGTATGATAGATCAAGGGATGATATCAACT
MMP-13			
rs2252070	GA	PCRU	TTTATATTTCCCTCAAATTCTACCAC
		PCRL	ATTACCTTTACTTTTATAGGCCTGC
		SNPU	CGACTGTAGGTGCGTAACTCTAAGCATGTTTACCTTCAAGTGACT
TIMP1			
rs4898	CT	PCRU	TTTCTCCTTAGGAAAACTGCAG
		PCRL	TAGGTCTTGGTGAAGCCC
		SNPU	AGATAGAGTCGATGCCAGCTTCTTGCACATCACTACCTGCAGTTT
TIMP3			
rs96193114	CT	PCRU	AGTTTTGGATCAGCTCACCC
		PCRL	GTAGAAAGGCAAGAGGAAGTGG
		SNPU	AGAGCGAGTGACGCATACTACAAATCCCTTGCTGAAGGGTGRAGC
ELN			
rs17855987	GA	PCRU	TTTGGTGTCGGAGTCGGA
		PCRL	GGAAATGCCAACTCCCGG
		SNPU	GGATGGCGTTCCGTCCTATTTATCCCTGGAGTCGCAGGTGTCCCT

PCR, Polymerase chain reaction; *PCRU*, oligonucleotide forward; *PCRL*, oligonucleotide reverse; *SNPU*, oligonucleotide primer extension.
[a]All oligonucleotide sequences are in 5′–3′ direction.

Table B, online only. PCR and hybridization oligonucleotide for electronic microchip analysis

SNP ID	*Type of oligonucleotides*	*Sequences*	*Ta (°C)*	*Tb (°C)*
MMP3	Forward PCR	5′-gattacagacatgggtcacg-3′	61	
rs3025058	Reverse PCR	5′-*biotin*-gaattcacatcactgccacc-3′		
	Stabilizer	5′-atgtcttgtcctgattgaaatacagggaaaatatttggcc-3′		48
	Reporter WT	5′-*Cy3*-ctgagtccgaacattgagtttgggggggaaaaacc-3′		
	Reporter MUT	5′-*Cy5*-gcagtatatcgcttgacaccgggggggaaaaaacc-3′		
MMP9	Forward PCR	5′-*biotin*-gcctggcacatagtaggccc-3′	66	
rs3918242	Reverse PCR	5′-cttcctagccagccggcatc-3′		
	Stabilizer	5′-tgcgccaccacgcctggctaaatttttgtatt-3′		38
	Reporter WT	5′-*Cy3*-ctgagtccgaacattgagaaggtattataggcg-3′		
	Reporter MUT	5′-*Cy5*-gcagtatatcgcttgacaaatggtattataggca-3′		

PCR, Polymerase chain reaction; *Ta*, annealing temperature; *Tb*, optimal temperature for thermal stringency.