ORIGINAL RESEARCH

Genetic Variation at the *ADAMTS7* Locus is Associated With Reduced Severity of Coronary Artery Disease

Kenneth Chan, MRCP; Xiangyuan Pu, MD, PhD; Pratik Sandesara, MD; Robin N. Poston, MD, FRCPath; Iain A. Simpson, MD; Arshed A. Quyyumi, MD;* Shu Ye, MD, PhD, FRCPath;* Riyaz S. Patel, MD*

***Background*—**Genome-wide association studies identified ADAMTS7 as a risk locus for coronary artery disease (CAD). Functional studies suggest that ADAMTS7 may promote cellular processes in atherosclerosis. We sought to examine the association between genetic variation at ADAMTS7 and measures of atherosclerosis using histological, angiographic, and clinical outcomes data.

***Methods and Results*—**The lead CAD-associated single-nucleotide polymorphism rs3825807 at the ADAMTS7 locus was genotyped. The G allele (reduced ADAMTS7 function) was associated with a smaller fibrous cap (P=0.017) and a smaller percentage area of α-actin (smooth muscle cell marker) in the intima (P=0.017), but was not associated with calcification or plaque thickness, following ex vivo immunohistochemistry analysis of human coronary plaques (n=50; mean age 72.2±11.3). In two independent cohorts (Southampton Atherosclerosis Study [n=1359; mean age 62.5±10.3; 70.1% men] and the Emory Cardiovascular Biobank [EmCAB; n=2684; mean age 63.8±11.3; 68.7% men]), the G allele was associated with 16% to 19% lower odds of obstructive CAD (Southampton Atherosclerosis Study: odds ratio, 0.81; 95% confidence interval, 0.67–0.98; EmCAB: odds ratio, 0.84; 95% confidence interval, 0.75–0.95) with similar effects for multivessel, left anterior descending, and proximal CAD. Furthermore, each copy of the G allele was associated with lower angiographic severity Gensini score (Southampton Atherosclerosis Study, P=0.026; EmCAB, P<0.001), lower Sullivan Extent score (Southampton Atherosclerosis Study, P=0.029; EmCAB, P<0.001), and a 23% lower risk of incident revascularization procedures (EmCAB: hazard ratio, 0.76; 95% confidence interval, 0.59–0.98). There were no associations with all-cause mortality or incident myocardial infarction.

***Conclusions*—**Genetic variation at the *ADAMTS7* locus is associated with several complementary CAD phenotypes, supporting the emerging role of ADAMTS7 in atherosclerosis and may represent a potential drug target. **(*J Am Heart Assoc*. 2017;6:e006928. DOI: 10.1161/JAHA.117.006928.)**

Key Words: angiography • atherogenesis • coronary artery disease • genetic association

Genome-wide association studies have revealed a robust association between genetic variation in the *ADAMTS7* (a disintegrin and metalloprotease with thrombospondin motif 7) gene on chromosome 15q25 and clinical phenotypes of coronary artery disease (CAD).[1–3] One of the lead single nucleotide polymorphisms (SNPs), rs3825807, is an adenine (A) to guanine (G) substitution located in exon 4 of the *ADAMTS7* gene, with the G allele conferring a reduced risk of CAD,[1–3] and is found to be in linkage disequilibrium with other SNPs such as rs1994016[2] and rs7178051[4] commonly studied at the ADAMTS7 loci. This nonsynonymous SNP leads to an amino acid change in the prodomain of the

From the Institute of Cardiovascular Sciences, University College London, London, United Kingdom (K.C., R.S.P.); Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, United Kingdom (K.C.); William Harvey Research Institute, Queen Mary University of London, United Kingdom (X.P., R.N.P.); Department of Medicine, Emory University School of Medicine, Atlanta, GA (P.S., A.A.Q.); Wessex Regional Cardiac Unit, Southampton University Hospital, Southampton, United Kingdom (I.A.S.); Department of Cardiovascular Sciences, University of Leicester, United Kingdom (S.Y.); Shantou University Medical College, Shantou, China (S.Y.); Barts Heart Centre, St Bartholomew's Hospital, London, United Kingdom (R.S.P.).

Accompanying Tables S1 through S3 and Figure S1 are available at http://jaha.ahajournals.org/content/6/11/e006928/DC1/embed/inline-supplementary-material-1.pdf

*Dr Quyyumi, Dr Ye and Dr Patel are joint last authors.

Correspondence to: Shu Ye, MD, PhD, FRCPath, Department of Cardiovascular Sciences, University of Leicester, Leicester LE3 9QP, United Kingdom. E-mail: sy127@le.ac.uk

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Clinical Perspective

What Is New?

- Variation in the ADAMTS7 gene has been associated with risk of coronary artery disease; however, its pathological and clinical consequences have yet to be determined.
- We found that a genetic variant associated with reduced function of ADAMTS7 is associated with less severe histological features of human atherosclerotic plaques, lower angiographic coronary artery disease severity in 2 independent cohorts, and lower risk of incident revascularization during follow-up.

What Are the Clinical Implications?

- These findings are strongly consistent with the hypothesis that ADAMTS7 activity promotes atherosclerosis and therefore may represent a potential pathway for drug targeting.

ADAMTS7 protein, a metalloproteinase that plays a role in proteolysis and degradation of extracellular matrix in connective tissues.[5] Functional studies have revealed that this substitution affects maturation of ADAMTS7, resulting in reduced vascular smooth muscle cell migration.[6] Furthermore, in vivo animal studies show that *ADAMTS7* deficiency confers reduced atherosclerotic lesion formation and neointima thickening.[7,8]

These data therefore suggest an important role of normally functioning *ADAMTS7* in driving atherosclerosis and plaque development. We sought to further explore this hypothesis by examining whether genetic variation in *ADAMTS7* (and therefore reduced functional activity of the gene product) would be associated with lower measures of quantifiable atherosclerosis (in vivo and ex vivo) and clinically relevant outcomes.

Methods

Immunohistochemical Analysis of Ex Vivo Human Coronary Atherosclerotic Plaques

Human coronary arteries were obtained from authorized hospital postmortems for research purposes (n=50). DNA was extracted from tissues using the Wizard Genomic DNA purification kit (Promega).[6] Formaldehyde-fixed paraffin-embedded sections were deparaffinized, rehydrated, and incubated in sodium citrate for antigen retrieval. The sections were then double stained with anti-human smooth muscle α-actin antibody (Sigma A5691) and anti-human ADAMTS7 antibody (Abcam, ab28557). Morphology of the section was determined by one investigator (K.C.) in accordance with standard American Heart Association classification.[9] These were independently verified by an expert pathologist (R.N.P.) who was blinded to the findings, with complete agreement. For American Heart Association type V or VI classified plaques (fibroatheromatous), the intima and fibrous cap thicknesses were measured in the most representative area on a standardized scale and power field. The percentage area of positive α-actin stain in the intima was calculated in both fibroatheromatous and fibrous plaques. Calcification of the atherosclerotic plaque was assessed as a binary measure—presence or absence—and by semiquantitative assessment on a scale of 0 to 3. All measurements were performed using Image-Pro 7.0.[6]

Southampton Atherosclerosis Study

Consecutive white patients undergoing interventional or diagnostic coronary angiography were recruited in the Wessex Cardiothoracic Unit of the Southampton General Hospital from 1999 to 2002 as part of the SAS (Southampton Atherosclerosis Study).[10] The study was approved by the local research ethics committee, and all participants provided written consent. Demographic and clinical data were recorded including age, sex, weight, height, occupation, smoking habit, and number of cigarettes smoked per day by each smoker, the presence or absence of hyperlipidemia (defined as cholesterol >5.2 mmol/L and/or triglyceride >3 mmol/L), current medications particularly the use of statins and fibrates, hypertension (defined as diastolic blood pressure >95 mm Hg and/or systolic blood pressure >160 mm Hg), type 1 or type 2 diabetes mellitus, previous myocardial infarction (MI), and coronary heart disease in first-degree relatives younger than 65 years. DNA was extracted from peripheral blood samples using the "salting out" method.[11] ADAMTS7 rs3825807 was genotyped as part of the CARDIoGRAM project using the Sequenom platform.[3]

Emory Cardiovascular Biobank

The Emory Cardiovascular Biobank (EmCAB) consists of 3600 consecutive patients aged 18 to 90 years, enrolled before undergoing elective or emergency cardiac catheterization across 3 Emory Healthcare sites in Atlanta between 2003 and 2009. The study was approved by the institutional review board of Emory University, Atlanta, GA, and all participants provided written informed consent. Patients were excluded if they had a history of heart transplantation. Full details of the cohort have been previously published.[12] Demographic characteristics; risk factors such as hypertension, dyslipidemia, and diabetes mellitus; and medication usage were recorded. Laboratory data were collected at the time of enrollment. Genotyping of SNPs including rs3825807 was performed at

deCODE genetics in Reykjavik, Iceland, using the Centaurus (Nanogen) Platform.[12]

Coronary Angiography Evaluation

Coronary angiograms were systematically characterized by a consultant cardiologist in SAS. In EmCAB, angiograms were reported by 2 independent observers with good interobserver agreement and intraclass correlation coefficient of 0.88 (95% confidence interval [CI], 0.74–0.95).[13] Observers in both studies were blinded to genotype. The angiogram reports were then characterized for several anatomic and morphological criteria: (1) number of major epicardial vessels with >50% stenosis, where multivessel disease was defined as ≥2 vessels involvement; (2) anatomical location of the lesions using a 17-segment modified American Heart Association model[14]; and (3) the percentage diameter stenosis for each lesion graded into <25%, 26% to 50%, 51% to 74%, 75% to 94%, 95% to 99%, and 100% stenosis. Proximal disease was defined as lesions located in left main, proximal left anterior descending (LAD), proximal right coronary artery, and proximal left circumflex arteries.

A semiquantitative scoring system was used to grade the angiographic burden of CAD. The Gensini score is a nonlinear scale weighting lesions by prognostic significance, using location, ranging from 0.5 to 5.0, and degree of stenosis (<25%=2, 26–50%=4, 51–75%=8, 76–90%=16, 91–99%=32, and 100%=64); thus, left main and severe proximal lesions confer higher scores.[15] The Sullivan Extent score quantifies the proportional surface area of the coronary tree affected by atheroma, with the right coronary artery divided into 4 segments (25% per segment) and the LAD and left circumflex each divided into 3 segments (33% per segment).[16]

In both cohorts, only native vessel disease was scored. Stented segments were counted as diseased (>75%), while lesions or stents within arterial or venous grafts were not included.

Incident Outcomes

Outcome data were available for the EmCAB cohort only. Patients were followed prospectively for determination of incident all-cause mortality (defined as death from any cause), incident nonfatal MI (defined using standard criteria for MI), and incident coronary artery revascularization (defined as native vessel revascularization with stenting or first coronary artery bypass grafting). Follow-up was performed by personnel blinded to genotype data through telephone interview, chart review, and linkage with the Social Security Death Index and State records. Medical records were accessed or requested to validate all self-reported events. Definitions and details about follow-up and outcome ascertainment have been previously published.[17]

Statistical Analysis

Continuous variables are presented as means (SDs) and categorical variables as proportions (percentages) with 1-way analysis of variance and χ^2 tests used to determine differences by genotype. Variables were tested for normality with Kolmogorov-Smirnov statistics and (+1 natural log) transformed where appropriate for parametric analyses and reverse transformed for interpretation of the effect estimate. Non-normally distributed variables were tested using appropriate nonparametric tests (Kruskal–Wallis). Haploview 4.0 (Broad Institute) was used to compute Hardy–Weinberg equilibrium and minor allele frequency for rs3825807. Power calculations were performed using G-Power package 3.1 (Heinrich-Heine-Universität Düsseldorf).

Logistic and linear regression models were constructed to test the additive effect of the rs3825807 SNP on CAD phenotypes including severity and extent, with the SNP coded as 0, 1, and 2, based on the number of minor alleles (G). Analyses were repeated after adjustment for traditional risk factors for CAD including age, sex, smoking status, diabetes mellitus, and hypertension.

We also conducted a meta-analysis of the summary estimates for the genotype-CAD association analysis from each cohort under a random-effects model. We calculated pooled statistics as odds ratios (ORs) with 95% CIs and overall z statistics. Cochran's Q, tau^2, and I^2 index were used to assess heterogeneity between the 2 cohorts.

Outcome and survival data were analyzed using Cox proportional hazards regression models adjusted for age and sex, and further adjusted for other risk factors including smoking status, diabetes mellitus, and hypertension. Schoenfeld residuals were examined to check for violation of the proportional hazards assumption. Patients with heart transplants or coronary bypass grafting at baseline were excluded from the outcome analysis. A 2-tailed $P<0.05$ was considered significant. Statistical analyses were performed using SPSS version 18.0 (SPSS Inc).

Results

ADAMTS7 Association With Atherosclerotic Plaque Characteristics

Examining all 50 ex vivo human coronary atherosclerotic plaques together (mean age of patients 72.2±11.3 years, 47.6% men, all white), there was no statistically significant association between whole intima or media thickness with the G allele of the ADAMTS7 SNP (Table 1).

Table 1. Ex Vivo Coronary Atherosclerotic Plaque Characteristics by ADAMTS7 rs3825807 Genotype

All Coronary Plaques (n=50)	ADAMTS7 Genotype			β (SE)	*P* Value
	AA (n=22)	AG (n=17)	GG (n=11)		
Whole intima thickness	699.4 (66.8)	793.5 (83.3)	667.8 (75.9)	17.3 (62.6)	0.54
Media thickness	134.6 (13.9)	156.4 (14.9)	167.7 (25.7)	17.1 (12.2)	0.38
AHA Class V or VI Plaques (n=28)	AA (n=14)	AG (n=9)	GG (n=5)		
Intima thickness	741.4 (69.4)	712.6 (88.6)	615.8 (67.8)	−23.6 (63.1)	0.35
Fibrous cap thickness	329.4 (51.4)	217.0 (36.1)	157.7 (8.8)	−88.8 (33.9)	0.017
Fibrous cap:intima thickness ratio	0.47 (0.06)	0.33 (0.06)	0.24 (0.04)	−0.11 (0.05)	0.039
Percent area α-actin stain in intima	36.0 (4.51)	29.2 (4.51)	17.0 (1.64)	−9.1 (3.5)	0.017
Calcification (binary), %	53.3	26.7	20		0.71
Calcification (quantitative)	1.46 (0.37)	1.00 (0.44)	1.40 (0.40)	−0.09 (0.31)	0.78

Coronary plaque features for the 50 ex vivo samples, presented by genotype of rs3825807 single nucleotide polymorphism. Intima, media, and fibrous cap thicknesses were measured in a.u. and expressed as mean (SEM). Characteristics are also shown for the subset of plaques falling into American Heart Association (AHA) class V or VI fibroatheroma and suitable for further characterization. Calcification was characterized as presence or absence (binary) or graded on a scale of 0 to 3 depending on burden of calcification (quantitative).

However, among the 28 plaque samples deemed to be fibroatheromatous according to American Heart Association criteria and suitable for further plaque characterization, there was a significant association between the rs3825807 G allele and reduced fibrous cap thickness (β −88.8±33.9, *P*=0.017), reduced fibrous cap-to-intima thickness (β −0.11±0.05, *P*=0.039), and a lower percentage area α-actin in intima (β −0.08±0.03, *P*=0.029) under an additive genetic model (Table 1 and Figure 1). There were no associations between the SNP and intima thickness or the extent of plaque calcification.

ADAMTS7 Association With CAD

A total of 1359 white patients from SAS and 2684 white patients from EmCAB were genotyped for the ADAMTS7 SNP

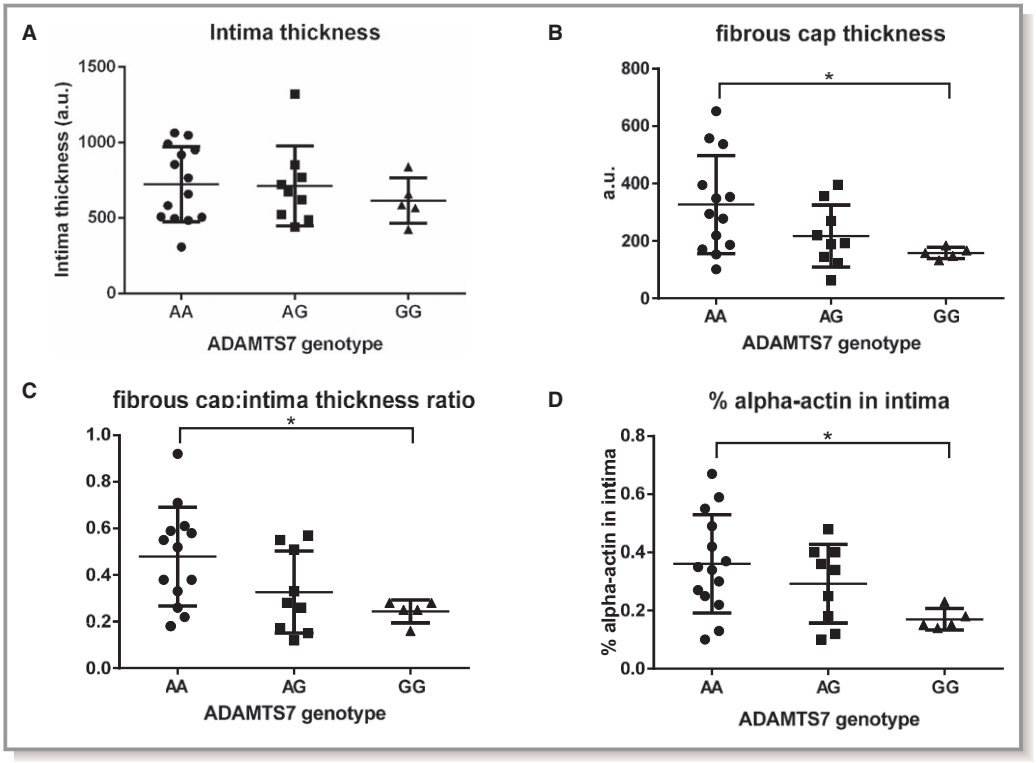

Figure 1. Associations between human atherosclerotic plaque morphology and ADAMTS7 genotypes. A, Intima thickness, (B) fibrous cap thickness, (C) fibrous cap:intima thickness, (D) percentage α-actin in intima.* denotes p<0.05

rs3825807 and included in the analysis. Baseline patient characteristics for both cohorts are presented in Table 2. The mean age and proportion of men were similar in both SAS (62.8 [10.2] years and 70%) and EmCAB (63.8 [11.1] years and 68%) cohorts. However, there were some differences, including more smokers and fewer patients with diabetes mellitus in the SAS cohort (70% and 12%, respectively) compared with in the EmCAB cohort (60% and 30%, respectively). Significant (>50% stenosis) angiographic CAD was recorded in 79.2% of patients in SAS and 63.7% of patients in EmCAB, with the remainder having normal or nonobstructive disease. The observed genotype frequencies were consistent with Hardy–Weinberg equilibrium, and the minor allele frequency for rs3825807 was 0.43 for both cohorts, similar to previously published reports.[1]

The G allele of the ADAMTS7 SNP was associated with a protective effect on CAD in both cohorts, with a 16% to 19% lower odds of disease per allele after adjustment for age and sex (SAS: OR, 0.81 [95% CI, 0.67–0.98]; EmCAB: OR, 0.84 [95% CI, 0.75–0.95]). These estimates persisted after adjustment for further CAD risk factors. Consistent with prior reports, there was no significant association between the G allele and MI (SAS: OR, 1.01 [95% CI, 0.86–1.18]; EmCAB: OR, 0.85 [95% CI, 0.70–1.02]).

Similarly, we found that in both cohorts, the G allele was modestly associated with lower odds of multiple-vessel disease (SAS: OR, 0.86 [95% CI, 0.74–1.00]; EmCAB: 0.81 [95% CI ,0.72–0.92]), as well as LAD disease (SAS: OR, 0.84 [95% CI, 0.72–0.98]; EmCAB: OR, 0.80 [95% CI, 0.71–0.89]) in both cohorts, and with proximal disease in EmCAB (Table 3).

Combining data for both cohorts through meta-analysis revealed that the G allele conferred a pooled OR of 0.84 (95% CI, 0.77–0.93) for obstructive CAD, 0.85 (95% CI, 0.78–0.93) for multivessel disease, 0.83 (95% CI, 0.76–0.91) for LAD disease, and 0.84 (95% CI, 0.76–0.93) for proximal disease (Figure S1). There was no significant heterogeneity Tau^2 and I^2=0% between the cohorts for the pooled analyses.

In addition, we identified an association between the G allele of the ADAMTS7 SNP and semiquantitative angiographic scores. In both cohorts, there was an ≈5-point reduction in median Gensini score and a 10-point reduction in median Sullivan score between those with 2 copies of the G allele compared with those with none. Clinically, this difference can be interpreted as equivalent to, for example, a 50% discrete lesion in the proximal LAD (Gensini score=5) or detectable atheroma affecting half of the right coronary artery (Sullivan score=10). After adjustment for age and sex, the Gensini score was lower for each copy of the G allele in both SAS (P=0.026) and EmoryCAB (P<0.001), while Sullivan Extent score was also similarly lower in both cohorts (SAS P=0.029, EmCAB P<0.001). These associations persisted after adjustment for further risk factors (Table 4).

Table 2. Patient Characteristics by ADAMTS7 rs3825807 Genotype

		SAS					EmCAB			
		ADAMTS7 Genotype					ADAMTS7 Genotype			
Patient Characteristics	Total	AA	AG	GG	*P* Value	Total	AA	AG	GG	*P* Value
No.	1359	472	595	292		2684	858	1336	490	
Age, y	62.5 (10.3)	62.2 (10.5)	62.8 (10.2)	62.2 (10.2)	0.63	63.8 (11.4)	63.9 (11.1)	64.0 (11.5)	63.3 (11.6)	0.56
Men, %	70.1	67.8	71.3	71.2	0.42	67.7	66.0	67.7	71.1	0.15
Body mass index, kg/m^2	27.6 (4.4)	27.8 (4.4)	27.5 (4.4)	27.5 (4.4)	0.41	29.4 (6.1)	29.6 (6.2)	29.4 (6.2)	29.0 (5.8)	0.32
Diabetes mellitus, %	11.8	13.9	11.1	9.6	0.17	30.2	30.2	30.2	29.9	0.99
Hypertension, %	42.6	40.9	44.8	40.6	0.36	68.3	68.0	68.5	68.4	0.97
Hypercholesterolemia, %	79.2	82.2	78.0	77.1	0.14	70.0	70.7	69.1	71.3	0.59
Smoker, %	72.3	74.3	70.6	72.5	0.41	60.5	58.5	61.4	61.3	0.37
Statin use, %	49.1	45.1	51.4	50.7	0.10	24.9	24.3	25.3	24.5	0.86
Prior myocardial infarction, %	38.4	37.2	38.8	39.5	0.83	33.7	33.0	33.6	34.9	0.77
Normal coronaries, %	20.8	19.7	19.2	26.0	0.04	27.7	23.4	28.9	32.4	0.001
Angiographic CAD >50%, %	79.2	78.5	80.4	72.8	0.05	63.7	67.7	63.2	59.4	0.02

Data are presented as mean (SD) or percentage unless indicated by rs3825807 genotype for each study. CAD indicates coronary artery disease; EmCAB, Emory Cardiovascular Biobank; SAS, Southampton Atherosclerosis Study.

Table 3. Association Between Genetic Variation at ADAMTS7 (rs3825807) and Binary CAD Phenotypes

	OR (95% CI)*	Adjusted OR (95% CI)†
Obstructive CAD		
SAS	0.81 (0.67–0.98)	0.82 (0.67–0.99)
EmCAB	0.84 (0.75–0.95)	0.84 (0.74–0.95)
Multivessel disease		
SAS	0.86 (0.74–1.00)	0.87 (0.74–1.02)
EmCAB	0.81 (0.72–0.92)	0.82 (0.73–0.93)
LAD disease		
SAS	0.84 (0.72–0.98)	0.84 (0.71–0.98)
EmCAB	0.80 (0.71–0.89)	0.81 (0.72–0.91)
Proximal disease		
SAS	0.92 (0.75–1.13)	0.86 (0.69–1.07)
EmCAB	0.80 (0.72–0.90)	0.80 (0.71–0.90)

Odds ratio (OR) with 95% confidence interval (CI) derived for association between the G allele of rs3825807 and obstructive coronary artery disease (CAD) vs no CAD, multivessel disease vs single-vessel disease; left anterior descending (LAD) disease vs non-LAD disease, and proximal disease vs distal disease. EmCAB indicates Emory Cardiovascular Biobank; SAS, Southampton Atherosclerosis Study.
*Adjusted for age and sex only.
†Adjusted for age, sex, smoking status, hypertension, and diabetes mellitus.

Effect of ADAMTS7 on Cardiovascular Outcomes

Within the EmCAB cohort, after excluding those with prior coronary artery bypass grafting or heart transplants, 1929 patients with available follow-up data were included in the outcome analysis, with a median follow-up of 3.0±2.3 years. During this time, there were 184 (9.5%) all-cause deaths, 68 (3.5%) MI events, and 114 (5.9%) revascularization procedures. Cox regression analysis adjusted for traditional risk factors showed that there was no significant association for all-cause mortality (hazard ratio, 1.13; 95% CI, 0.94–1.38) or MI (hazard ratio, 0.85; 95% CI, 0.61–1.18) during the follow-up period (Table 5). However, each additional G allele conferred a significantly lower risk of incident native vessel revascularization (hazard ratio, 0.76; 95% CI, 0.59–0.98) (Figure 2).

Discussion

In this study, using (1) histological samples, (2) coronary angiographic phenotypes, and (3) clinical outcomes data, we demonstrate that a nonsynonymous SNP resulting in loss of function of ADAMTS7 is associated with reduced CAD burden. Collectively, our findings provide further support for the emerging role of this protease in promoting atherosclerosis.

Early genome-wide association studies first identified variants in the ADAMTS7 gene as associating with prevalent CAD and MI. In an important analysis, Reilly et al[2] identified this variant as associating with CAD but not MI, suggesting that the gene primarily drives atherosclerosis as its mechanism of risk. The adenine (A) to guanine (G) substitution of rs3825807 results in a serine-to-proline substitution in the prodomain of ADAMTS7,[6] and functional studies using cultured vascular smooth muscle cell model have revealed that this affects ADAMTS7 function not by influencing its expression but rather its maturation and thrombospondin-5 cleavage.[6] Recent in vivo studies using ADAMTS7-null knockout mouse models have shown that ADAMTS7−/− mice have less significant neointima thickening, likely through reduced vascular smooth muscle cell migration.[8] Normally functioning ADAMTS7 might also impair re-endothelialization by degrading thrombospondin-1, thereby inhibiting endothelial cell proliferation and migration.[18] These results thus indicate that ADAMTS7 may ordinarily promote atherosclerosis while impairment of this protein could halt the atherosclerotic process. However, this has not yet been assessed with detailed in vivo or ex vivo measures of atherosclerosis in humans.

Table 4. Association Between Genetic Variation at ADAMTS7 (rs3825807) and Angiographic CAD Scores

	ADAMTS7 Genotype					
Angiographic Scores	AA	AG	GG	β (SE)	*P* Value*	*P* Value†
Gensini						
SAS	26.3 (7–57)	28 (7–57)	20 (2–52)	−0.12 (0.06)	0.026	0.049
EmCAB	15 (15–63)	11 (0–48)	10 (0–44)	−0.18 (0.05)	<0.001	<0.001
Sullivan						
SAS	40 (20–50)	40 (20–50)	30 (20–50)	−0.09 (0.05)	0.029	0.053
EmCAB	40 (20–60)	35 (0–60)	30 (0–55)	−0.15 (0.04)	<0.001	<0.001

CAD indicates coronary artery disease; EmCAB, Emory Cardiovascular Biobank; SAS, Southampton Atherosclerosis Study.
*Adjusted for age and sex only.
†Adjusted for age, sex, smoking status, hypertension, and diabetes mellitus; β (SE) for the ln+1 transformed values.

Table 5. Association Between Genetic Variation at ADAMTS7 (rs3825807) and Incident Outcomes in the EmCAB Cohort

Events, No. (%)	ADAMTS7 Genotype			HR (95% CI)*	HR (95% CI)†
	AA	AG	GG		
All-cause mortality	73 (12.3)	116 (12.4)	43 (12.5)	1.12 (0.92–1.35)	1.13 (0.94–1.38)
Myocardial infarction	29 (4.9)	39 (4.2)	10 (3.0)	0.84 (0.60–1.17)	0.85 (0.61–1.18)
Revascularization	50 (8.4)	67 (7.2)	14 (4.2)	0.77 (0.60–0.98)	0.76 (0.59–0.98)

Hazard ratio (HR) and 95% confidence interval (CI) shown for the association between the G allele of rs3825807 and risk of incident events. EmCAB indicates Emory Cardiovascular Biobank.

*Adjusted for age and sex only.

†Adjusted for age, sex, smoking status, hypertension, and diabetes mellitus.

In an analysis of the MESA (Multi-Ethnic Study of Atherosclerosis) cohort, an association was reported between a different SNP in ADAMTS7 and coronary artery calcification, but only in a Hispanic population subset, and no association was found with carotid intima-media thickness.[19] Similarly, ADAMTS7 was not associated with carotid intima-media thickness in Spanish patients with rheumatoid arthritis.[20] A positive association with CAD was, however, recently reported in a Chinese cohort using angiographic data, although the association was modest, used broad phenotypic definitions, was restricted to a Chinese population, and was not replicated in a smaller Portuguese study.[21]

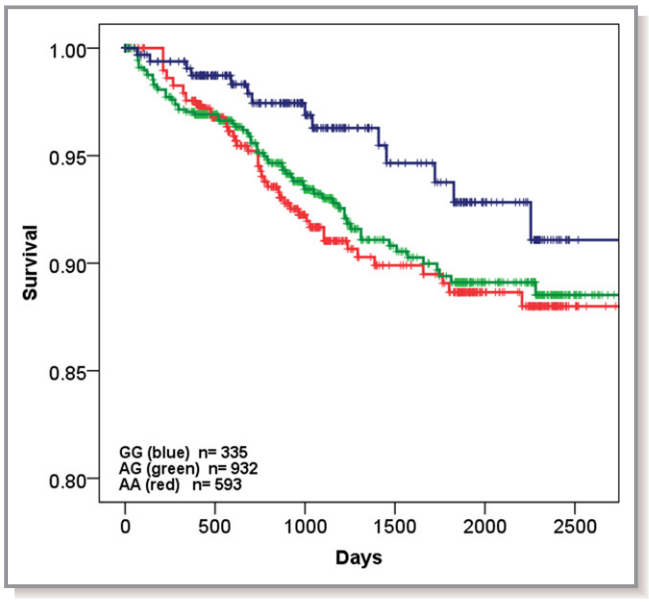

Figure 2. Association between genetic variation at ADAMTS7 (rs3825807) and incident revascularization events in the Emory Cardiovascular Biobank (EmCAB) cohort. Kaplan–Meier curve showing association between ADAMTS7 genotype and risk of incident native vessel revascularization (percutaneous or surgical coronary intervention), among participants within the EmCAB cohort. Genotype GG=blue; AG=green; AA=red.

Study Strengths and Limitations

Our study builds on some of these reports and adds important novel data. First, using ex vivo plaque samples, we show that with each G allele there is a trend to lower intima-media thickness and other plaque characteristics indicative of atheroma development. Second, using clinical coronary angiographic data, we demonstrate that each copy of the G allele confers lower odds of having both obstructive disease and severe manifestations of CAD such as multivessel and proximal disease. This is also reflected with lower semiquantitative scores of CAD burden, a more refined and validated phenotype using Gensini and Sullivan scoring systems.[12]

We also confirmed that while ADAMTS7 variation was associated with CAD, it was not associated with MI per se, using the approach described by Reilly et al.[2] This has been used as evidence that ADAMTS7 confers risk through atherosclerosis and not by plaque rupture or thrombotic mechanisms, which may drive an MI. To further explore this hypothesis, we examined the association with incident outcomes and found that the protective G allele of the ADAMTS7 variant did not impact rates of all-cause death or MI. This is in line with a recent study of 1100 patients with known CAD, in whom no association was found with the same SNP and all-cause mortality over a median of 5 years, although there was an association with cardiovascular death (P=0.025).[22] Intriguingly, in our study, where nonfatal events were also available, the rate of native vessel revascularization following enrollment was significantly lower in those carrying the protective allele, an indirect reflection of lower atheroma development and progression, and again supporting the concept that ADAMTS7 ordinarily drives progressive atherosclerosis.

Finally, a recent study reported that of the 45 loci known to associate with CAD risk, a variant at ADAMTS7 rs7178051, which is in modest linkage disequilibrium with rs3825807 (LD=0.52), exhibited an important gene-smoking interaction in 61 000 cases with coronary heart disease and 80 000 controls.[4] The protective effect of ADAMTS7 genetic

variation on CAD risk was found to be lower in persons who smoked than in nonsmokers, while exposure of human coronary cell lines to cigarette smoke led to induction of ADAMTS7 activity. We examined this observation in our study and while there were some nonsignificant trends, we were unable to identify any significant interactions or differences in association between the rs3825807 SNP and CAD phenotypes among smokers and nonsmokers (Tables S1 through S3). However, it is worth noting that our study was within two cohorts *with* coronary heart disease, rather than a case-control design with coronary heart disease–free controls. Thus a much higher prevalence of smoking in our cohort (64.4% versus 56.9%) and smaller overall sample size (3981 versus 141 162) might have contributed to the lack of interactions with smoking found in the current study. Nonetheless, we believe our findings are complementary to the narrative emerging on the atherogenic role of ADAMTS7.

Our study is unique in that we were able to draw on several complementary sources of data and phenotypes related to atherosclerosis, overcoming limitations associated with each individually. Despite our consistent and promising findings, some limitations need to be considered. First, only a small number of ex vivo samples were available to study and these may have been prone to measurement error despite attempts to reduce potential variation with a priori measurement standard. Second, there is a degree of selection bias with both SAS and EmCAB cohorts enrolling only those undergoing coronary angiography and with CAD, which could have distorted genotype distribution. However, allele frequency was similar to those reported in general populations, suggesting that this was not likely to have been a major factor. Finally, the association with revascularization, while exciting and supportive of the overall hypothesis, should be interpreted with caution given these data were from a single center and revascularization as an end point is susceptible to clinical practice variation. However, together, our data provide consistent evidence for the role of ADAMTS7 in promoting atherosclerosis.

Conclusions

Genetic variation at the *ADAMTS7* locus is associated with several complementary CAD phenotypes. Collectively, these findings support the emerging role of ADAMTS7 in promoting atherosclerosis and may represent a potential antiatherosclerosis drug target.

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Disclosures

None.

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Supplemental Material

Table S1. Association between genetic variation at ADAMTS7 (rs3825807) and obstructive CAD phenotype stratified by smoking status

Presence of CAD	n	OR (95% CI)*	p value*	Interaction p value
SAS				
Ever-smoked	974	0.98 (0.75-1.27)	0.85	0.92
Never-smoked	373	0.73 (0.52-1.01)	0.06	
EmCAB				
Ever-Smoked	1593	0.80 (0.68-0.94)	0.01	0.63
Never-Smoked	1041	0.84 (0.70-1.01)	0.07	

Odds ratio (OR) with 95% confidence interval (95% CI) derived for association between the G allele of rs3825807 and obstructive CAD vs no CAD, stratified by smoking status. *adjusted for age and sex.

Table S2. Association between genetic variation at ADAMTS7 (rs3825807) and angiographic CAD scores stratified by smoking status

Angiographic scores			n	β (SE)	p-value*	Interaction p-value
Gensini Score	SAS	Ever-smoked	974	-0.12 (0.06)	0.048	0.11
		Never-smoked	373	-0.14 (0.11)	0.21	
	Emory CAB	Ever-smoked	1593	-0.19 (0.06)	0.002	0.35
		Never-smoked	1041	-0.14 (0.08)	0.08	
Sullivan Extent Score	SAS	Ever-smoked	974	-0.09 (0.04)	0.037	0.15
		Never-smoked	373	-0.08 (0.08)	0.28	
	Emory CAB	Ever-smoked	1593	-0.15 (0.05)	0.002	0.94
		Never-smoked	1041	-0.13 (0.06)	0.04	

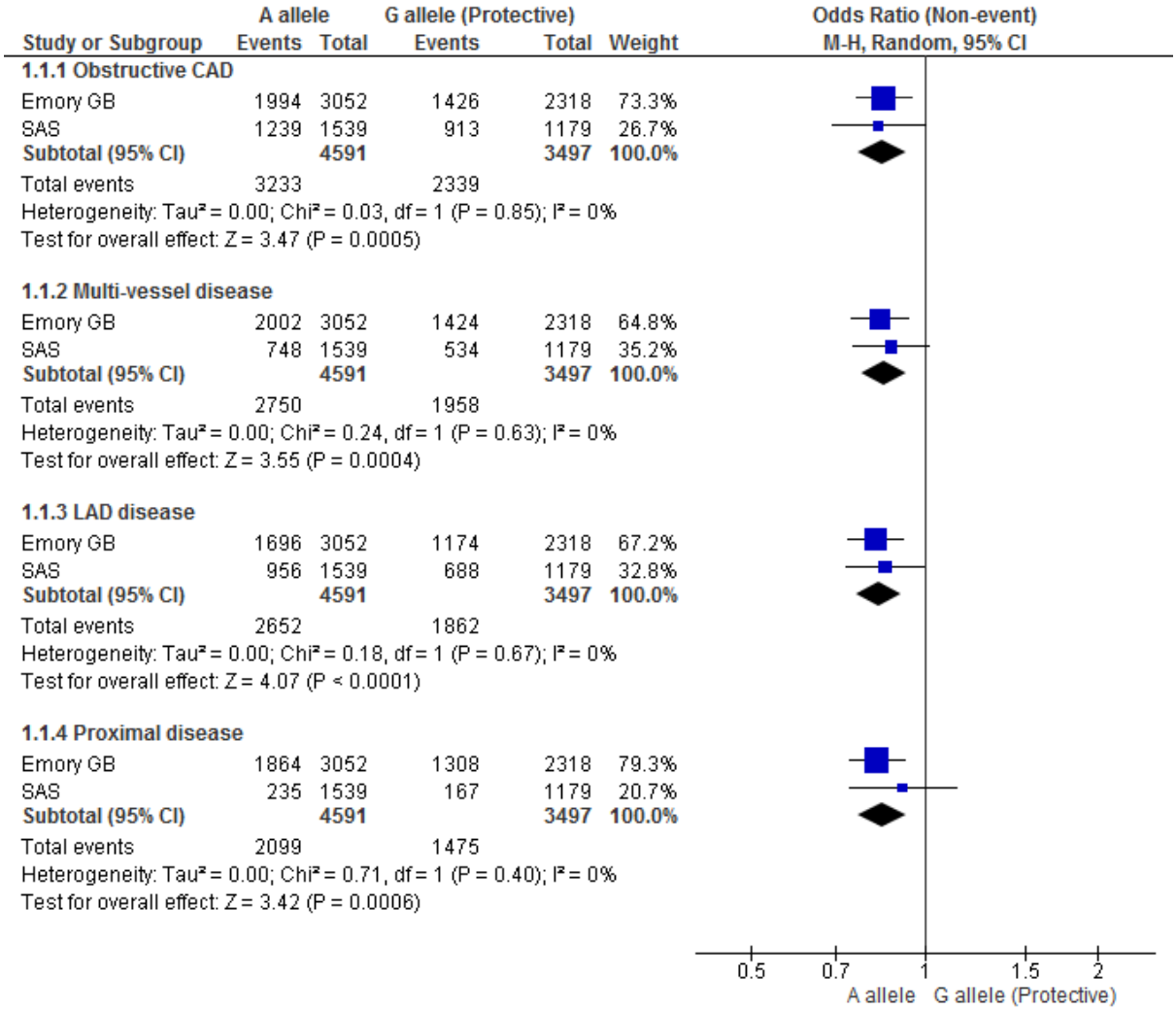
SAS - Southampton Atherosclerosis Study; EmCAB – Emory Cardiovascular Biobank; CAD – Coronary Artery Disease; β (SE) for the ln+1 transformed value; *adjusted for age and sex. P-value interaction analysed using general linear model (univariate)

Table S3. Association between genetic variation at ADAMTS7 (rs3825807) and incident outcomes in the EmCAB cohort stratified by smoking status

	Ever-smoked (n=1115)	Non-smoker (n=783)	
	HR (95% CI)*	**HR (95% CI)***	**P for interaction**
All-cause mortality	1.27 (1.00-1.59)	0.89 (0.64-1.25)	0.11
MI	0.97 (0.64-1.47)	0.65 (0.38-1.12)	0.24
Revascularization	0.73 (0.53-0.99)	0.85 (0.55-1.32)	0.57

Hazard Ratio (HR) and 95% Confidence Interval (CI) shown for association between the G allele of rs3825807 and risk of incident events, stratified by smoking status; *adjusted for age and sex only;

Figure S1. Meta-analysis with pooled estimates for association between genetic variation at ADAMTS7 (rs3825807) and CAD phenotypes



Meta-analysis of association between the G allele of rs3825807 and CAD phenotypes, under an additive genetic model

SAS - Southampton Atherosclerosis Study; EmCAB – Emory Cardiovascular Biobank; CAD – Coronary Artery Disease; LAD – Left Anterior Descending