Association of a matrix metallopeptidase 1 gene polymorphism with long-term outcome of thoracic aortic aneurysm

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Abstract. Although genetic variants are thought to contribute to the development of thoracic aortic aneurysm including dissection (TAA), it remains unclear whether gene polymorphisms are associated with the long-term outcome of TAA. The purpose of the present study was to identify genetic variants associated with the long-term outcome of medically treated patients with TAA. A total of 103 medically-treated patients with TAA (13 aneurysms and 90 dissections) were retrospectively studied for their outcomes (mean follow-up period, 24 months). The genotypes for 95 polymorphisms of 89 candidate genes were determined by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology. Evaluation of genotype distributions by the Chi-square test and subsequent multivariable logistic regression analysis with adjustment for covariates revealed that the -340A→G polymorphism (rs514921) of the matrix metallopeptidase 1 gene (*MMP1*) was significantly (P=0.0288) associated with the outcome of TAA, with the minor *G* allele being related to a favorable outcome. The aneurysm diameter was significantly (P=0.0167) smaller in the combined group of the *AG* and *GG* genotypes for this polymorphism than in subjects with the *AA* genotype. Kaplan-Meier survival curves constructed according to *MMP1* genotypes showed a more favorable outcome of TAA (log-rank P=0.0146) in subjects with the *G* allele of rs514921. Determination of genotype for this polymorphism may prove informative for assessment of the long-term outcome of TAA.

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Introduction

Thoracic aortic aneurysm including dissection (TAA) is a serious condition that results from aortic atherosclerosis and is a leading cause of mortality (1). Recent studies on the genetic basis of familial TAA have focused on its relation to systemic connective tissue disorders such as the Marfan syndrome (2) and the Ehlers-Danlos syndrome (3). However, up to 19% of individuals with non-syndromic TAA referred for surgery have been found to have affected first-degree relatives (4). In addition to conventional risk factors for TAA including age, arteriosclerosis, hypertension, and inflammatory or autoimmune diseases, genetic epidemiological studies have suggested that genetic variants contribute to the initiation and progression of this condition (5,6). It has remained unclear, however, whether gene polymorphisms are associated with the long-term outcome of TAA.

Although computed tomography (7) parameters are currently applied to prediction of the risk for TAA rupture, only the relative, not the individual, rupture risk can be determined (8). The identification of gene polymorphisms related to the long-term outcome of medically-treated TAA may therefore lead to a better understanding of the factors relevant to the progression and rupture of TAA, and consequently may better inform the selection of patients as candidates for surgical therapy because of a higher risk of rupture.

We have now performed an association study for 95 polymorphisms of 89 candidate genes and TAA in 103 Japanese patients with this condition. The purpose of the present study was to identify genetic variants associated with the long-term outcome of medically-treated patients with TAA and thereby to contribute to prediction of the outcome of this condition.

Subjects and methods

Study subjects. The present retrospective study (mean follow-up period ± SD, 24±35 months) examined the relation of genetic variants to unfavorable or favorable outcomes of acute TAA. The

Table I. The 95 polymorphisms of 89 genes examined in the study.

Locus	Gene	Symbol	Polymorphism	dbSNP
1q21	Proteasome (prosome, macropain) subunit, β type, 4	*PSMB4*	A→G	rs7172
1q21.1	Gap junction protein, α 5, 40 kDa	*GJA5*	-44G→A	rs35594137
1q24	RAB GTPase activating protein 1-like	*RABGAP1L*	C→G	rs12078839
1q32.1	Protein phosphatase 1, regulatory (inhibitor) subunit 12B	*PPP1R12B*	G→T	rs930734
1p35.1	Gap junction protein, α 4, 37 kDa	*GJA4*	1019C→T (Pro319Ser)	rs1764391
1p36.3	Tumor protein p73	*TP73*	C→G	rs12027041
1p36.31-p36.11	Acyl-CoA thioesterase 7	*ACOT7*	A→C	rs3789485
1q44	Olfactory receptor, family 13, subfamily G, member 1	*OR13G1*	A→G (Ile132Val)	rs1151640
2p11	Fatty acid binding protein 1, liver	*FABP1*	A→G (Thr94Ala)	rs2241883
2p24	Rho-associated, coiled-coil containing protein kinase 2	*ROCK2*	125592C→A (Thr431Asn)	rs9808232
2q14	Bridging integrator 1	*BIN1*	C→G	rs754107
2q32.1	Zinc finger, SWIM-type containing 2	*ZSWIM2*	A→G	rs6726153
2q36	Insulin receptor substrate 1	*IRS1*	3494G→A (Gly972Arg)	rs1801278
3p21	Chemokine (C-C motif) receptor 2	*CCR2*	190G→A (Val64Ile)	rs1799864
3p21	Chemokine (C-C motif) receptor 5	*CCR5*	59029G→A	rs1799987
3p21.2-14.1	Inter-α (globulin) inhibitor H4 (plasma Kallikrein-sensitive glycoprotein)	*ITIH4*	IVS17+8C→T	rs3821831
3p25	Peroxisome proliferator-activated receptor γ	*PPARG*	34C→G (Pro12Ala)	rs1801282
3p25	Peroxisome proliferator-activated receptor γ	*PPARG*	-681C→G	rs10865710
3pter-p21	Chemokine (C-X3-C motif) receptor 1	*CX3CR1*	C→T (Thr280Met)	rs3732378
3q13.33	Transmembrane protein 39A	*TMEM39A*	C→T	rs2282170
3q27	Adiponectin, C1Q and collagen domain containing	*ADIPOQ*	-11377C→G	rs266729
3q28	Adiponectin, C1Q and collagen domain containing	*ADIPOQ*	G→T in intron 2 (SNP-276)	rs1501299
4p15.1	Peroxisome proliferator-activated receptor γ, coactivator 1 α	*PPARGC1A*	G→A (Gly482Ser)	rs8192678
4p16.1	Sortilin-related VPS10 domain containing receptor 2	*SORCS2*	A→G	rs2285780
4p16.3	Phosphatidylinositol glycan anchor biosynthesis, class G	*PIGG*	C→T	rs4499656
4p16.3	Regulator of G-protein signaling 12	*RGS12*	C→T	rs2236052
5q13	Phosphoinositide-3-kinase, regulatory subunit 1 (α)	*PIK3R1*	G→A (Met326Ile)	rs3730089
5q35.1-q35.2	Endoplasmic reticulum-golgi intermediate compartment (ERGIC) 1	*ERGIC1*	A→G	rs2339745
6p21.1	p53-associated parkin-like cytoplasmic protein	*PARC*	A→G	rs9462875
6p21.3	Tumor necrosis factor (TNF superfamily, member 2)	*TNF*	-863C→A	rs1800630
6q22	c-ros oncogene 1, receptor tyrosine kinase	*ROS1*	G→A (Asp2213Asn)	rs529038
6q22-q23	Ectonucleotide pyrophosphatase/phosphodiesterase 1	*ENPP1*	97A→C (Lys121Gln)	rs1044498
6q24-q25	A kinase (PRKA) anchor protein (gravin) 12	*AKAP12*	A→G	rs756009
6q25.1	Estrogen receptor 1	*ESR1*	-1989T→G	rs2071454
7p15.1	Neuropeptide Y	*NPY*	C→T	rs5574
7p22	Eukaryotic translation initiation factor 2-α kinase 1	*EIF2AK1*	C→G	rs10263017
7q11.2	Elastin (supravalvular aortic stenosis, Williams-Beuren syndrome)	*ELN*	659G→C	rs8326
7q11.23	LIM domain kinase 1	*LIMK1*	-916G→A	rs6460071
7q11.23	LIM domain kinase 1	*LIMK1*	-428G→A	rs710968
7q11.23-q21.11	Protein phosphatase 1, regulatory (inhibitor) subunit 3A	*PPP1R3A*	G→T (Ser883Arg)	rs1800000
7q21.3	Paraoxonase 1	*PON1*	584G→A (Gln192Arg)	rs662
7q22.1	Collagen, type I, α 2	*COL1A2*	G→C (Ala459Pro)	rs42524
7q22.1	Cytochrome P450, family 3, subfamily A, polypeptide 5	*CYP3A5*	6986A→G in intron 3	rs776746
7q32	Interferon regulatory factor 5	*IRF5*	A→C	rs3807306
7q32	Paired box 4	*PAX4*	C→T (Arg121Trp)	rs114202595
7q36	Protein tyrosine phosphatase, receptor type, N polypeptide 2	*PTPRN2*	C→T	rs1638021
9q22.3	WNK lysine deficient protein kinase 2	*WNK2*	G→T	rs16936752
9q22-q31	ATP-binding cassette, sub-family A (ABC1), member 1	*ABCA1*	2583A→G (Ile823Met)	rs4149313
9q32	Zinc finger protein 618	*ZNF618*	C→T	rs1330171

Table I. Continued.

Locus	Gene	Symbol	Polymorphism	dbSNP
9q32-q33.3	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	*PTGS1*	A→T	rs10306135
9q33.3	Mitogen-activated protein kinase associated protein 1	*MAPKAP1*	A→G	rs10986769
9q34	Zinc finger protein 79	*ZNF79*	C→T	rs10819291
10q11.2	Arachidonate 5-lipoxygenase	*ALOX5*	G→A (Glu254Lys)	rs2228065
10q11.2	Protein kinase, cGMP-dependent, type I	*PRKG1*	C→T	rs12247775
10q21-q22	ER lipid raft associated 1	*ERLIN1*	C→T	rs1324694
11q12.2	Coiled-coil domain containing 86	*CCDC86*	G→T	rs480081
11q13	Uncoupling protein 3 (mitochondrial, proton carrier)	*UCP3*	-55C→T	rs1800849
11q13.4	NAD synthetase 1	*NADSYN1*	C→T	rs3814731
11q22.3-23.1	Acetyl-coenzyme A acetyltransferase 1 (acetoacetyl coenzyme A thiolase)	*ACAT1*	-77G→A	rs11545566
11q22-q23	Matrix metallopeptidase 1 (interstitial collagenase)	*MMP1*	-340A→G	rs514921
11q23	Apolipoprotein A-V	*APOA5*	-1131T→C	rs662799
11q23	Apolipoprotein A-V	*APOA5*	-3A→G	rs651821
11q23.3	C1q and tumor necrosis factor related protein 5	*C1QTNF5*	T→A	rs9640
11q23.3-q25	Heat shock 70 kDa protein 8	*HSPA8*	-110A→C	rs1008438
12q22	Leukotriene A4 hydrolase	*LTA4H*	C→T	rs2540475
13q34	Protein Z, vitamin K-dependent plasma glycoprotein	*PROZ*	79G→A	rs3024735
14q11.2	Solute carrier family 7 (cationic amino acid transporter, y^+ system), member 8	*SLC7A8*	C→G	rs1998055
15q14-q15	Isovaleryl coenzyme A dehydrogenase	*IVD*	A→G	rs2075624
15q21	Spectrin, β, non-erythrocytic 5	*SPTBN5*	A→G	rs4923918
15q22	Promyelocytic leukemia	*PML*	C→T	rs3784562
16p11	Interleukin 21 receptor	*IL21R*	C→T	rs3093412
16p11.2	Vitamin K epoxide reductase complex, subunit 1	*VKORC1*	2255T→C	rs2359612
16p13.1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	*ABCC1*	A→G	rs9635480
16p13.3	Transducin (β)-like 3	*TBL3*	A→C	rs8053843
16q23.3	Oxidative stress induced growth inhibitor 1	*OSGIN1*	C→G	rs824400
17p11.1	A kinase (PRKA) anchor protein 10	*AKAP10*	2073A→G (Ile646Val)	rs203462
17pter-p12	Glycoprotein Ib (platelet), α polypeptide	*GP1BA*	-5T→C	rs2243093
17q11.2-q12	Chemokine (C-C motif) ligand 5	*CCL5*	-403G→A	rs2107538
17q23-qter	Apolipoprotein H (β-2-glycoprotein I)	*APOH*	341G→A (Ser88Asn)	rs1801692
19p13.3	Resistin	*RETN*	62G→A	rs3745368
19p13.3-p13.2	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	*ICAM1*	G→A (Glu469Lys)	rs5498
19q13.13	Spectrin, β, non-erythrocytic 4	*SPTBN4*	C→T	rs7258094
19q13.2	Apolipoprotein E	*APOE*	3932T→C (Cys112Arg)	rs429358
19q13.3	RuvB-like 2 (*E. coli*)	*RUVBL2*	C→T	rs753307
20p11.2-p11.1	Phosphorylase, glycogen; brain	*PYGB*	C→T	rs2474777
20q13.3	Cadherin 4, type 1, R-cadherin (retinal)	*CDH4*	A→G	rs11698886
20q13.33	TAF4 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 135 kDa	*TAF4*	G→T	rs2296086
22q11.2-q13.2	Phospholipase A2, group III	*PLA2G3*	A→G	rs5753472
22q12	Heme oxygenase (decycling) 1	*HMOX1*	G→C (Asp7His)	rs2071747
22q13	Sterol regulatory element binding transcription factor 2	*SREBF2*	1784G→C (Gly595Ala)	rs4822063
22q13.3	Cadherin, EGF LAG seven-pass G-type receptor 1 (flamingo homolog, *Drosophila*)	*CELSR1*	A→C	rs9615362
22q13.3	Cadherin, EGF LAG seven-pass G-type receptor 1 (flamingo homolog, *Drosophila*)	*CELSR1*	C→T	rs4044210
22q13.3	Cadherin, EGF LAG seven-pass G-type receptor 1 (flamingo homolog, *Drosophila*)	*CELSR1*	C→T	rs6007897
22q13.33	Pannexin 2	*PANX2*	A→G	rs6010152
22q13.33	SET binding factor 1	*SBF1*	A→G	rs2236030

Table II. Primers, probes and other conditions for genotyping of single nucleotide polymorphisms (SNPs) for genes *MMP1*, *LIMK1* and *TBL3* related (P<0.01) to thoracic aortic aneurysm by the Chi-square test.

	MMP1	*LIMK1*	*TBL3*
SNP	A→G (rs514921)	G→A (rs6460071)	A→C (rs8053843)
Sense primer	ACAGCCATCAGGTGCGCAGTGTTA	CAACAGAGCGAGACCGAATCTAA	GGTTGCCGTTGCTCCTTCAGC
Antisense primer	ACACAGGTCAAAGAGTACTCCATG	CAGCTCCAACTCTAGTCATCATG	CTCGTGGCCTGGCAGCTCTGA
Probe 1	GTTCTGATGGTCATAAAGTGCTAC	CTTTGCAGACATGCCTAGAC	CCCAGATGATACATATCTTGTC
Probe 2	GTAGCACCTTATGACCATCAG	CTTTGCAGACGTGCCTAGA	GCCGTGACAAGATCTGTATCA
Annealing (˚C)	60	60	60
Cycles	50	50	50

Primers and probes are listed in the 5'→3' direction.

103 subjects with TAA comprised unrelated Japanese individuals (65 men, 38 women; mean age ± SD, 63.3±11.9 years) who were admitted to the emergency ward of the participating hospital and were examined initially on the basis of a chest X-ray and echocardiography followed by contrast medium-enhanced chest CT. The 103 subjects included 13 patients with an aortic aneurysm who were treated with conservative therapy because of the small aneurysm size (<50 mm), 27 patients with an aortic dissecting aneurysm of Stanford type A for whom surgical repair was contraindicated because of severe complications and advanced age, and 63 patients with an aortic dissecting aneurysm of Stanford type B who were treated with conservative therapy.

Aortic aneurysm was defined as a permanent localized dilation of the aorta with a ≥50% increase in diameter relative to the expected normal diameter of the artery or with a diameter of >5 cm (9). Aortic dissection was defined as a separation of the layers of the aortic wall, resulting in the formation of a true or false lumen, or as intramural hematoma revealed by enhanced chest CT (10). Individuals with Marfan syndrome, Ehlers-Danlos syndrome, traumatic aneurysm, a bicuspid aortic valve, arteritis, pseudoaneurysm, mycotic aneurysm, connective tissue disorders, congenital malformations of the heart or vessels, a familial history of other aortic disease, or renal or endocrinological diseases that cause secondary hypertension were excluded from the study.

An unfavorable outcome was defined as: i) death from cardiovascular causes or aneurysm rupture, ii) progression of aortic aneurysm, iii) conversion to surgical repair or iv) the occurrence of cardiovascular events after initial hospitalization. All patients were followed up with enhanced chest CT according to the standard care for aortic aneurysm. The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University Graduate School of Medicine and Gifu Prefectural Tajimi Hospital. Each patient gave written informed consent in order to participate in the study.

Selection of polymorphisms. The 95 polymorphisms of 89 genes examined in the present study (Table I) were selected either with the use of public databases [including PubMed (NCBI) and Online Mendelian Inheritance in Man (NCBI)] from polymorphisms of candidate genes that might be related to vascular diseases, or on the basis of the results (P-value for allele frequency of $<1.0\times10^{-7}$) of genome-wide association studies of ischemic stroke or myocardial infarction performed with the GeneChip Human Mapping 500K Array Set (Affymetrix, Santa Clara, CA) (11,12).

Genotyping of polymorphisms. Venous blood (7 ml) was collected into tubes containing 50 mmol/l EDTA (disodium salt), and genomic DNA was isolated with the use of a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 95 polymorphisms were determined at G&G Science (Fukushima, Japan) with a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with analysis by suspension array technology (Luminex 100 flow cytometer; Luminex, Austin, TX). Primers, probes, and other conditions for genotyping of polymorphisms related to TAA by the Chi-square test are shown in Table II. Detailed methodology for genotyping was previously described (13).

Statistical analysis. Quantitative data were compared between subjects with a favorable or unfavorable outcome of TAA with the unpaired Student's t-test. Categorical data were compared with the Chi-square test. Allele frequencies were estimated by the gene counting method. In the initial screen, the genotype distribution of each polymorphism was compared between subjects with favorable or unfavorable outcomes of TAA with the Chi-square test (3x2). Given the multiple comparisons of genotypes with the outcome of TAA, the false discovery rate (FDR) was calculated from the distribution of P-values for the 95 polymorphisms. Polymorphisms with a P-value of <0.01 in the Chi-square test were further examined by multivariable logistic regression analysis with adjustment for covariates, with the outcome of TAA as a dependent variable and independent variables including age, gender (0, woman; 1, man), body mass index (BMI), smoking status (0, nonsmoker; 1, current or former smoker), past medical history (0, no history of diabetes mellitus, hypercholesterolemia, hypertension, chronic kidney disease or prior cardiac surgery; 1, positive history), clinical presentation (0, no presentation with chest or back pain, hypotension or shock, or aortic dissecting aneurysm of Stanford type A; 1, positive presentation), and genotype of

Table III. Baseline characteristics of the study subjects with thoracic aortic aneurysm including dissection (TAA) classified according to long-term outcome.

Characteristic	Favorable outcome (n=37)	Unfavorable outcome (n=66)	P-value
Age (years)	61.1±11.7	64.6±11.9	0.1683
Female	13 (35.1)	25 (37.9)	0.7816
Body mass index (kg/m^2)	23.7±3.6	23.3±3.3	0.5213
Stanford type A	5 (13.5)	22 (33.3)	**0.0093**
Past medical history			
Smoking	16 (43.2)	32 (48.5)	0.6089
Hypertension	35 (94.6)	53 (80.3)	**0.0485**
Dyslipidemia	21 (56.8)	44 (66.7)	0.3192
Diabetes mellitus	12 (32.4)	13 (19.7)	0.1529
Chronic kidney disease	8 (21.6)	23 (34.9)	0.1603
Prior cardiac surgery	1 (2.7)	10 (15.2)	**0.0310**
Family history of aneurysm	3 (8.1)	4 (6.1)	0.6953
Clinical presentation			
Chest or back pain	35 (94.6)	51 (77.3)	**0.0231**
Migrating pain	11 (29.7)	20 (30.3)	0.9514
Radiating pain	3 (8.1)	11 (16.7)	0.2240
Any pulse deficit	1 (2.7)	4 (6.1)	0.4468
Hypotension or shock	1 (2.7)	9 (13.6)	**0.0492**
Inferior myocardial infarction	1 (2.7)	3 (4.6)	0.6332
Pericardial effusion	2 (5.4)	11 (16.7)	0.0987
Stroke	1 (2.7)	6 (9.1)	0.1859
Paraplegia	2 (5.4)	1 (1.5)	0.2600
Acute renal failure	2 (5.4)	11 (16.7)	0.0987
Mesenteric ischemia	2 (5.4)	4 (6.1)	0.8917
Limb ischemia	3 (8.1)	3 (4.6)	0.4589
ECG abnormalities	8 (21.6)	21 (31.8)	0.2696
Maximum aneurysm diameter (mm)	38.3±6.3	51.4±12.5	**<0.0001**
Reference aortic diameter (mm)	32.0±4.9	34.7±8.0	0.0856

Data are presented as the number of patients (%) or as mean ± SD. P<0.05 are shown in bold.

each polymorphism; P-values, odds ratios, and 95% confidence intervals were calculated. Genotype was assessed according to dominant, recessive, and additive genetic models. Additive models included the additive 1 (heterozygotes vs. wild-type homozygotes) and additive 2 (variant homozygotes vs. wild-type homozygotes) models, which were analyzed simultaneously with a single statistical model. We also performed a stepwise forward selection procedure to examine the effects of genotypes as well as of other covariates on the long-term outcome of TAA. The receiver operating characteristic (ROC) curve was determined to assess the predictive power based on the calculation of the area under the curve (AUC). Differences in survival were evaluated by Kaplan-Meier curves and the log-rank test. With the exception of the initial screen by the Chi-square test (FDR <0.05), a P-value of <0.05 was considered to denote statistical significance. Statistical significance was examined by two-sided tests, which were performed with JMP version 5.1 software (SAS Institute, Cary, NC).

Results

The baseline characteristics of the study subjects are shown in Table III. The proportion of subjects with an aortic dissecting aneurysm of Stanford type A, the prevalence of prior cardiac surgery or of presentation with hypotension or shock, and the maximum aneurysm diameter were greater, whereas the prevalence of hypertension or of presentation with chest or back pain was lower, in subjects with an unfavorable outcome of TAA than in those with a favorable outcome. Evaluation of genotype distributions with the Chi-square test revealed that three polymorphisms were related (P<0.01) to the outcome of TAA. Among these polymorphisms, the -340A→G polymorphism (rs514921) of the matrix metallopeptidase 1 gene (*MMP1*) was significantly (FDR <0.05) associated with the outcome of TAA (Table IV).

Multivariate logistic regression analysis with adjustment for age, gender, BMI, smoking status, and the prevalence of

Table IV. Genotype distributions of polymorphisms related to the outcome of thoracic aortic aneurysm including dissection (TAA) as determined with the Chi-square test (P<0.01).

Gene	Polymorphism	dbSNP	Favorable outcome[a]	Unfavorable outcome[a]	Genotype P-value	Genotype FDR
MMP1	A→G	rs514921			0.0008	0.0499
	AA		17 (46.0)	54 (81.8)		
	AG		18 (48.7)	10 (15.2)		
	GG		2 (5.4)	2 (3.0)		
LIMK1	G→A	rs6460071			0.0066	0.1987
	GG		35 (94.6)	49 (74.2)		
	GA		1 (2.7)	16 (24.2)		
	AA		1 (2.7)	1 (1.5)		
TBL3	A→C	rs8053843			0.0073	0.1987
	AA		27 (73.0)	61 (92.4)		
	AC		10 (27.0)	5 (7.6)		
	CC		0 (0.0)	0 (0.0)		

[a]Numbers denote the number of subjects (%) with polymorphism among those with favorable (n=37) or unfavorable (n=66) outcome. FDR, false discovery rate.

diabetes mellitus, hypercholesterolemia, hypertension, chronic kidney disease, prior cardiac surgery, chest or back pain, hypotension or shock, and aortic dissecting aneurysm of Stanford type A revealed that rs514921 of *MMP1* (dominant and additive 1 models) was significantly associated with the outcome of TAA, with the minor *G* allele being protective against an unfavorable outcome (Table V).

We performed a stepwise forward selection procedure to examine the effects of *MMP1* genotype as well as age, gender, BMI, smoking status, diabetes mellitus, hypercholesterolemia, hypertension, chronic kidney disease, prior cardiac surgery, chest or back pain, hypotension or shock, and aortic dissecting aneurysm of Stanford type A on the outcome of TAA. *MMP1* genotype was examined according to a dominant model on the basis of statistical significance in the multivariable logistic regression analysis. In descending order of statistical significance, *MMP1* genotype [dominant model; contribution rate (R^2)=0.0879, P=0.0019], aortic dissecting aneurysm of Stanford type A (R^2=0.0528, P=0.0161), and chronic kidney disease (R^2=0.0267, P=0.0478) were significant and independent determinants of the outcome of TAA.

We determined the AUC of the ROC curve to assess the predictive power of the maximum aneurysm diameter for the outcome of TAA. The AUC for maximum aneurysm diameter was 0.8483±0.0402 (mean ± SD). For the prediction of an unfavorable outcome, the optimal cut-off value for maximum aneurysm diameter from the ROC curve was 43.5 mm. A maximum aneurysm diameter of >43.5 mm showed a sensitivity of 81.0%, specificity of 85.3%, positive predictive value of 91.1%, negative predictive value of 70.7%, accuracy of 82.5%, and odds ratio of 3.11 for prediction of an unfavorable outcome of TAA.

We examined the relation of rs514921 of *MMP1* to the maximum aneurysm diameter in all subjects with TAA. The maximum aneurysm diameter was significantly (P=0.0167) smaller in the combined group of the *AG* and *GG* genotypes (42.3±14.0 mm, mean ± SD) than in subjects with the *AA* genotype (48.8±11.2 mm).

Finally, we generated Kaplan-Meier survival curves for all TAA patients according to *MMP1* genotype (Fig. 1). The log-rank test revealed that the curve for the combined group of the *AG* and *GG* genotypes differed significantly from that for the *AA* genotype (P=0.0146).

Discussion

Degenerative and dissecting aneurysms of the thoracic aorta develop as a result of progressive weakening of the aortic wall. They are associated with characteristic histological features including medial degeneration, which is referred to as cystic medial necrosis and involves degeneration and fragmentation of elastic fibers as well as loss of smooth muscle cells and an accumulation of basophilic ground substances (14). Studies on the pathophysiological mechanism of TAA have focused on elastin degradation, smooth muscle cell depletion, and increased expression and tissue localization of elastin- and collagen-degrading enzymes-in particular, matrix metallopeptidases (MMPs) (15). The up-regulation of MMPs has been found to contribute to the degeneration of elastic fibers and the deterioration of aortic contraction and mechanical properties associated with the pathogenesis of TAA (16).

MMP1 is located at chromosome 11q22-q23, encodes an enzyme that initiates the breakdown of interstitial collagen, including types I, II, and III, and is expressed in a wide variety of cell types such as stromal fibroblasts, macrophages, and epithelial cells (17). MMP1 is thus a major peptidase of the MMP family and plays a prominent role in collagen degradation, specifically that of collagen type I, which is important for remodeling of the extracellular matrix and cell-matrix interactions (18). The serum concentrations of MMP1, MMP3,

Table V. Multivariable logistic regression analysis of polymorphisms related to the outcome of thoracic aortic aneurysm including dissection (TAA) by the Chi-square test.

		Dominant		Recessive		Additive 1		Additive 2	
Gene	Polymorphism (dbSNP)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
MMP1	A→G (rs514921)	**0.0288**	0.24 (0.06-0.83)	0.4631		**0.0375**	0.11 (0.06-0.89)	0.3178	
LIMK1	G→A (rs6460071)	0.9205		0.1599		0.9053		0.9224	
TBL3	A→C (rs8053843)	0.0701				0.0701			

Multivariable logistic regression analysis was performed with adjustment for age, gender, body mass index, smoking status, the prevalence of diabetes mellitus, hypercholesterolemia, hypertension, chronic kidney disease, prior cardiac surgery, chest or back pain, hypotension or shock, and aortic dissecting aneurysm of Stanford type A. OR, odds ratio; CI, confidence interval. P<0.05 are shown in bold.

Figure 1. Kaplan-Meier event-free survival curves for comparison in a dominant model of genotypes for the -340A→G polymorphism (rs514921) of *MMP1*.

and MMP9 have been found to be increased in patients with acute aortic dissection (19). Expression of *MMP1* and *MMP9* was also shown to be increased in tissue affected by aortic aneurysm or dissection (20). Such up-regulation of *MMP1* and *MMP9* expression may result in increased proteolysis and matrix degradation, which is characteristic of aortic aneurysms (21). The level of *MMP1* expression is influenced by polymorphism in the promoter region of *MMP1* (22). Such polymorphism affects both transcription of *MMP1* and enzyme activity (23). Indeed, the -340A→G polymorphism (rs514921) of *MMP1* affects the binding of transcription factors and influences the level of *MMP1* expression (22,24). The *G* allele of this polymorphism was associated with a lower level of *MMP1* expression and was found to be protective against the development of myocardial infarction (24). We have now shown that the *G* allele of rs514921 in *MMP1* was significantly associated with a favorable long-term outcome of TAA and with a smaller maximum aneurysm diameter. This association might be attributable to the reduced level of enzyme activity also associated with the *G* allele compared with the *A* allele, although the underlying molecular mechanism remains to be elucidated.

The maximum aneurysm diameter is a well-established determinant of the prognosis of TAA, being one of the most important predictors for the development of adverse clinical events after medical treatment in patients with this condition. The combination of genetic analysis and non-invasive vascular imaging thus holds promise for the stratification of TAA patients according to risk for progression and rupture. We have now shown that risk stratification of TAA patients according to maximum aneurysm diameter (>43.5 mm) and the *AA* genotype of *MMP1* may be useful for prediction of an unfavorable outcome of TAA.

Although we have provided evidence for an association of the *MMP1* genotype with the long-term outcome of TAA, there are several limitations to our study: i) measurement of parameters of structural remodeling in the aorta was not performed in TAA patients; ii) given the small number of

subjects with TAA studied, it is not possible to avoid type I or II error completely; iii) it is also possible that rs514921 is in linkage disequilibrium with polymorphisms in the same gene or nearby genes that are actually responsible for the outcome of TAA; iv) the functional relevance of the association of rs514921 with TAA outcome remains to be determined; v) given that the results of the present study were not replicated, validation of our findings will require their replication with independent subject panels.

In conclusion, our present results suggest that the *G* allele of rs514921 in *MMP1* is associated with a favorable outcome for Japanese patients with TAA. Determination of genotype for this polymorphism may prove informative for assessment of the long-term outcome of TAA and may contribute to the appropriate and timely selection of stage-adapted therapy including surgical or endovascular repair.

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