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Polymorphisms of the MMP-9 gene and abdominal aortic aneurysm

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Abstract

Background—Increased matrix metalloproteinase-9 (MMP-9) activity has been implicated in the formation of abdominal aortic aneurysms (AAAs). The aim of the present study was to explore the association between potentially functional variants of the MMP-9 gene and AAA.

Method—The –1562C>T and –1811A>T variants of the MMP-9 gene were genotyped in 678 men with AAAs (>30mm in diameter) and 659 controls (aortic diameter 19–22mm) recruited from a population-based trial of screening for AAAs. The levels of MMP-9 were measured in a random subset of 300 cases and 84 controls. The association between genetic variants (including haplotypes) and AAA was assessed using multivariate logistic regression.

Results—There was no association between the MMP-9 –1562C>T (OR 0.70 95%CI 0.27, 1.82) or –1811A>T (OR 0.71, 95%CI 0.28, 1.85) genotypes, or the most common haplotype (OR 0.81 95%CI 0.62, 1.05), and AAA. The serum MMP-9 concentration (ng/mL) was higher in cases than controls and in minor allele carriers in cases and controls although the differences were not statistically significant.

Conclusion—The results suggest that a genetic tendency to have higher levels of circulating MMP-9 is not associated with AAAs.

Introduction

Proteolysis is a key process in the development of abdominal aortic aneurysms (AAAs). Although various matrix metalloproteinases (MMPs) have been implicated in the pathogenesis of AAA, one of the most important appears to be MMP-9 (92 kD type IV collagenase) 1· 2. MMP-9 is expressed in the walls of AAAs and the level increases with diameter 1· 3 and in the event of rupture 4. Circulating levels of MMP-9 appear to be elevated in aneurysmal disease 5 and may be associated with both the rate of expansion 6 and with rupture 7. Aneurysm formation is inhibited in MMP-9-deficient mice models 8· 9 and there is evidence of differential expression of MMP-9 in the abdominal aorta 10.

Although the walls of aneurysmal aortas may be a source of MMP-9 5, it is not known if up-regulation of MMP-9 initiates the onset of AAA formation or is part of the response to the

disease (ie reverse causation). As MMP-9 activity may be under genetic control 11, its role in the causal pathway can be explored by comparing the frequency of genetic variants associated with increased MMP-9 activity in subjects with and without AAAs. Functional variants of the MMP-9 gene (specifically -1562C>T) have been implicated in AAA in some but not all studies 12, 13. The aim of the present study was to explore the association between two single nucleotide polymorphisms (SNPs) of the MMP-9 gene (-1562C>T and -1811T>A) and AAA in a large population-based cohort. These SNPs were chosen as they are located in the promoter region of the MMP-9 gene, and therefore have the potential to influence MMP-9 activity 11.

Methods

Subject recruitment

Details of the Western Australian trial of screening for AAAs are described elsewhere 14,15. Men attending for screening completed a cardiovascular risk factor questionnaire. Height, weight, girth at hips and waist, and blood pressure were measured. The maximum diameter of the infra-renal aorta was measured using a Capasee ultrasound machine with a 3.75 MHz probe (Toshiba Australia, North Ryde, NSW). All staff performing ultrasound measurements participated in regular quality control exercises and 95% of differences between repeat measures were less than 3mm.

Between 1996 and 1998, 12,203 men aged 65–83 years attended for baseline screening and 875 with AAAs (aortic diameters >30mm) were identified. Men with large AAAs (generally >50mm) were referred for surgical assessment and were not actively recruited into surveillance and follow-up. All men with small AAAs (aortic diameter 30–50mm) were invited to attend a follow-up study (1997–2004) involving repeat ultrasound scans at intervals of 6–12 months 16. At the first follow-up visit, a sample of blood was obtained from all consenting men. Men without AAAs were reviewed as part of a separate study in 2001–4 which also included blood sampling 17. The controls for the study consisted of randomly selected age-matched men with aortic diameters in a previously defined reference range of 19–22mm 18. The study was approved by the Human Research Ethics Committee of the University of Western Australia.

Laboratory Methods

Genomic DNA was extracted from whole blood using a standard Triton X-100 procedure. Two SNPs in the MMP-9 promoter region were chosen for study: MMP-9 -1562 C>T (rs3918242) was selected on the basis that it may affect MMP-9 mRNA levels 11; MMP-9 -1811 T>A (rs3918241) also lies in the promoter region, although its effect on mRNA expression has not been formally examined. Both SNPs are found at >10% frequency in the Caucasian population (dbSNP; <http://www.ncbi.nlm.nih.gov/SNP>). MMP-9 -1562 was genotyped using restriction-fragment length polymorphism analysis. MMP9 -1811 was genotyped using fluorescent Taqman probes (Applied Biosystems). Aliquoted serum collected during follow-up was stored at -80°C, as previously described 19, 20. Thawed serum was analysed at completion of the study using ELISA to measure MMP-9 concentrations in a randomly selected subset of 300 cases and 84 controls (DuoSet, R&D Systems). Inter-assay coefficient of variations were between 3 and 6%.

Statistical Methods

The primary binary outcome of this association analysis is the presence or absence of AAA (aortic diameter >30mm). The principal explanatory variables are the two polymorphisms from the MMP-9 gene, -1562C>T and -1811T>A. All loci were tested for deviations from Hardy-Weinberg equilibrium by means of an exact test, using only controls. Univariate

logistic regression was initially used to model the effects of genotype on AAA case-control status. This was followed by multivariate analyses, with independent variables selected via step-wise regression based on prior evidence of their involvement in AAA development. Only those covariates that were statistically significant predictors of the outcome were retained for multivariate modelling. The two bi-allelic MMP-9 polymorphisms were then sequentially added under a co-dominant model coded into three classes, with the most common homozygote as the baseline. Further genetic modelling (dominant, recessive or additive) was then guided by the coefficients for the co-dominant model.

Haplotypes were inferred using the Expectation Maximization (EM) algorithm ²¹ and a global test of haplotype association was initially performed taking the most common haplotype as baseline and comparing all other haplotypes simultaneously. Any haplotypes that showed statistical significance in this analysis were then explored individually. All analyses and haplotype estimations were performed under a generalized linear model framework ²².

Natural logarithmic transformations of MMP-9 serum concentrations were undertaken to ensure that residuals were approximately Normally distributed in regression models. Linear regression was used to assess the association between genotype and $\log_e$ MMP-9, which is presented as a geometric mean comparing those who were heterozygotes or minor allele homozygotes for the two MMP-9 genotypes to major allele homozygotes. R and SimHap were used to manage and analyse the data ^{23, 24}. Statistical significance was defined at the 5% level.

Results

Genotyping results were available from 678 cases, representing 77% of the 875 men found to have AAAs at baseline screening. There were 535 men with AAAs between 30–39mm in diameter, 118 between 40–49mm, 25 of 50+mm and 659 controls (aortic diameters between 19–22mm). The characteristics and risk factors of the cases and controls are shown in Table 1. Genotyping was successful in the following percentages of cases and controls respectively: MMP-9 –1562 C>T (97.4%, 98.2%) and MMP-9 –1811 A>T (96.3%, 98.9%). Genotype frequencies in cases and controls are presented in Table 2. Both SNPs in control subjects were in Hardy-Weinberg equilibrium.

There was no association (OR 0.70, 95%CI 0.27, 1.82) between the MMP-9 –1562C>T variant and AAA in the multivariate modelling (Table 3). Exclusion of the 25 men with larger AAAs (>50mm) did not alter this result substantially (OR 0.66, 95%CI 0.25, 1.77). Similar modelling also revealed no association with MMP-9 –1811A>T (OR 0.71, 95%CI 0.28, 1.85). Using the most common haplotype (h.TC) as a reference, there was no evidence of an association between remaining haplotypes and AAA (Table 4).

The serum MMP-9 concentration (ng/mL) was higher in cases than controls although the difference was not statistically significant ($P=0.12$): geometric mean 750.2 (95%CI 707.2, 795.7) in cases ($n=300$) and 681.1 (95%CI 617.7, 751.0) in controls ($n=84$). The geometric mean serum MMP-9 concentrations were not significantly different across genotypes in either cases or controls for either SNP, in both a univariate and multivariate modelling framework (Table 5).

Discussion

This study has found no evidence of an association between the putatively functional MMP-9 –1562 C>T variant and screen-detected small AAAs in men. In addition no association was found with another promoter gene variant (MMP-9 –1811A>T) or with

haplotypes of these SNPs. There was a non-significant trend towards higher levels of MMP-9 in the minor allele carriers for both SNPs - and in men with AAAs.

There is ample evidence that MMP-9 is important in the pathogenesis of AAAs. MMP-9 activity is up-regulated in the walls of AAAs, particularly large AAAs and at the site of rupture 1, 2, 4, 25. Various animal model studies also support a key role for MMP-9 in aneurysm formation 8-10. Several studies have documented elevated levels of circulating MMP-9 in patients with AAAs 5, 26, 27. Reports that levels fall after successful endoluminal stenting of AAAs is further indirect evidence that MMP-9 activity is up-regulated in AAAs 28, 29.

Although the animal model studies support a causal role for MMP-9 in aneurysmal disease, observational studies in humans based on analyses of MMP-9 levels in the aortic wall or the circulation are subject to residual confounding and reverse causation. The advantage of genetic studies is that they minimise this problem as genotype precedes adult pathology. The present study focussed on the MMP-9 -1562 C>T variant as it appears to be of functional significance. In vitro studies have shown that the T allele of MMP-9 -1562 C>T results in higher promoter activity as a transcription repressor is preferentially bound to the C allele 11. The variant is associated with up-regulation of MMP-9 RNA and protein in the aortic wall and increased stiffness of large arteries 30.

In a study of 414 cases of AAA and 203 controls, Jones et al initially reported a significant association (OR 2.41, 95%CI 1.44,4.02) between the T allele of MMP-9 1562 C>T and AAA 12. A subsequent study involving two separate populations (involving a total of 387 cases and 425 controls) failed to confirm this result 13. In another study of 455 patients with small AAAs, there was no association between the T allele and rate of expansion 31. Although a recent meta-analysis of these studies suggested a marginally positive association (OR 1.09, 95%CI 1.10,1.18) this was probably due to the weighting (57.8%) of the study by Jones et al 32.

The strengths of this study are its relatively large sample size and the use of an age-matched controls who have all had an ultrasound scan to exclude an AAA. Furthermore it is the only study of AAA to include an assessment of both MMP-9 genotype and protein concentration. The negative result may be due to the relatively small influence of both SNPs on MMP-9 levels and the possibility of other unidentified variants playing a greater role. There is some evidence that MMP-9 may be particularly important in larger AAAs 1 and it is possible that a significant association would have been found if a greater proportion of large AAAs had been included. This was not possible as the cases were recruited from a population-based screening study rather than a selected surgical series. However the discovery of factors causing early disease remains a priority for our understanding of AAA formation and prevention and, as such, the study of small AAAs is relevant.

The findings of this study should not be interpreted as evidence against the role of MMP-9 as either a biomarker or a proteolytic mediator in aneurysmal disease. However, the results do suggest that a genetic tendency to have higher levels of circulating MMP-9 is not associated with AAAs.

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Table 1

Baseline characteristics of the AAA cases and the controls. Age, height, weight, BP and diameter are all expressed as means $\pm$ 1SD. Remaining variables are percentages (number).

	AAA (n=678)	Controls (n=659)
Age (years)	73.3 (4.4)	72.3 (3.9)
Aortic diameter (mm)	35.7 (6.8)	21.2 (1.1)
BMI	27.2 (3.7)	26.6 (3.2)
Waist Hip Ratio		
< 1.00	66.5 (451)	75.7 (499)
≥ 1.00	33.5 (227)	24.3 (160)
Systolic BP (mmHg)	157.1 (22.0)	155.0 (19.4)
Diastolic BP (mmHg)	90.8 (12.2)	89.9 (10.8)
Place of Birth		
Australia	57.7 (391)	63.7 (420)
Northern Europe	29.2 (198)	26.3 (173)
Mediterranean	6.0 (41)	4.4 (29)
Other	7.1 (48)	5.6 (37)
History of stroke	10.9 (74)	4.9 (32)
History of myocardial infarction	26.4 (179)	8.5 (56)
History of diabetes	9.9 (67)	5.8 (38)
History of coronary artery surgery	15.9 (108)	5.9 (39)
Father had AAA	1.8 (12)	1.1 (7)
History of/treatment for hypertension	53.4 (362)	35.5 (234)
History of/treatment for angina	29.6 (201)	13.1 (86)
History of/treatment for high cholesterol	44.1 (299)	31.6 (208)
Smoking Status		
Never smoked	14.5 (98)	36.1 (238)
Ex-smoker	67.7 (459)	54.6 (360)
Current smoker <25/day	10.3 (70)	5.6 (37)
Current smoker $\geq$ 25/day	7.5 (51)	3.6 (24)
Vigorous exercise	19.9 (135)	31.1 (205)
Non-vigorous exercise	63.4 (430)	70.4 (464)

Alternative Table 1A

Baseline characteristics of the AAA cases and the controls. Age, height, weight, BP and diameter are all expressed as means $\pm$ 1SD. Remaining variables are percentages (number). T-test and chi-square tests were used to compare continuous and binary/categorical variables respectively.

	AAA (n=678)	Controls (n=659)	P-Value
Age (years)	73.3 (4.4)	72.3 (3.9)	<0.001
Aortic diameter (mm)	35.7 (6.8)	21.2 (1.1)	<0.001
BMI	27.2 (3.7)	26.6 (3.2)	0.002
Waist Hip Ratio			
< 1.00	66.5 (451)	75.7 (499)	<0.001
≥ 1.00	33.5 (227)	24.3 (160)	
Systolic BP (mmHg)	157.1 (22.0)	155.0 (19.4)	0.06
Diastolic BP (mmHg)	90.8 (12.2)	89.9 (10.8)	0.16
Place of Birth			
Australia	57.7 (391)	63.7 (420)	0.13
Northern Europe	29.2 (198)	26.3 (173)	
Mediterranean	6.0 (41)	4.4 (29)	
Other	7.1 (48)	5.6 (37)	
History of stroke	10.9 (74)	4.9 (32)	<0.001
History of myocardial infarction	26.4 (179)	8.5 (56)	<0.001
History of diabetes	9.9 (67)	5.8 (38)	0.008
History of coronary artery surgery	15.9 (108)	5.9 (39)	<0.001
Father had AAA	1.8 (12)	1.1 (7)	0.46
History of/treatment for hypertension	53.4 (362)	35.5 (234)	<0.001
History of/treatment for angina	29.6 (201)	13.1 (86)	<0.001
History of/treatment for high cholesterol	44.1 (299)	31.6 (208)	<0.001
Smoking Status			
Never smoked	14.5 (98)	36.1 (238)	<0.001
Ex-smoker	67.7 (459)	54.6 (360)	
Current smoker <25/day	10.3 (70)	5.6 (37)	
Current smoker $\geq$ 25/day	7.5 (51)	3.6 (24)	
Vigorous exercise	19.9 (135)	31.1 (205)	<0.001
Non-vigorous exercise	63.4 (430)	70.4 (464)	0.008

Table 2

Distribution of MMP-9 genotypes.

SNP	n (%)			p Value*
MMP-9 1811 A>T rs3918241	TT	TA	AA	
AAAs	491 (73.4)	166 (24.8)	12 (1.8)	0.4
Controls	458 (70.0)	182 (27.8)	14 (2.1)	
MMP-9 1562 C>T rs3918242	CC	CT	TT	
AAAs	495 (73.2)	169 (25.0)	12 (1.8)	0.5
Controls	456 (70.3)	179 (27.6)	14 (2.2)	

* AAAs vs controls

Table 3

Results of the multivariate modelling including the MMP-9 1562 C>T genotype.

Variable		OR (95% CI)
Age (yrs)		1.08 (1.04, 1.11)
Waist Hip Ratio		1.45 (1.09, 1.93)
Diastolic BP (mmHg)		1.02 (1.00, 1.03)
History stroke		1.88 (1.12, 3.14)
History myocardial infarction		2.88 (1.94, 4.29)
History coronary artery surgery		2.34 (1.48, 3.68)
History of /treatment for hypertension		1.60 (1.23, 2.09)
Smoking status	Never Smoked	-----
	Ex-smoker	3.21 (2.33, 4.42)
	Current smoker <25 /day	5.54 (3.20, 9.61)
	Current smoker ≥25 /day	5.34 (2.84, 10.04)
Vigorous exercise		0.53 (0.39, 0.72)
Non-vigorous exercise		0.74 (0.56, 0.97)
MMP-9-1562	CC	-----
	CT	0.80 (0.60, 1.09)
	TT	0.70 (0.27, 1.82)

Table 4

MMP-9 haplotypes and their association with AAA in multivariate modelling. All haplotypes with frequencies <1% were grouped together in the rare category.

	1811T>A	1562C>T	Frequency	OR (95%CI)
h.TC	T	C	0.837	---
h.AT	A	T	0.151	0.81 (0.62, 1.05)
Rare	C	T	0.013	0.91 (0.49, 1.68)

Table 5

Geometric means of MMP-9 levels by genotype for cases and controls, after adjusting for significant predictors.

SNP	Alleles	Controls (n= 84)		Cases (n = 300)	
		Geometric Mean (95% CI)	P-Value	Geometric Mean (95% CI)	P-Value
MMP-9 1811 T>A	TT	656.1 (583.8, 737.4)	--	718.8 (667.8, 773.6)	--
	TA	756.9 (627.7, 912.7)	0.21	735.2 (644.8, 838.2)	0.77
	AA	608.9 (322.9, 1148.4)	0.82	940.9 (642.5, 1377.8)	0.18
MMP-9 1562 C>T	CC	660.1 (586.5, 742.9)	--	720.8 (670.7, 774.5)	--
	CT	756.9 (627.4, 913.0)	0.23	725.7 (639.2, 824.0)	0.93
	TT	608.9 (322.4, 1150.2)	0.81	942.0 (647.8, 1369.7)	0.17