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Original
Multiple sites of vascular dilation or aneurysmal disease and matrix metalloproteinase genetic variants in patients with abdominal aortic aneurysm / Fiotti, N., Calvagna, C., Sgorlon, G., Altamura, N., Pitacco, P., Zamolo, F., Di Girolamo, F.G., Chiarandini, S., Biolo, G., Adovasio, R.. - In: JOURNAL OF VASCULAR SURGERY. - ISSN 0741-5214. - STAMPA. - 67:6(2018), pp. 1727-1735. [10.1016/j.jvs.2017.09.047]

Availability:
This version is available at: 11368/2918450 since: 2018-03-07T11:45:22Z
Publisher:

Published
DOI:10.1016/j.jvs.2017.09.047
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06 October 2026

arTs Università degli Studi di Trieste
Archivio della ricerca – postprint

Multiple sites of vascular dilation or aneurysmal disease and matrix metalloproteinase genetic variants in patients with abdominal aortic aneurysm

Nicola Fiotti, MD,[a] Cristiano Calvagna, MD,[b] Giada Sgorlon, MD,[b] Nicola Altamura, MD,[a] Paola Pitacco,[a] Francesca Zamolo, MD,[b] Filippo Giorgio Di Girolamo, PharmD, PhD,[a] Stefano Chiarandini, MD,[b] Gianni Biolo, MD, PhD,[a] *and* Roberto Adovasio, MD,[b] *Trieste, Italy*

ABSTRACT

Objective: The objective of this study was to assess whether functional genetic polymorphisms of matrix metalloproteinases (MMPs) 1, 3, 9, and 12 are associated with arterial enlargements or aneurysms of the thoracic aorta or popliteal arteries in patients with abdominal aortic aneurysm (AAA).

Methods: The associations between *MMP1* (−1607 G in/del, rs1799750), *MMP3* (−1171 A in/del rs35068180), *MMP9* (13-26 CA repeats around −90, rs2234681, rs917576, rs917577), and *MMP12* (C/T missense variation, rs652438) polymorphisms and enlargements or aneurysms of the thoracic aorta and popliteal arteries were tested in 169 consecutive AAA patients.

Results: Thoracic aorta enlargement or aneurysm (TE/A; maximum diameter, >35 mm) was detected in 34 patients (20.1% prevalence). *MMP9* rs2234681 microsatellite was the only genetic determinant of TE/A in AAA patients ($P = .003$), followed by hypercholesterolemia and antiplatelet use. Carriers of both alleles with ≥22 CA repeats had a 5.9 (95% confidence interval, 1.9-18.6; $P < .0001$) increased odds of TE/A, and a score considering all three variables showed 98% negative predictive value and 30% positive predictive value for thoracic aortic aneurysm detection. Eighty-two popliteal artery enlargements or aneurysms (diameter >10 mm) occurred in 55 patients (33.1% prevalence). Carriers of *MMP12* rs652438 C allele showed an 18% ($P = .006$) increased diameter in popliteal arteries and a 2.8 (95% confidence interval, 1.3-6; $P = .008$) increased odds of popliteal artery enlargement or aneurysm compared with TT genotype.

Conclusions: Among patients with AAA, carriers of homozygous ≥22 CA repeats in *MMP9* rs12234681 and of C allele in *MMP12* rs652438 have a substantial risk of carrying thoracic and popliteal enlargements, respectively. (J Vasc Surg 2018;67:1727-35.)

Abdominal aortic aneurysm (AAA) is a degenerative vascular dilation of the infrarenal aorta occurring in 5% of men older than 60 years, probably caused by a combination of risk factors (hypertension, smoking habits, atherosclerosis, and others) and poorly defined genetic conditions. All genetic variants recognized so far account for around 40% of AAA occurrence variability,[1] although heritability inferred from family studies is around 70%.[2] Beyond the exact weight of inheritance on the occurrence of AAA, profound differences in vascular gene expression pattern can be identified in AAA patients compared with controls,[3,4] both within and outside AAA tissue. Such a pattern might induce abnormal reactions to environmental stimuli, ultimately leading to altered remodeling in virtually the whole vascular tree.

The crucial mechanism of aneurysm development in animal models is the protease-mediated degradation of the extracellular matrix (ECM). Some members of the matrix metalloproteinase (MMP) family, a class of around 26 neutral endoproteases able to regulate turnover of all ECM components, are alleged to influence aneurysmal growth.[5]

In humans, histopathologic studies that show a high MMP concentration within the AAA tissue corroborate but do not prove the causal role of some MMPs in the development of aneurysms in real life. Functional genetic polymorphisms, the main genetic features able to induce differential MMP expression, should then be excellent candidates to prove the association between AAA and MMPs. Even with undisputable laboratory and ex vivo evidence, identification of MMP polymorphisms accounting for increased susceptibility has been, so far, disappointing.[6,7] A possible explanation for such a discrepancy is that all experimental models of aneurysm induction require a mandatory chemical or mechanical trigger that first modifies expression of genes and transcription factors[8] and then the MMP expression and matrix degradation. If so, genetic and environmental triggers of aneurysms induce MMP expression, and their functional genetic variants (polymorphisms) might not induce the aneurysm formation but, likely, determine

From the Unit of Clinica Medica,[a] and Unit of Vascular Surgery,[b] Department of Medical, Surgical and Health Sciences, University of Trieste.

The study was funded by Medtronic Italia, which was not involved in the study design, data collection, data interpretation, article preparation, or decision to submit the manuscript.

Author conflict of interest: none.

Correspondence: Nicola Fiotti, MD, Clinica Medica, Ospedale di Cattinara, Strada di Fiume 447, Cattinara, Trieste 34149, Italy (e-mail: fiotti@units.it).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

features (size, speed of growth) of an established AAA and, possibly, positive vascular remodeling in other different sites, ultimately leading to vascular enlargements or aneurysm formation.

This study addresses the hypothesis that in patients with AAA, functional genetic polymorphisms of MMPs previously identified in AAA tissue,[9-15] inducing positive vascular remodeling, increase the prevalence of enlargements or aneurysms in thoracic aorta or popliteal arteries.

To support the interpretation of the results and to rule out spurious association with occurrence of atherosclerosis in AAA patients, genetic and allelic frequencies of MMP polymorphisms were compared with those obtained from an age- and sex-matched population composed of patients with coronary or cerebrovascular disease from a previously published study.[16] Such a comparison would precisely identify the association between genetic variants and features of aneurysms, overcoming the eventual association with atherosclerosis.

ARTICLE HIGHLIGHTS

- **Type of Research:** Single-center retrospective case-control study
- **Take Home Message:** In 169 patients with an abdominal aortic aneurysm, thoracic aortic aneurysm or enlargement was found in 20% and popliteal artery aneurysm or enlargement in 33%, and these changes were associated with carriers of homozygous ≥22 CA repeats in *MMP9* rs2234681 and of C allele in *MMP12* rs652438.
- **Recommendation:** This study suggests that in patients with an abdominal aortic aneurysm, carriers of specific *MMP9* and *MMP12* genes have an increased risk of thoracic and popliteal artery aneurysms or enlargements.

METHODS

Study population

The aims of the study were explained to all consecutive patients with documented AAA admitted to the vascular surgery unit of the University of Trieste Teaching Hospital or attending there as outpatients for preoperative or postoperative intervention follow-up between July 15, 2010, and June 15, 2014. All patients willing to participate were asked to undergo a physical examination and a noninvasive peripheral vascular assessment, to provide a blood sample for genotyping, and to give a written informed consent. Past medical history (risk factors, demographics, clinical conditions, drug therapy) and all vascular assessments were obtained from medical records. Medical personnel reviewed the records unaware of the genetic status of the patients. Clinical conditions are defined under Statistical Analysis.

Control population

Age- and sex-matched control subjects with carotid or coronary atherosclerosis were selected among participants of an already published study[16] (Clinical Trial registration NCT00484406). Matching was carried out in a 1:1 mode from the database according to the privacy regulations, selecting patients with ±3-year difference in age and allowing a maximum sex difference of 5% between the two populations. Clinical and genetic data were then extracted, completed, or modified to match the actual definitions of risk factors and embedded in the database together with AAA patients. Patients in this last group were not screened for AAA at the time of enrollment. All patients in both groups were white individuals. All diagnostic and therapeutic procedures followed institutional guidelines, and the study did not interfere with patients' treatment. The institutional ethical committee approved the study; it complies with the Helsinki Declaration.

Vascular measurements

Abdominal aorta. In all patients, diagnosis of AAA was made when the maximum diameter at diagnosis was ≥35 mm on computed tomography angiography (CTA) scans.

Thoracic aorta. Occurrence of thoracic aorta enlargement or aneurysm (TE/A) was initially screened through a standard chest radiograph and subsequently confirmed (or ruled out) by CTA scan. The evaluation of the diameter of the descending thoracic aorta was conducted with dedicated software (3mensio Vascular; Pie Medical, Bilthoven, The Netherlands). The diameter was measured at the level of the maximal dilation of the aorta. Enlargement was considered when the diameter of any segment of the thoracic aorta was between 35 and 44 mm, and aneurysms were diagnosed when the size was ≥45 mm. The ratio between maximal extension of the TE/A in the aorta tract and its maximal diameter measured from CTA scans could provide more details on the shape of the TE/A. This was collected and compared with the MMP polymorphisms. Abdominal and thoracic aorta CTA imaging for all patients was obtained at the radiology unit of the Teaching Hospital of Cattinara, Italy.

Popliteal arteries. Popliteal artery diameters of all patients were obtained with the Acuson S1000 ultrasound device (Siemens Healthcare, Hoffman Estates, Ill) during either diagnosis or follow-up examinations at the vascular surgery unit of the Teaching Hospital of Cattinara by two expert vascular surgeons (C.C. and G.S.) within the time window of observation. Popliteal artery enlargement or aneurysm (PE/A) was defined when any popliteal artery tract was ≥10 mm and subdivided into enlargement (between 10 and 14 mm) and aneurysm (≥15 mm). If the patient's lower limb was amputated or underwent popliteal aneurysm repair, the

Table I. Allelic frequency and position of polymorphisms analyzed in the study[a]

Gene	rs number	Allelic frequency, %	Position
MMP1	rs1799750	G−: 43.7 G+*: 56.3	chr11:102799765-102799765
MMP3	rs35068180	5A*: 21.2 6A: 78.8	chr11:102845217-102845216
MMP9	rs2234681	<22 CA: 67.9 ≥22 CA*: 32.1	chr20:46008773-46008774
MMP9	rs17576	A: 60.5 G*: 39.5	chr20:46011586-46011586
MMP9	rs17577	G: 78.9 A*: 21.1	chr20:46014472-46014472
MMP12	rs652438	T: 92.5 C*: 7.5	chr11:102865911-102865911

MMP, Matrix metalloproteinase.
[a]According to http://genome.ucsc.edu/index.html. CA repeats in rs2234681 have been grouped, and the G allele in rs652438 (allelic frequency of 0.0026%) has been neglected. The *asterisks* indicate alleles presumably at risk.

preintervention maximal popliteal diameter was sought in the patient's medical records.

DNA extraction, polymorphism analysis, and sequencing

DNA was extracted from a venous blood sample with a suitable extraction kit on a Maxwell 16 system (Promega Italia, Milan, Italy). For *MMP1*, *MMP3*, and *MMP9* rs2234681, in/del or variable number of tandem repeat variants, the forward primers were fluoresceinated, and the amplicon size (informative of genotype) was assessed through capillary electrophoresis according to the methods already published.[15] For *MMP9* rs917576 and rs917577 and *MMP12* rs652438, which are substitution variants, commercial kits (TaqMan technology, assays C_11655953_10, C_11655948_1_, and C_785907_10; Life Technologies, Carlsbad, Calif) assessed real-time polymerase chain reaction (Bio-Rad, Hercules, Calif) according to the manufacturer's instructions. Details on investigated single-nucleotide polymorphisms are reported in Table I.

Genetic material was handled according to the Italian guidelines for treatment of genetic material (General Authorization No. 8/2013 for the Processing of Genetic Data, from the *Garante per la protezione dei dati personali*; http://www.garanteprivacy.it/web/guest/home/docweb/-/docweb-display/docweb/2818993).

Statistical analysis

Categorical or continuous variables are reported by the number of observations and prevalence or median and interquartile range (IQR) values, respectively. The relatively small number of observations and the asymmetric data distribution called for nonparametric statistics; therefore the association between genotype and vessel or aneurysm diameter has been investigated with the Mann-Whitney or Kruskal-Wallis test.

Allelic and genetic frequencies of the genetic variants between patients and controls were compared with χ^2 test.

Multivariate logistic regression (forward conditional method) was carried out to determine the clinical and genetic variables associated with the occurrence of TE/A or PE/A within AAA patients. Independent clinical variables for regression analyses were age, sex, number of affected arteries (abdominal, thoracic aorta, popliteal arteries), smoking habit (never, former, current smoker of more than three cigarettes/day), hypertension (systolic and diastolic pressure >140/85 mm Hg on more than one measurement), hypercholesterolemia (total cholesterol concentration >200 mg/dL or high-density lipoprotein cholesterol concentration <40 mg/dL in women or <35 mg/dL in men), diabetes (fasting blood glucose concentration >116 mg/dL), weight, height, body mass index, coronary heart disease, peripheral arterial obstructive disease, use of antiplatelet or anticoagulant agents, antihypertensive drugs, and lipid-lowering drugs. Genetic variables were *MMP1* rs1799750, *MMP3* rs35068180, *MMP9* rs2234681, *MMP9* rs17576, *MMP9* rs17577, and *MMP12* rs652438 polymorphisms. For *MMP9* rs2234681 (variable number of tandem repeats polymorphism), the repeats have been grouped according to a cutoff between 21 and 22 per allele and analyzed as a categorical variable <22/<22, <22/≥22, and ≥22/≥22.[16] The B values of the three independent variables identified by multivariate logistic regression together with the constant value were used to calculate the equation value for each patient according to the general rule (logit = constant + β1X1 + β 2X2 +…+ β iXi). Such a value was then dichotomized and used to infer the risk of TE/A or PE/A.

The result of the regression equation was tested on a receiver operating characteristic curve with occurrence of TE/A as a categorical variable and dichotomized according to the Youden method. According to this approach, positive vs negative occurrence of the enlargement was predicted by selecting the value (cut point) that maximized the distance of the curve from the chance (45-degree) line on the receiver operating characteristic curve. The odds ratios and 95% confidence interval (CI) for association between aneurysm occurrence and all dichotomous variables were calculated. For PE/A, we analyzed the association in two ways, as legs nested within patients and as legs independent.

In designing the study, we assumed, for the most restrictive comparison (TE/A and MMP genotypes), a prevalence of one of four patients carrying TE/A (ratio, 0.33) and a difference in prevalence of genotypes at risk of around 20%. These data determined that around 140 patients were required for an α level of .05 and a power of 80%. In post hoc re-evaluation, the actual power achieved was 77%. Therefore, our study has a reasonable power to detect the observed differences. A two-tailed *P* value <.05 or 95% CI not encompassing the 1.0 value

was considered statistically significant. The whole statistical analysis was conducted with SPSS 21.0 (Statistical Package for Social Sciences; IBM Corp, Armonk, NY).

RESULTS

There were 169 patients who were studied and genotyped. Compared with the control population, only allelic frequency of 5A in *MMP3* rs35068180 polymorphism and >22 CA repeats in rs2234681 were higher in AAA patients. TEs/As were detected in 34 patients, whereas PEs/As were identified in 82 legs of 55 patients. Eight legs were amputated, and their diameters were either not available or not reliable; these limbs have been excluded from the analysis. Thirteen patients had enlargements or aneurysms in all three considered areas. The main clinical characteristics and MMP genotyping of the study group and the matched control group (ie, 169 patients with coronary or cerebrovascular disease) are reported in Table II. No differences could be found in the main demographic, clinical, and pharmacologic characteristics of the patients according to their genetic profile, including smoking habit and hypertension (*P* values for χ^2 test ranging from 0.197 to 0.276). Comparisons of the patients according to the sites of the enlargements or aneurysms also did not show any difference, except for a marginal increase in the prevalence of antiplatelet prescription in patients with more than one dilation (χ^2 test, $P = .045$).

TEs/As. TEs/As were detected in 34 cases (4 of them were type IV aneurysms, 20% prevalence). In multivariate logistic regression, hypercholesterolemia (negatively), antiplatelet use, and *MMP9* rs2234681 microsatellite (positively) accounted for the occurrence of thoracic aneurysms (Table III). Compared with <22/<22 repeats, odds ratios for occurrence of TE/A were 1.6 (95% CI, 0.6-4; $P = .333$) and 7.3 (95% CI, 2.0-26.1; $P = .001$) for heterozygous and ≥22/≥22 patients, respectively, and 2.1 (95% CI, 0.87-5.1; $P = .093$) for the dominant model. The area under the receiver operating characteristic curve (C statistics) from the multivariate logistic regression equation in Table III was 0.759 (95% CI, 0.667-0.851; $P = .00002$), 0.660 (95% CI, 0.547-0.773; $P = .007$) for *MMP9* polymorphism rs2234681 (≥22/≥22), 0.607 (95% CI, 0.503-0.710; $P = .074$) for antiplatelet use, and 0.393 (95% CI, 0.277-0.510; $P = .075$) for hypercholesterolemia (Fig 1). A cutoff value of 2.65 obtained from the multivariate logistic regression was set according to the Youden index to discriminate TE/A-likely from non-TE/A-likely patients. Patients above such a cutoff value had a 24.6 (95% CI, 3.2-186; $P < .0001$) increased odds of having a thoracic aneurysm. Such a cutoff value had 96.5% sensitivity, 46.4% specificity, 29.79% positive predictive value, 98.31 negative predictive value, 1.8 positive likelihood ratio, 0.07 negative likelihood ratio, and 55.3% overall accuracy.

The ratio maximal extension/maximal diameter of the enlargement did not differ significantly according to the *MMP9* rs2234681 polymorphism. Nonetheless, <22/<22 displayed a 50% increase in diameter (1.4 [0.8-2.4] vs 2.2 [1.5-2.8; $P = .275$]), although nonsignificant.

Popliteal arteries. The popliteal artery diameter median value was 8.1 mm (IQR, 6.9-9.8 mm). On multivariate logistic regression analysis, the occurrence of popliteal aneurysm in an AAA patient was correlated to *MMP12* rs652438 and sex (Table III) and to *MMP12* rs652438, sex, and anticoagulant use considering each leg independently (Table III). The popliteal diameter was 8.1 mm (IQR, 6.9-9.8 mm) in *MMP12* TT genotype and 9.55 mm (IQR, 7.6-11.2 mm) in TC genotype (+18%; $P = .006$); it was 8.3 mm (IQR, 7-10 mm) in men (n = 268 arteries) and 6.9 mm (IQR, 6.1-8.1 mm; $P = .008$) in women (n = 30 arteries); and it was 9.35 mm (IQR, 7.5-10.6 mm) in anticoagulated patients vs 8 mm (IQR, 6.9-9.5 mm; $P = .035$) in nonanticoagulated patients. Table IV reports the prevalence of PE/A according to these variables. Fig 2 describes the popliteal artery diameters according to the occurrence of male sex, *MMP12* rs652438 C variant, and being anticoagulated at observation. The number of legs with diameter >1 cm was around 0%, 25%, 50%, and 100% according to the occurrence of none, one, two, or three of these conditions, respectively. Multiple logistic regression considering as dependent variables all popliteal diameters available provided similar results (B value, 0.259 [$P < .001$] for *MMP12* rs652438 C carrier; 0.171 [$P = .001$] for male sex; 0.199 [$P < .001$] for anticoagulant use; −0.053 [$P = .045$] for *MMP9* rs17577).

DISCUSSION

The main result of our study is that in patients with documented AAA, occurrence of vascular enlargements or aneurysms in the thoracic aorta and popliteal arteries is associated with genetic variants of *MMP9* and *MMP12*, respectively.

A recent review of Bradley et al[1] reported that of 263 genes investigated for genetic susceptibility for AAA, polymorphisms of 87 genes proved to be associated with AAA, but only 8 were supported by meta-analyses, *MMP3* polymorphism being among them.

Even if it was not the aim of our study, we can confirm higher prevalence of *MMP3* 5A variant in AAA patients compared with patients with symptomatic coronary or carotid atherosclerosis. The 5A allele does not bind an inhibitor, and the consequent increased expression adds to the association with increased ECM breakdown and positive vascular remodeling.

Arterial gene expression profile[3] and hemodynamic pattern[17] differ between AAA patients and controls even when examined outside the AAA segment. This suggests that beyond the site of the aneurysm, the whole vascular tree could be prone to vascular enlargements.

Table II. Demographic characteristics and genetic profile of abdominal aortic aneurysm (*AAA*) patients and the control group

	Controls	AAA patients	*P*
General			
No	169	169	
Age at observation, years	75 (67-80)	74 (69-78)	.549
Male/female	155/14	152/18	
Type of intervention	N/A	54/112/3	
Body mass index, kg/m^2	26 (24-29)	26 (23-28)	.669
Coronary heart disease	111/58	46/123	<.001
Peripheral arterial obstructive disease	41/128	39/130	.798
Risk factors			
Smoking habit	40/88/41	11/103/55	<.001
Hypertension	129/40	146/23	.018
Hypercholesterolemia	100/69	106/63	.504
Diabetes	40/129	30/139	.18
Drugs			
Antiplatelet	160/9	129/40	.045
Antihypertensive	122/47	136/33	.073
Lipid lowering	66/103	84/85	.05
Genetic profile			
MMP1 rs1799750			
G−/G−	40 (23.7)	41 (24.2)	.512
G−/G+	97 (57.4)	88 (52.1)	
G+/G+	32 (18.9)	40 (23.7)	
MMP3 rs35068180			
5A/5A	56 (33.1)	52 (30.8)	.019[a,b]
5A/6A	70 (41.4)	92 (54.4)	
6A/6A	43 (25.4)	25 (14.8)	
MMP9 rs2234681			
<22/<22	85 (50.3)	68 (40.2)	.02[c,d]
<22/≥22	61 (36.1)	86 (50.9)	
≥22/≥22	23 (13.6)	15 (8.9)	
MMP9 rs17576			
A/A	57 (33.7)	75 (44.4)	.092
A/G	88 (52.1)	69 (40.8)	
G/G	24 (14.2)	25 (14.8)	
MMP9 rs17577			
GG	125 (74)	111 (65.7)	.072
GA	40 (23.7)	46 (27.2)	
AA	4 (2.3)	12 (7.1)	
MMP12 rs652438			
TT	146 (86.4)	152 (90.0)	.414
TC	22 (13.0)	17 (10.0)	
CC	1 (0.6)	0 (0.0)	

MMP, Matrix metalloproteinase; *N/A*, not applicable.
Unless otherwise specified, the figures are presence/absence of the specific condition and, in parentheses, the percentage; type of intervention: open repair/endovascular/in follow-up; smoking habit: never a smoker/former smoker/current smoker.
[a] $P < .015$ in χ^2 test in 5A dominant mode between patients and controls.
[b] $P < .016$ homozygous vs heterozygous patients and controls.
[c] $P = .063$ ≥22 CA repeats dominant model.
[d] $P = .017$ homozygous vs heterozygous patients and controls.

Table III. Multivariate logistic regression and univariate analysis for thoracic aorta and popliteal artery aneurysm in abdominal aortic aneurysm (AAA) patients

	Multivariate				Univariate		
	B	ES	*P*	Exp (B)	Odds ratio	95% CI	*P*
TE/A							
MMP9 rs2234681	1.291	.394	.001	3.636	5.0	1.6-15.5	.003
Hypercholesterolemia	−1.044	.478	.029	.352	0.44	0.21-0.95	.035
Antiplatelet use	1.472	.779	.059	4.356	3.9	1.1-13.5	.023
Constant	−4.554	1.034	.000	.011			
PE/A, legs							
MMP12 rs652438	1.371	.409	.001	3.939	3.1	1.4-6.6	.002
Sex	−1.427	.646	.027	0.24	3.48	1.08-11.8	.033
Antiplatelet use	0.870	.387	.024	2.387	2.11	1.03-4.36	.039
Constant	−0.187	.687	.785	1.206			
PE/A, legs nested within patient							
MMP12 rs652438	1.219	.566	.031	3.385	2.74	0.96-7.81	.052
Sex	−1.521	.809	.060	0.218	3.94	0.86-17.8	.059
Constant	0.833	.857	.331	2.300			

CI, Confidence interval; *ES*, effect size; *MMP*, matrix metalloproteinase; *PE/A*, popliteal artery enlargement or aneurysm; *TE/A*, thoracic aorta enlargement or aneurysm.
Independent variables are reported in the Methods section. Interpretation: *MMP9* rs223468: ≤21/≤21 = 1, ≤21/≥22 = 2, ≥22/≥22 = 3. For univariate analysis, homozygous long microsatellite (≥22/≥22) carriers were compared with the other genotypes; the figures for the dominant model were 2.1 (0.87-5.1; *P* = .093). *MMP12* rs652438: 0 = TT, 1 = CT genotype. Sex: 1 = man, 2 = woman. Hypercholesterolemia: 0 = no, 1 = yes. Antiplatelet: 0 = no, 1 = yes.

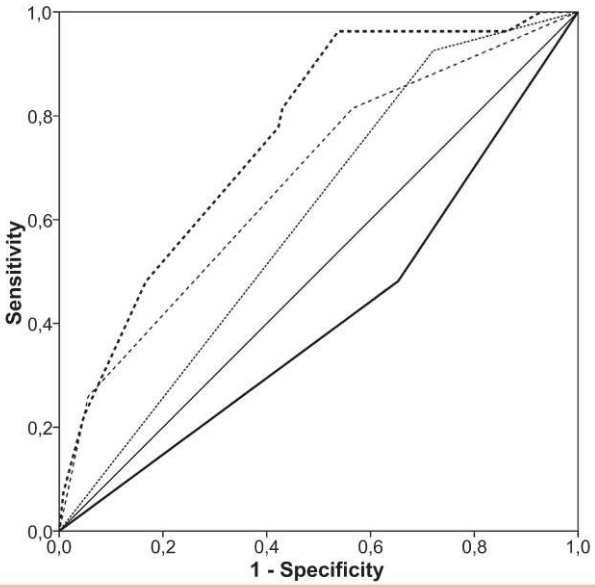

Fig 1. Receiver operating characteristic curve of binary logistic regression analysis equation, rs223468 *MMP9* polymorphism and antiplatelet use for occurrence of thoracic aortic aneurysm (TAA). Multivariate logistic regression analysis (*thick dashed line*), *MMP9* rs2234681 polymorphism (*thin dashed line*), antiplatelet use (*thin dotted line*), reference line (*thin solid line*), and hypercholesterolemia (*thick solid line*).

Embryologic origin[18] and occurrence of atherosclerotic lesions[19] of the considered artery also might play a role is determining positive remodeling.

Table IV. Popliteal artery enlargement or aneurysm (*PE/A*) according to *MMP12* rs652438, sex, and anticoagulation status

MMP12 rs652438	TT	TC	*P*
No PE/A	212 (68.8)	16 (5.2)	.003
PE/A	65 (21.1)	15 (4.9)	
Sex	**Men**	**Women**	
No PE/A	219 (66.4)	29 (8.8)	.033
PE/A	79 (23.9)	3 (0.9)	
Anticoagulation	**Yes**	**No**	
No PE/A	22 (5.7)	226 (68.5)	.039
PE/A	14 (4.2)	68 (20.6)	

MMP, matrix metalloproteinase.
Values are reported as number (%).

The genetics of thoracic aneurysms is known only in part. Fibrillin 1 (*FBN1* gene, associated with the classic form of Marfan syndrome), genes (*ACTA2* and *MYH11*) encoding contractile proteins of the smooth muscle cell (SMC), or other factors involved in the transforming growth factor β pathway (*TGFBR1*, *TGFBR2*, *SMAD3*) have been associated with the occurrence of thoracic aortic aneurysm (TAA). Despite this list of genes, their alterations fail to account for all familial forms of TAAs, and 80% remain unexplained at the molecular level.[20]

In nonsyndromic thoracic aneurysms, MMP-9 is the most represented and studied protease,[20] but to the best of our knowledge, this is the first time that

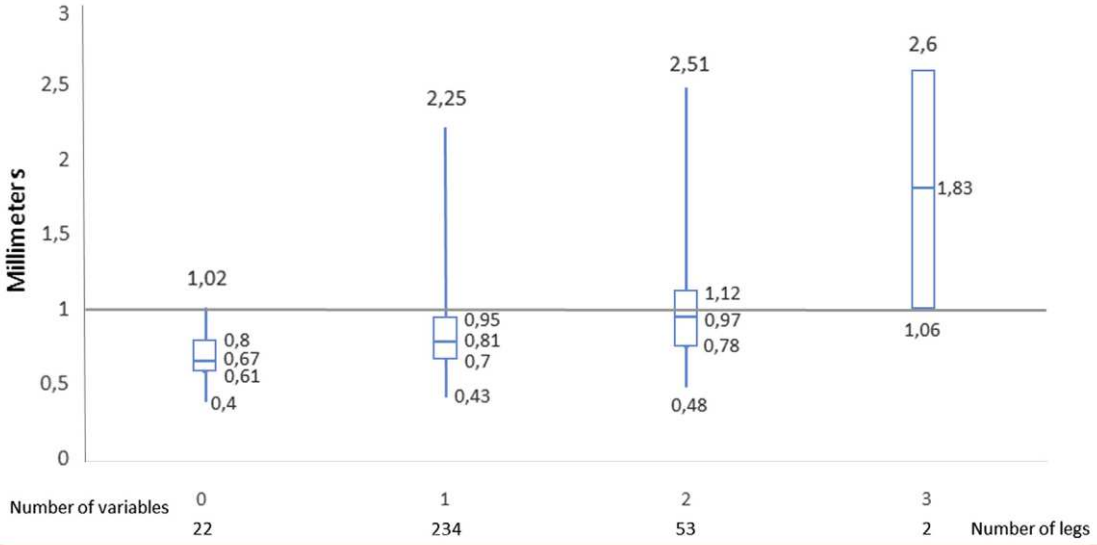

Fig 2. Relationship between popliteal artery diameter and male sex, *MMP12* rs652438 C variant, and anticoagulant therapy. Legs observed (*in abscissa*) are grouped according to the presence of none, one, two, or three of the determinants. The *bars* report median and interquartile range values.

rs2234681 has been associated with the diagnosis of TAA in humans. MMP-9 expression is higher in TAA compared with AAA,[20] particularly within the media of the anterior part of the thoracic aorta,[20] and the cell type mainly accounting for such expression is the SMC.[21]

The different embryologic origins of aortic SMCs may contribute to the site of aortic positive vascular remodeling. Aortic SMCs originate from three distinct developmental lineages; the ascending aorta and arch are neural crest derived and show higher MMP-9 expression than the descending aortic SMCs, originating from paraxial and somitic mesoderm.[22] In such a scenario, a polymorphic microsatellite (13-26 CA repeats around −90, rs2234681), with the larger number of repeats showing higher MMP-9 transcription levels,[23] could increase MMP-9 concentration and directly induce the breakdown of matrix components in thoracic (ascending and arch) segments and enhance local remodeling and dilation. The enlargement could alternatively or additionally be supported by MMP-9-dependent migration of SMCs.[24] The observation that putative higher MMP-9 expression is associated with circumferential enlargement, more than extension along the vessels, suggests that SMCs remodel preferentially along the cell longitudinal axis, with laterolateral binding proteins being less susceptible to MMP-9 activity.

Given the embryologic origin-derived MMP-9 regulation within the aorta and the microRNA (miRNA)-based MMP-9 modulation observed by Duellman et al[25] in wild-type but not in genetic variants, we also tested the hypothesis that two *MMP9* genetic variants docking two miRNAs could be associated with the susceptibility of thoracic aorta enlargement. The negative findings of our study do not rule out the hypothesis of an miRNA-based regulation of MMP expression within the aorta, although we can conclude that polymorphisms rs17675 and rs17676 are hardly associated with an increased prevalence of TEs/As.

Hypercholesterolemia (as a protective factor) and antiplatelet use are the other two variables associated with TAA. The reasons for such a role in determining TAA risk are speculative. The first could probably represent, in our population, a predictor of low atherosclerosis occurrence in the thoracic aorta. To support the hypothesis, we examined, ex post, the atherosclerosis burden of the affected segments from the computed tomography scans but found no difference in the atherosclerotic involvement between patients with or without hypercholesterolemia (data not shown). The use of statins also was not included in the logistic regression equation, apparently ruling out the pleiotropic effect of statins on inflammation. Patients taking antiplatelet drugs also seem to have higher prevalence of TE/A; even considering singularly the different antiplatelet (acetylsalicylic acid or others) or anticoagulant (warfarin) therapy, there were no obvious explanations. Therefore, we can conclude that genetic variant rs2234681 (raising MMP-9 expression), hypercholesterolemia, and antiplatelet intake increase the risk of a TAA in patients with AAA. If validated, the proposed score based on *MMP9* rs2234681, hypercholesterolemia, and antiplatelet use could rule out a thoracic aneurysm with a 98% negative predictive value and 55% accuracy in AAA patients without any invasive radiologic workup.

Popliteal aneurysms and AAAs are often associated but have different histopathologic features. In our study, one AAA patient of three had one or two PEs/As associated, and the risk of being a PE/A carrier was three times higher with male sex and C carriers of *MMP12* rs652438 and twice with anticoagulation. More than 50% of our

patients with at least two conditions were carriers of a popliteal aneurysm.

The histopathologic evaluation of the popliteal aneurysm shows iron deposits (likely expression of previous hemorrhages) and inflammatory infiltration mainly composed of macrophages in the intimal layer (and not in the adventitial layer, as in AAA).[26] The *MMP12* rs652438 causes a missense change of asparagine to serine at amino acid position 357, located in exon 8, which is part of the hemopexin domain. Such a variant could directly influence MMP-12 in vivo activity, modifying substrate affinity and binding[27] and macrophage migration.[28] Therefore, the combination of matrix composition (influenced by sex?) and *MMP12* rs652438 could influence macrophage mobility and induce positive remodeling within the intimal layer of popliteal arteries.[27] The association of anticoagulants with the development of PEs/As, not observed in nested legs within patients, is intriguing but at the moment speculative. The histopathologic evaluation of PE/A of these patients with potentially larger iron deposits should indicate the role of anticoagulants in inducing intra-arterial hemorrhages and vascular enlargements. The relevance of such an observation could be in the possibility of detecting a vascular risk profile and developing a strategy to monitor popliteal aneurysm growth in patients with AAA.

This study has some limitations that deserve to be acknowledged. The first is the lack of biologic sample analysis to support the results; the second is the limited number of genetic variants investigated. Whereas the first point would be difficult to carry out in a clinical study, the second would have increased type I statistical error by increasing the number of performed comparisons. Because this is an association study, we cannot infer a role of a polymorphism or of an MMP in the development of an aneurysm. More mechanistic investigation is needed to support such a role.

This study considered only white patients. Therefore, given the differences in MMP genetic profiles across the different ethnic groups, the results should apply only to such a population. Moreover, we focused on enlargements and aneurysms of the thoracic aorta and popliteal artery and did not consider the intracranial ones, for which an association (unrelated to MMPs) has been demonstrated between AAA and the internal carotid artery.

CONCLUSIONS

In patients with an already demonstrated AAA, *MMP9* and *MMP12* genetic variants are associated with the occurrence of enlargements or aneurysmal disease outside the abdominal aorta.

AUTHOR CONTRIBUTIONS

Conception and design: NF, RA
Analysis and interpretation: NA, FD, GB
Data collection: CC, GS, PP, FZ, SC
Writing the article: NF, NA, PP, FZ, RA
Critical revision of the article: NF, CC, GS, FD, SC, GB
Final approval of the article: NF, CC, GS, NA, PP, FZ, FD, SC, GB, RA
Statistical analysis: NF, FD
Obtained funding: RA
Overall responsibility: NF

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accepted Sep 25, 2017.