

Identification of *EGFLAM*, *SPATC1L* and *RNASE13* as novel susceptibility loci for aortic aneurysm in Japanese individuals by exome-wide association studies

YOSHIJI YAMADA^{1,2}, JUN SAKUMA^{2,4}, ICHIRO TAKEUCHI^{2,4,5}, YOSHIKI YASUKOCHI^{1,2}, KIMIHIKO KATO^{1,6}, MITSUTOSHI OGURI^{1,7}, TETSUO FUJIMAKI⁸, HIDEKI HORIBE⁹, MASA-AKI MURAMATSU¹⁰, MOTOJI SAWABE¹¹, YOSHINORI FUJIWARA¹², YU TANIGUCHI¹², SHUICHI OBUCHI¹³, HISASHI KAWAI¹³, SHOJI SHINKAI¹⁴, SEIJIRO MORI¹⁵, TOMIO ARAI¹⁶ and MASASHI TANAKA¹⁷

¹Department of Human Functional Genomics, Advanced Science Research Promotion Center, Mie University, Tsu, Mie 514-8507; ²CREST, Japan Science and Technology Agency, Kawaguchi 332-0012;

³Computer Science Department, College of Information Science, University of Tsukuba, Tsukuba 305-8573;

⁴RIKEN Center for Advanced Intelligence Project, Tokyo 103-0027; ⁵Department of Computer Science,

Nagoya Institute of Technology, Nagoya 466-8555; ⁶Department of Internal Medicine, Meitoh Hospital,

Nagoya 465-0025; ⁷Department of Cardiology, Kasugai Municipal Hospital, Kasugai 486-8510; ⁸Department of

Cardiovascular Medicine, Inabe General Hospital, Inabe 511-0428; ⁹Department of Cardiovascular Medicine,

Gifu Prefectural Tajimi Hospital, Tajimi 507-8522; ¹⁰Department of Molecular Epidemiology, Medical Research Institute,

Tokyo Medical and Dental University, Tokyo 101-0062; ¹¹Section of Molecular Pathology,

Graduate School of Health Care Sciences, Tokyo Medical and Dental University, Tokyo 113-8510;

¹²Research Team for Social Participation and Community Health, ¹³Research Team for Promoting Support System

for Home Care, and ¹⁴Research Team for Social Participation and Health Promotion, Tokyo Metropolitan

Institute of Gerontology; ¹⁵Center for Promotion of Clinical Investigation, ¹⁶Department of Pathology, and

¹⁷Department of Clinical Laboratory, Tokyo Metropolitan Geriatric Hospital, Tokyo 173-0015, Japan

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Abstract. We performed an exome-wide association study (EWAS) to identify genetic variants - in particular, low-frequency or rare variants with a moderate to large effect size - that confer susceptibility to aortic aneurysm with 8,782 Japanese subjects (456 patients with aortic aneurysm, 8,326 control individuals) and with the use of Illumina HumanExome-12 DNA Analysis BeadChip or Infinium Exome-24 BeadChip arrays. The correlation of allele frequencies for 41,432 single nucleotide polymorphisms (SNPs) that passed quality control to aortic aneurysm was examined with Fisher's exact test. Based on Bonferroni's correction, a P-value of $<1.21 \times 10^{-6}$ was considered statistically significant. The EWAS revealed 59 SNPs that were significantly associated with aortic aneurysm. None of these

SNPs was significantly ($P < 2.12 \times 10^{-4}$) associated with aortic aneurysm by multivariable logistic regression analysis with adjustment for age, gender and hypertension, although 8 SNPs were related ($P < 0.05$) to this condition. Examination of the correlation of these latter 8 SNPs to true or dissecting aortic aneurysm separately showed that rs1465567 [T/C (W229R)] of the EGF-like, fibronectin type III, and laminin G domains gene (*EGFLAM*) (dominant model; $P = 0.0014$; odds ratio, 1.63) was significantly ($P < 0.0016$) associated with true aortic aneurysm. We next performed EWASs for true or dissecting aortic aneurysm separately and found that 45 and 19 SNPs were significantly associated with these conditions, respectively. Multivariable logistic regression analysis with adjustment for covariates revealed that rs113710653 [C/T (E231K)] of the spermatogenesis- and centriole associated 1-like gene (*SPATC1L*) (dominant model; $P = 0.0002$; odds ratio, 5.32) and rs143881017 [C/T (R140H)] of the ribonuclease A family member 13 gene (*RNASE13*) (dominant model; $P = 0.0006$; odds ratio, 5.77) were significantly ($P < 2.78 \times 10^{-4}$ or $P < 6.58 \times 10^{-4}$, respectively) associated with true or dissecting aortic aneurysm, respectively. *EGFLAM* and *SPATC1L* may thus be susceptibility loci for true aortic aneurysm and *RNASE13* may be such a locus for dissecting aneurysm in Japanese individuals.

Correspondence to: Professor Yoshiji Yamada, Department of Human Functional Genomics, Advanced Science Research Promotion Center, Mie University, 1577 Kurima-machiya, Tsu, Mie 514-8507, Japan
E-mail: yamada@gene.mie-u.ac.jp

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Introduction

Aortic aneurysm is a serious condition that results from an atherosclerotic aorta and is a leading cause of mortality in humans (1). Studies on the genetic basis of familial aortic aneurysm have centered on the relationship between the condition to systemic connective tissue disorders such as Marfan syndrome (2) and Ehlers-Danlos syndrome (3). Although the molecular mechanism underlying nonsyndromic aortic aneurysm is complex and has not been determined definitively, several risk factors, including age, arteriosclerosis, hypertension and inflammatory or autoimmune diseases that affect the aorta, have been identified clinically (4,5). In addition to these conventional risk factors, recent studies have shown the importance of genetic factors in the development of sporadic aortic aneurysm by revealing a heritability of ~70% (6). Genome-wide association studies (GWASs) have uncovered several loci and genes that confer susceptibility to aortic aneurysm in European ancestry populations (7-12), but genetic variants that contribute to the development of this condition in Japanese individuals have not been identified definitively.

Genetic variants previously associated with aortic aneurysm typically have a minor allele frequency (MAF) of >10% and a small individual effect size (7-12). Given that these common variants explain only a small fraction of disease heritability, low-frequency (MAF of 0.5-5%) or rare (MAF of <0.5%) variants with a larger effect size may contribute to the genetic architecture of aortic aneurysm (13).

In the present study, we performed exome-wide association studies (EWASs) with the use of exome array-based genotyping methods to identify single nucleotide polymorphisms (SNPs) - in particular, low-frequency or rare coding variants with a moderate to large effect size - that confer susceptibility to aortic aneurysm in Japanese individuals. Given that most low-frequency or rare variants were not included in the arrays of previous GWASs, we used Illumina HumanExome-12 DNA Analysis BeadChip or Infinium Exome-24 BeadChip arrays, which provide coverage for functional SNPs including low-frequency or rare variants in entire exons.

Materials and methods

Study population. A total of 8,782 Japanese individuals (456 patients with aortic aneurysm, 8,326 controls) was examined. The subjects were recruited from individuals who visited outpatient clinics of or were admitted to participating hospitals (Gifu Prefectural Tajimi Hospital, Tajimi; Gifu Prefectural General Medical Center, Gifu; Japanese Red Cross Nagoya First Hospital, Nagoya; Inabe General Hospital, Inabe; Hirosaki University Hospital and Hirosaki Stroke and Rehabilitation Center, Hirosaki) either because they were experiencing various symptoms or for an annual health checkup between 2002 and 2014; from community-dwelling individuals recruited to a population-based cohort study in Inabe between 2010 and 2014 or in Tokyo or Kusatsu between 2011 and 2015; or from individuals who underwent autopsy at Tokyo Metropolitan Geriatric Hospital from 1995 to 2012.

True aortic aneurysm was defined as a permanent localized dilation of the aorta with a $\geq 50\%$ increase in diameter relative to the expected normal size of the artery or with a diameter

of >5 cm (14). Dissecting aortic aneurysm was defined as separation of the aortic wall layers with resulting true and false lumens or as intramural hematoma (15). The subjects with aortic aneurysm (279 with true aneurysm and 181 with dissecting aneurysm (four had both conditions) were examined by chest and abdominal X-ray and echocardiography followed by contrast medium-enhanced computed tomography. Some subjects were also examined by aortic angiography. Individuals with Marfan syndrome, Ehlers-Danlos syndrome, bicuspid aortic valve disease, aortitis syndrome, connective tissue disorder, congenital malformations of the heart or vessels, pseudoaneurysm, or traumatic aneurysm were excluded from the study. The control individuals had no history of aortic, coronary, or peripheral arterial disease; ischemic or hemorrhagic stroke; intracranial aneurysm; or other atherosclerotic, thrombotic, embolic or hemorrhagic disorders. Autopsy cases without aortic aneurysm were excluded from controls.

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, Hirosaki University Graduate School of Medicine, Tokyo Metropolitan Institute of Gerontology, and participating hospitals. Written informed consent was obtained from each participant or from families of the deceased subjects.

EWASs. Venous blood (5 or 7 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), peripheral blood leukocytes were isolated, and genomic DNA was extracted from these cells either with the use of a DNA extraction kit (Genomix supplied by Talent, Trieste, Italy, or SMITEST EX-R&D supplied by Medical and Biological Laboratories, Nagoya, Japan) or by standard protocols based on phenol-chloroform extraction and spin columns. In autopsy cases, genomic DNA was extracted from kidneys. EWASs were performed for the 456 subjects with aortic aneurysm (or for the 279 subjects with true aneurysm or 181 subjects with dissecting aneurysm) and the 8,326 control subjects with the use of a HumanExome-12 v1.1 or v1.2 DNA Analysis BeadChip or an Infinium Exome-24 v1.0 BeadChip (Illumina, San Diego, CA, USA). These exome arrays include putative functional exonic variants selected from >12,000 individual exome or whole-genome sequences. The exonic content consists of ~244,000 SNPs representing diverse populations, including European, African, Chinese and Hispanic individuals (16). SNPs contained in only one of the exome arrays (~3.6%) were excluded from analysis. We performed quality control (17) as follows. i) Genotyping data with a call rate of <97% were discarded, with the mean call rate for the remaining data being 99.9%. ii) Gender specification was checked for each sample, with samples for which the gender designation in the clinical records was inconsistent with genetic sex being discarded. iii) Duplicated samples and cryptic relatedness were checked by calculation of identity by descent; all pairs with DNA samples showing identity by descent of >0.1875 were inspected and one sample from each pair was excluded. iv) The frequency of heterozygosity of SNPs was calculated for all samples, with those found to have extremely low or high heterozygosity (>3 standard deviations from the mean) being discarded. v) SNPs in sex chromosomes or mitochondrial DNA were excluded from the analysis, as

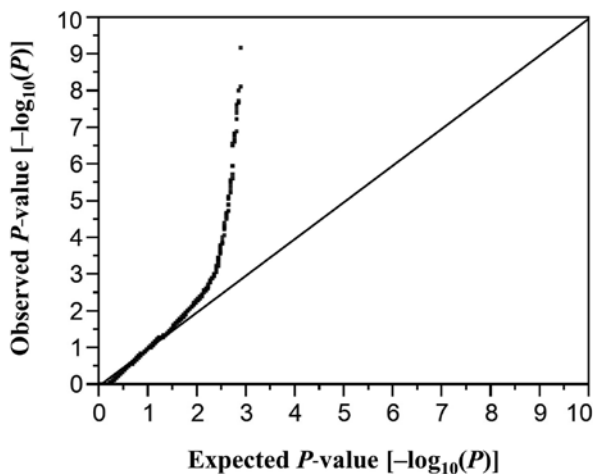


Figure 1. Quantile-quantile plot for P-values of allele frequencies in the exome-wide association study (EWAS) of aortic aneurysm. The observed P-values (y-axis) are compared with the expected P-values (x-axis) under the null hypothesis, with the values being plotted as $-\log_{10}(P)$.

were nonpolymorphic SNPs or SNPs with a MAF of $<0.1\%$. vi) SNPs whose genotype distributions in control individuals deviated significantly ($P<0.001$) from Hardy-Weinberg equilibrium were excluded. vii) The genotype data for each EWAS were examined for population stratification by principal components analysis (18), and population outliers were excluded from the analysis. A total of 41,432 SNPs passed quality control and was subjected to analysis.

Statistical analysis. Quantitative data for characteristics of the study subjects were compared between patients with aortic aneurysm and control individuals with the unpaired Student's t test. Categorical data were compared between the two groups with Fisher's exact test. Allele frequencies were estimated by the gene counting method, and Fisher's exact test was applied to identify departure from Hardy-Weinberg equilibrium. Allele frequencies of SNPs were compared between patients with aortic aneurysm and control subjects with Fisher's exact test. Multivariable logistic regression analysis was performed with aortic aneurysm as a dependent variable and independent variables including age, gender (0, woman; 1, man), the prevalence of hypertension (0, no history of this condition; 1, positive history), and genotype of each SNP. Genotypes of SNPs were assessed according to dominant [0, AA; 1, AB+BB (A, major allele; B, minor allele)], recessive (0, AA+AB; 1, BB), and additive genetic models, and the P-value, odds ratio, and 95% confidence interval were calculated. Additive models comprised additive 1 (0, AA; 1, AB; 0, BB) and additive 2 (0, AA; 0, AB; 1, BB) models, which were analyzed simultaneously with a single statistical model. To compensate for multiple comparisons of genotypes with aortic aneurysm, we applied Bonferroni's correction for statistical significance of association. Given that 41,432 SNPs were finally examined, a P-value of $<1.21 \times 10^{-6}$ ($0.05/41,432$) was considered statistically significant. A quantile-quantile plot for P-values of allele frequencies in the EWAS for aortic aneurysm is shown in Fig. 1. The inflation factor (λ) was 1.57. P-values for other comparisons were similarly adjusted by Bonferroni's correc-

Table I. Characteristics of the 8,782 study subjects.

Characteristics	Aortic aneurysm	Control	P-value
No. of subjects	456	8326	
Age (years)	74.7 \pm 13.5	57.3 \pm 13.5	<0.0001
Gender (male/female, %)	64.0/36.0	51.4/48.6	<0.0001
Body mass index (kg/m ²)	23.2 \pm 3.5	23.1 \pm 3.5	0.7068
Current or former smoker (%)	44.1	39.8	0.1777
Hypertension (%)	94.5	40.7	<0.0001
Diabetes mellitus (%)	44.7	14.7	<0.0001
Dyslipidemia (%)	67.9	56.8	0.0006
Chronic kidney disease (%)	41.9	17.5	<0.0001
Hyperuricemia (%)	25.7	16.1	0.0003

Quantitative data are expressed as means \pm SD and were compared between patients with aortic aneurysm and control individuals with the unpaired Student's t-test. Categorical data were compared with Fisher's exact test. Based on Bonferroni's correction, a P-value of <0.0056 ($0.05/9$) was considered statistically significant.

tion. Statistical tests were performed with JMP Genomics version 6.0 software (SAS Institute, Cary, NC, USA).

Results

Characteristics of the subjects. The characteristics of the subjects enrolled in the study are shown in Table I. Age, the frequency of males, and the prevalence of hypertension, diabetes mellitus, dyslipidemia, chronic kidney disease and hyperuricemia were significantly greater in patients with aortic aneurysm than in control individuals.

EWAS of aortic aneurysm. We examined the correlation of allele frequencies for 41,432 SNPs that passed quality control to aortic aneurysm using the Fisher's exact test. A Manhattan plot for the EWAS of aortic aneurysm is shown in Fig. 2. After Bonferroni's correction, 59 SNPs were found to be significantly ($P<1.21 \times 10^{-6}$) associated with aortic aneurysm (Table II). The genotype distributions of these SNPs were in Hardy-Weinberg equilibrium ($P>0.001$) both among patients with aortic aneurysm and among the control individuals (data not shown).

Multivariable logistic regression analysis of the correlation of SNPs to aortic aneurysm. The relation of the 59 identified SNPs to aortic aneurysm was examined further by multivariable logistic regression analysis with adjustment for age, gender and the prevalence of hypertension. Although 8 SNPs were related ($P<0.05$) to aortic aneurysm, no SNP was significantly [$P<2.12 \times 10^{-4}$ ($0.05/236$)] associated with this condition (Table III). We then examined the correlation of the 8 identified SNPs to true or dissecting aortic aneurysm separately. Five SNPs were related ($P<0.05$) to true aortic aneurysm (Table IV), among which rs1465567 [T/C (W229R)] of EGF-like, fibronectin type III, and laminin G domains gene (EGFLAM) was significantly [$P<0.0016$ ($0.05/32$)] associated with this condition, with the minor C allele representing a risk

Table II. The 59 single nucleotide polymorphisms (SNPs) significantly ($P < 1.21 \times 10^{-6}$) associated with aortic aneurysm in an exome-wide association study (EWAS).

Gene	dbSNP	Nucleotide (amino acid) substitution ^a	Chromosome: position	MAF (%)	P-value (allele)	Allele OR
<i>CATSPER4</i>	rs11247866	A/G (Q77R)	1:26191303	0.4	9.82×10^{-147}	1.81
<i>RNASE13</i>	rs143881017	C/T (R140H)	14:21033870	0.5	2.49×10^{-144}	2.77
<i>RNASE10</i>	rs202109789	G/A (G87S)	14:20510730	0.2	4.32×10^{-123}	0.47
	rs2582513	A/G	14:104948453	39.9	1.72×10^{-118}	0.86
<i>HEATR1</i>	rs193150310	T/A (V1975D)	1:236554752	0.3	3.09×10^{-118}	0.76
<i>KIAA1217</i>	rs10828663	G/A (A807T)	10:24524525	10.4	2.68×10^{-100}	1.00
<i>MTUS1</i>	rs3739407	G/A (R148C)	8:17755366	38.4	2.32×10^{-87}	0.98
<i>OR5W2</i>	rs75634103	G/A	11:55914523	10.4	2.74×10^{-87}	1.11
<i>ALPK1</i>	rs2074379	A/G (I732M)	4:112431743	32.0	1.03×10^{-86}	1.11
<i>ATAD5</i>	rs11657270	T/C (Y1419H)	17:30887369	18.1	3.24×10^{-64}	1.13
<i>ACAT2</i>	rs25683	A/G (K211R)	6:159775311	18.8	1.90×10^{-61}	0.86
<i>ZNF474</i>	rs201335566	G/A (R253Q)	5:122152748	0.5	4.22×10^{-44}	1.29
<i>ZNF804B</i>	rs6963781	A/G (M1105V)	7:89336295	5.1	1.85×10^{-42}	0.90
<i>LOC100506679</i>	rs5751416	G/A	22:43036820	26.3	1.62×10^{-41}	0.81
<i>SSPO</i>	rs191064068	G/A (R209H)	7:149777738	1.1	8.55×10^{-35}	1.13
<i>ARHGEF28</i>	rs536568	A/C	5:73935841	45.8	3.61×10^{-33}	1.03
<i>TMEM2</i>	rs142154818	G/A (T1062M)	9:71700645	1.0	6.41×10^{-33}	1.90
<i>HLA-DMB</i>	rs151719	A/G	6:32936123	25.7	1.11×10^{-30}	1.03
<i>CCDC66</i>	rs61747994	T/C (L802S)	3:56619399	9.8	3.77×10^{-30}	0.92
	rs3135365	T/G	6:32421478	18.9	1.62×10^{-28}	0.84
<i>NAA25</i>	rs12231744	C/T (R876K)	12:112039251	35.1	5.02×10^{-28}	1.07
<i>RALGAPA2</i>	rs142962992	G/C (E1676D)	20:20505435	0.9	1.77×10^{-26}	1.10
<i>NEU1</i>	rs13118	T/A	6:31859509	9.7	6.92×10^{-22}	1.20
<i>AXDND1</i>	rs41267592	C/T (T627M)	1:179468524	0.3	1.42×10^{-20}	0.63
<i>PHYKPL</i>	rs146105181	T/C (N88D)	5:178230016	0.2	2.15×10^{-20}	1.46
<i>PCDH8</i>	rs5030685	A/G (V743A)	13:52846209	0.3	9.92×10^{-20}	2.72
<i>SELE</i>	rs5361	T/G (S149R)	1:1169731919	3.3	2.49×10^{-17}	0.95
<i>MOV10L1</i>	rs760749	A/C (I454L)	22:50117257	27.8	2.97×10^{-17}	1.02
<i>HHLA1</i>	rs75623295	C/G (T90R)	8:132098893	2.9	1.23×10^{-16}	0.80
<i>TUBB1</i>	rs6070697	G/A (R307H)	20:59024347	12.1	2.17×10^{-16}	0.92
<i>ZNF708</i>	rs504280	C/T (R66Q)	19:21294577	7.4	2.16×10^{-15}	0.96
<i>TICRR</i>	rs79501973	G/A (V1373I)	15:89624427	14.7	2.51×10^{-15}	0.97
<i>ADNP</i>	rs148496595	C/G (D924E)	20:50891942	0.3	2.71×10^{-14}	0.68
<i>FCAR</i>	rs11666735	G/A (D113N)	19:54885501	3.2	3.10×10^{-14}	1.03
	rs2823962	G/A	21:16673913	32.8	7.80×10^{-14}	0.93
<i>EGFLAM</i>	rs1465567	T/C (W229R)	5:38370435	25.1	5.92×10^{-13}	1.19
	rs1480347	G/A	8:20489946	17.3	3.49×10^{-12}	1.07
<i>UBE4B</i>	rs180983516	G/A (R331H)	1:10106379	0.8	4.50×10^{-12}	0.60
	rs448705	A/G	8:17837193	12.4	5.42×10^{-12}	1.01
	rs11970286	C/T	6:118359211	17.3	1.38×10^{-11}	0.90
	rs10047727	T/C	13:21743051	42.7	6.67×10^{-10}	0.90
	rs507856	C/T	3:161736158	38.3	7.71×10^{-9}	0.93
<i>SLC1A6</i>	rs7253812	C/A	19:14982691	26.7	9.43×10^{-9}	0.99
<i>FGB</i>	rs1800789	G/A	4:154561591	13.3	1.86×10^{-8}	0.99
<i>SLC9A4</i>	rs1014286	A/G (S784G)	2:102532641	43.9	1.90×10^{-8}	1.13
<i>HECTD4</i>	rs2074356	C/T	12:112207597	25.4	2.22×10^{-8}	1.03
<i>PKDIL1</i>	rs66755489	G/A (P2021L)	7:47835032	2.9	2.50×10^{-8}	1.16
<i>CAMSAP1</i>	rs201291561	T/C (N1062S)	9:135821476	0.2	3.04×10^{-8}	1.14
<i>C7orf43</i>	rs3800952	C/T (R353Q)	7:100160331	6.3	4.08×10^{-8}	1.08

Table II. Continued.

Gene	dbSNP	Nucleotide (amino acid) substitution ^a	Chromosome: position	MAF (%)	P-value (allele)	Allele OR
<i>ZNF671</i>	rs3746207	G/A (A149V)	19:57721640	12.6	5.90x10 ⁻⁸	0.99
<i>RIN3</i>	rs7150931	T/C	14:92671696	46.2	1.26x10 ⁻⁷	1.03
	rs10805579	G/A	5:19127418	10.5	1.48x10 ⁻⁷	1.03
	rs12546220	T/C	8:69461493	29.1	1.82x10 ⁻⁷	0.96
<i>DRD2</i>	rs12363125	C/T	11:113415194	6.2	1.89x10 ⁻⁷	0.88
<i>MTUS2</i>	rs17571410	G/A	13:29007481	41.4	2.35x10 ⁻⁷	0.91
<i>GALNTL5</i>	rs11766982	A/G	7:151996417	27.6	2.56x10 ⁻⁷	1.05
<i>POLE</i>	rs5745022	C/T	12:132632393	20.6	2.82x10 ⁻⁷	0.96
<i>CHAT</i>	rs3810947	A/G	10:49613197	43.0	3.19x10 ⁻⁷	0.97
<i>LILRB5</i>	rs117421142	A/G (I420T)	19:54252383	1.0	1.16x10 ⁻⁶	1.29

Allele frequencies were analyzed with Fisher's exact test. ^aMajor allele/minor allele. MAF, minor allele frequency; OR, odds ratio.

Table III. Correlation of single nucleotide polymorphisms (SNPs) to aortic aneurysm as determined by multivariable logistic regression analysis.

SNP		Dominant		Recessive		Additive 1		Additive 2	
		P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
rs143881017	C/T (R140H)	0.0208	3.00 (1.20-6.80)	0.9665		0.0207	3.00 (1.20-6.79)	0.9667	
rs5751416	G/A	0.0351	0.79 (0.63-0.98)	0.2034		0.0697		0.1055	
rs142154818	G/A (T1062M)	0.0486	1.94 (1.00-3.50)	0.7044		0.0452	1.97 (1.02-3.54)	0.7078	
rs13118	T/A	0.0415	1.32 (1.01-1.71)	0.4182		0.0245	1.37 (1.04-1.78)	0.4879	
rs5030685	A/G (V743A)	0.0293	2.94 (1.13-6.76)	0.6067		0.0227	3.12 (1.19-7.22)	0.6090	
rs1465567	T/C (W229R)	0.0004	1.49 (1.19-1.85)	0.6932		0.0004	1.51 (1.20-1.90)	0.2807	
rs7253812	C/A	0.0834		0.0543		0.0189	1.31 (1.05-1.65)	0.1650	
rs7150931	T/C	0.5033		0.0224	1.36 (1.04-1.75)	0.9225		0.0586	

Multivariable logistic regression analysis was performed with adjustment for age, gender and the prevalence of hypertension. Based on Bonferroni's correction, a P-value of $<2.12 \times 10^{-4}$ (0.05/236) was considered statistically significant. OR, odds ratio; CI, confidence interval.

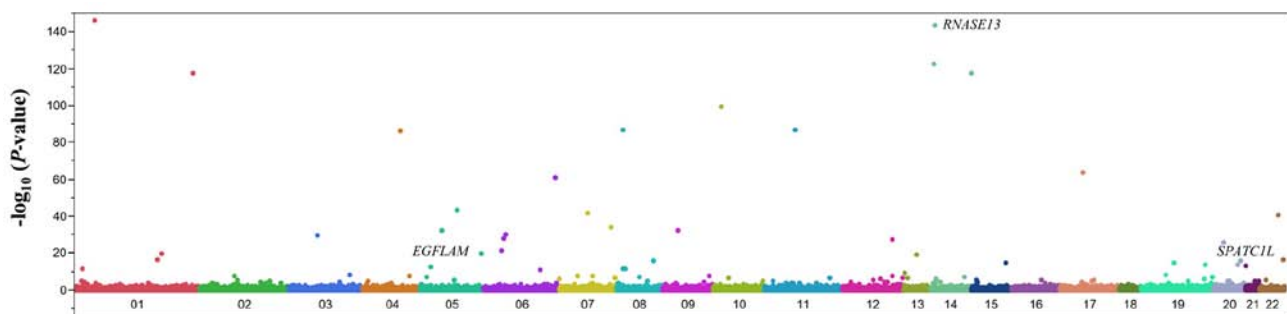


Figure 2. Manhattan plot for P-values of allele frequencies in the exome-wide association study (EWAS) of aortic aneurysm. The P-values (y-axis) are shown as $-\log_{10}(P)$ with respect to the physical chromosomal position of the corresponding single nucleotide polymorphism (SNP) (x-axis). The three SNPs ultimately found to be significantly associated with true aortic aneurysm (*EGFLAM*, *SPATC1L*) or dissecting aneurysm (*RNASE13*) are indicated.

factor. No SNP was found to be related to dissecting aortic aneurysm (data not shown).

EWASs of true or dissecting aortic aneurysm. We next examined the relation of allele frequencies for the total of

Table IV. Correlation of single nucleotide polymorphisms (SNPs) to true aortic aneurysm as determined by multivariable logistic regression analysis.

SNP		Dominant		Recessive		Additive 1		Additive 2	
		P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
rs143881017	C/T (R140H)	0.2899		ND		0.2899		ND	
rs5751416	G/A	0.0448	0.73 (0.54-0.99)	0.1221		0.1030		0.0663	
rs142154818	G/A (T1062M)	0.2214		0.8209		0.2154		0.8231	
rs13118	T/A	0.0253	1.51 (1.05-2.14)	0.6076		0.0167	1.57 (1.09-2.23)	0.6967	
rs5030685	A/G (V743A)	0.0779		0.7314		0.0667		0.7333	
rs1465567	T/C (W229R)	0.0014	1.63 (1.21-2.21)	0.6670		0.0014	1.66 (1.22-2.27)	0.2911	
rs7253812	C/A	0.0122	1.47 (1.09-1.99)	0.2265		0.0032	1.60 (1.17-2.18)	0.5864	
rs7150931	T/C	0.7329		0.0431	1.45 (1.01-2.04)	0.2737		0.2350	

Multivariable logistic regression analysis was performed with adjustment for age, gender and the prevalence of hypertension. Based on Bonferroni's correction, P-values of <0.0016 (0.05/32) were considered statistically significant and are shown in bold. OR, odds ratio; CI, confidence interval; ND, not determined.

41,432 SNPs to true or dissecting aortic aneurysm separately with the use of Fisher's exact test. After Bonferroni's correction, 45 or 19 SNPs were found to be significantly ($P < 1.21 \times 10^{-6}$) associated with true (Table V) or dissecting (Table VI) aortic aneurysm, respectively. The genotype distributions of these SNPs were in Hardy-Weinberg equilibrium ($P > 0.001$) both among patients with true or dissecting aortic aneurysm and among control individuals (data not shown).

Multivariable logistic regression analysis of the correlation of SNPs to true or dissecting aortic aneurysm. The correlation of the 45 identified SNPs to true aortic aneurysm was examined further by multivariable logistic regression analysis with adjustment for age, gender and the prevalence of hypertension. Among these SNPs, rs113710653 [C/T (E231K)] of the spermatogenesis and centriole-associated 1-like gene (*SPATC1L*) was significantly [$P < 2.78 \times 10^{-4}$ (0.05/180)] associated with true aortic aneurysm, with the minor T allele representing a risk factor for this condition (Table VII). The correlation of the 19 identified SNPs to dissecting aortic aneurysm was also further examined by multivariable logistic regression analysis with adjustment for age, gender and the prevalence of hypertension. The SNP rs143881017 [C/T (R140H)] of the ribonuclease A family member 13 gene (*RNASE13*) was significantly [$P < 6.58 \times 10^{-4}$ (0.05/76)] associated with dissecting aortic aneurysm, with the minor T allele representing a risk factor for this condition (Table VII).

Correlation of SNPs to intermediate phenotypes of aortic aneurysm. Finally, we examined the correlation of three SNPs (rs1465567, rs113710653 and rs143881017) to intermediate phenotypes (hypertension, diabetes mellitus, hypertriglyceridemia, hypo-HDL-cholesterolemia, hyper-LDL-cholesterolemia, chronic kidney disease, obesity and hyperuricemia) of aortic aneurysm. No SNP was found to be significantly [$P < 0.0021$ (0.05/24)] associated with intermediate phenotypes (data not shown).

Discussion

True and dissecting aneurysms of the aorta develop as a result of progressive weakening of the vessel wall. They are associated with characteristic histological features including medial degeneration, which involves degeneration and fragmentation of elastic fibers as well as loss of smooth muscle cells and an accumulation of basophilic ground substances (19). In the present study, we showed that rs1465567 [T/C (W229R)] of *EGFLAM* and rs113710653 [C/T (E231K)] of *SPATC1L* were significantly associated with true aortic aneurysm, whereas rs143881017 [C/T (R140H)] of *RNASE13* was significantly associated with dissecting aortic aneurysm, in Japanese individuals. The minor alleles of these SNPs were all risk factors for these conditions.

The *EGFLAM* is located at chromosomal region 5p13.2-p13.1 (NCBI Gene, <https://www.ncbi.nlm.nih.gov/gene>) and is expressed in various tissues and organs including vascular smooth muscle (The Human Protein Atlas, <http://www.protein-atlas.org>). *EGFLAM* is an extracellular matrix-like protein that colocalizes with both dystrophin and dystroglycan to the synaptic cleft of the photoreceptor ribbon synapse in the retina and which directly interacts with dystroglycan. It plays an important role in interactions between the photoreceptor ribbon synapse and bipolar dendrites (20,21), and it is implicated in defective photoreceptor synaptic function associated with congenital muscular dystrophies such as muscle-eye-brain disease caused by defective glycosylation of α -dystroglycan (22). A genome-wide pharmacogenomics study identified *EGFLAM* as a potential susceptibility locus for citalopram-induced side effects (23). We have now shown that rs1465567 [T/C (W229R)] of *EGFLAM* was significantly associated with true aortic aneurysm, with the minor C allele representing a risk factor for this condition, although the molecular mechanism underlying this association remains unclear.

The *SPATC1L* is located at chromosomal region 21q22.3 (NCBI Gene) and is expressed in various tissues

Table V. The 45 single nucleotide polymorphisms (SNPs) significantly ($P < 1.21 \times 10^{-6}$) associated with true aortic aneurysm in an exome-wide association study (EWAS).

Gene	dbSNP	Nucleotide (amino acid) substitution ^a	Chromosome: position	MAF (%)	P-value (allele)	Allele OR
<i>KIAA1217</i>	rs10828663	G/A (A807T)	10:24524525	10.4	6.87×10^{-94}	1.14
<i>NRAP</i>	rs79461687	G/T (H1246Q)	10:113606247	1.3	1.58×10^{-90}	1.67
<i>OR5W2</i>	rs75634103	G/A	11:55914523	10.4	4.84×10^{-81}	1.17
<i>ATAD5</i>	rs11657270	T/C (Y1419H)	17:30887369	18.1	9.29×10^{-70}	0.98
	rs9683944	A/G	4:137512008	10.8	1.65×10^{-56}	0.83
<i>TMPRSS3</i>	rs928302	C/T (V53I)	21:42389975	27.6	1.34×10^{-52}	0.99
<i>ZNF804B</i>	rs6963781	A/G (M1105V)	7:89336295	5.1	6.67×10^{-47}	1.01
<i>ZNF474</i>	rs201335566	G/A (R253Q)	5:122152748	0.5	5.79×10^{-42}	0.84
<i>LOC100506679</i>	rs5751416	G/A	22:43036820	26.3	2.46×10^{-26}	0.71
<i>ARHGEF28</i>	rs536568	A/C	5:73935841	45.8	3.10×10^{-23}	1.03
<i>RALGAPA2</i>	rs142962992	G/C (E1676D)	20:20505435	0.9	8.85×10^{-23}	0.72
<i>LYSMD1</i>	rs79024247	G/T (Q150K)	1:151160974	5.2	7.43×10^{-21}	0.88
<i>CNGA1</i>	rs192912733	C/T (R493Q)	4:47937223	0.7	8.13×10^{-21}	1.17
<i>MOV10L1</i>	rs760749	A/C (I454L)	22:50117257	27.8	1.23×10^{-20}	1.18
<i>HLA-DMB</i>	rs151719	A/G	6:32936123	25.7	1.96×10^{-19}	1.17
<i>TUBB1</i>	rs6070697	G/A (R307H)	20:59024347	12.1	3.20×10^{-18}	0.97
	rs3135365	T/G	6:32421478	18.9	3.94×10^{-17}	0.83
<i>CCDC33</i>	rs1484214	A/C	15:74288732	49.3	6.60×10^{-17}	1.09
<i>ZNF708</i>	rs504280	C/T (R66Q)	19:21294577	7.4	3.03×10^{-15}	0.98
	rs2823962	G/A	21:16673913	32.8	3.36×10^{-14}	0.97
<i>SGCZ</i>	rs1037934	G/A	8:14399065	9.0	3.44×10^{-13}	1.22
<i>DUOX1</i>	rs199549867	A/T (R569S)	15:45141997	0.2	1.58×10^{-10}	0.81
<i>CTSC</i>	rs3888798	T/C (I453V)	11:88294041	16.5	3.21×10^{-10}	1.06
<i>C10orf128</i>	rs118189413	C/G (H67D)	10:49166908	6.3	4.04×10^{-10}	0.84
<i>OR5V1</i>	rs9405124	A/G	6:29401036	19.9	4.15×10^{-10}	0.98
<i>UBAP2L</i>	rs143080179	T/C (S641P)	1:154255163	1.3	4.20×10^{-10}	0.66
<i>CAMSAP1</i>	rs201291561	T/C (N1062S)	9:135821476	0.2	5.67×10^{-10}	1.87
<i>TICRR</i>	rs79501973	G/A (V1373I)	15:89624427	14.7	7.76×10^{-10}	1.04
<i>NCAM1</i>	rs7111410	C/T	11:113178565	15.9	2.63×10^{-9}	0.80
<i>IRGQ</i>	rs3817	C/A	19:43586043	47.7	3.30×10^{-9}	0.92
<i>SSPO</i>	rs191064068	G/A (R209H)	7:149777738	1.1	2.35×10^{-8}	1.67
<i>SLC9A4</i>	rs1014286	A/G (S784G)	2:102532641	43.9	2.84×10^{-8}	1.11
	rs2138852	A/G	17:29376331	2.5	2.92×10^{-8}	0.77
<i>SPATC1L</i>	rs113710653	C/T (E231K)	21:46161921	1.9	3.91×10^{-8}	7.39
<i>DRD2</i>	rs12363125	C/T	11:113415194	6.2	4.99×10^{-8}	0.87
<i>CCT5</i>	rs201280643	C/G (S373C)	5:10262584	0.4	7.04×10^{-8}	0.99
<i>AXDND1</i>	rs41267592	C/T (T627M)	1:179468524	0.3	7.85×10^{-8}	1.03
	rs507856	C/T	3:161736158	38.3	1.10×10^{-7}	1.02
	rs962040	A/G	8:15454369	30.1	1.21×10^{-7}	0.96
	rs11970286	C/T	6:118359211	17.3	1.60×10^{-7}	0.83
<i>NINL</i>	rs199671123	C/T (A796T)	20:25476905	0.2	2.45×10^{-7}	0.85
	rs12531488	C/T	7:145194993	16.0	7.91×10^{-7}	1.14
<i>MROH7</i>	rs143029488	G/C (A1313P)	1:54710152	0.8	1.05×10^{-6}	1.34
<i>AFAP1</i>	rs28406288	G/C (C403S)	4:7800500	0.1	1.10×10^{-6}	ND
<i>GALM</i>	rs6741892	A/T (N190Y)	2:38689828	20.1	1.12×10^{-6}	0.93

Allele frequencies were analyzed with Fisher's exact test. ^aMajor allele/minor allele. MAF, minor allele frequency; OR, odds ratio; ND, not determined.

Table VI. The 19 single nucleotide polymorphisms (SNPs) significantly ($P < 1.21 \times 10^{-6}$) associated with dissecting aortic aneurysm in an exome-wide association study (EWAS).

Gene	dbSNP	Nucleotide (amino acid) substitution ^a	Chromosome: position	MAF (%)	P-value (allele)	OR
<i>ATXN7</i>	rs3774729	G/A (V862M)	3:63996406	46.8	3.81×10^{-35}	0.91
<i>KIAA1217</i>	rs10828663	G/A (A807T)	10:24524525	10.4	1.34×10^{-27}	0.78
<i>RNASE13</i>	rs143881017	C/T (R140H)	14:21033870	0.5	5.12×10^{-26}	4.48
<i>INPP5F</i>	rs3736822	A/G (I453V)	10:119806397	1.7	8.50×10^{-23}	0.62
	rs9683944	A/G	4:137512008	10.8	4.61×10^{-17}	1.17
	rs9610342	A/G	22:35734530	30.7	8.20×10^{-17}	0.79
<i>FAM98C</i>	rs3745962	C/A (T240K)	19:38405604	16.7	8.99×10^{-15}	0.96
<i>ZNF474</i>	rs201335566	G/A (R253Q)	5:122152748	0.5	1.02×10^{-12}	1.95
	rs3135365	T/G	6:32421478	18.9	1.21×10^{-12}	0.86
<i>DEPDC7</i>	rs34161108	G/A (A192T)	11:33027795	6.2	2.29×10^{-12}	0.74
<i>ARHGEF28</i>	rs536568	A/C	5:73935841	45.8	5.53×10^{-12}	1.04
<i>RALGPS1</i>	rs57728614	G/T (G383C)	9:127196583	9.5	5.73×10^{-11}	0.84
<i>AIM1L</i>	rs34370465	C/T (R847H)	1:26344118	23.9	3.63×10^{-10}	1.09
<i>ANXA7</i>	rs3750575	C/T (R419Q)	10:73378933	5.7	4.20×10^{-10}	1.06
<i>AXDND1</i>	rs41267592	C/T (T627M)	1:179468524	0.3	1.77×10^{-8}	ND
	rs2138852	A/G	17:29376331	2.5	1.49×10^{-7}	1.32
<i>CHAT</i>	rs78925077	C/G (S119R)	10:49622109	0.5	1.50×10^{-7}	1.93
<i>GPR156</i>	rs902790	A/T (E512D)	3:120167929	4.8	2.24×10^{-7}	0.71
<i>SELE</i>	rs5361	T/G (S149R)	1:169731919	3.3	3.58×10^{-7}	0.98

Allele frequencies were analyzed with Fisher's exact test. ^aMajor allele/minor allele. MAF, minor allele frequency; OR, odds ratio; ND, not determined.

Table VII. Relation of single nucleotide polymorphisms (SNPs) to true or dissecting aortic aneurysm as determined by multivariable logistic regression analysis.

SNP	Dominant		Recessive		Additive 1		Additive 2	
	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
True aortic aneurysm								
rs113710653 C/T (E231K)	0.0002	5.32 (2.33-11.14)	0.9263		0.0002	5.34 (2.34-11.18)	0.9299	
Dissecting aortic aneurysm								
rs143881017 C/T (R140H)	0.0006	5.77 (2.25-12.95)	0.9615		0.0006	5.48 (2.26-12.97)	0.9621	

Multivariable logistic regression analysis was performed with adjustment for age, gender and the prevalence of hypertension. Based on Bonferroni's correction, P-values of $< 2.78 \times 10^{-4}$ (0.05/180) or $< 6.58 \times 10^{-4}$ (0.05/76) were considered statistically significant for true or dissecting aortic aneurysm, respectively, and are shown in bold. OR, odds ratio; CI, confidence interval.

and organs including vascular smooth muscle (The Human Protein Atlas). *SPATC1L* is distributed in the cytoplasm, nucleus, and perinuclear region of cells, and it translocates to the sites of cell-cell junctions in response to stimulation of cells with the neuropeptide neurokinin A (24). Expression of *SPATC1L* was also found to modulate the response of cells to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and may thereby protect cells from cell death induced by this DNA-damaging

agent (25). We demonstrated that rs113710653 [C/T (E231K)] of *SPATC1L* was significantly associated with true aortic aneurysm, with the minor T allele representing a risk factor for this condition, although the functional relevance of this association remains to be elucidated.

RNASE13 is located at chromosomal region 14q11.2 (NCBI Gene) and is expressed at a high level in the epididymis (The Human Protein Atlas). A GWAS showed that an SNP

(rs3748348) located in the vicinity of *RNASE13* was associated with executive functioning resilience (26). Gene-based analyses also revealed a genome-wide significant association between *RNASE13* and executive functioning resilience (27). We now showed that rs143881017 [C/T (R140H)] of *RNASE13* was significantly associated with dissecting aortic aneurysm, with the minor T allele representing a risk factor for this condition, although the molecular mechanism underpinning this association remains unknown.

Previous GWASs identified the SNPs: rs10757278 of *CDKN2BAS*, rs7025486 of *DAP2IP*, rs1466535 of *LRP1*, rs2118181 of *FBN1*, rs6511720 of *LDLR* and rs599839 of *SORT1* as susceptibility loci for aortic aneurysm (7-12). The MAFs of these SNPs were >10%, and the odds ratios were 0.8-1.8 (5.7-12.28). We now identified three novel loci that may confer susceptibility to true or dissecting aortic aneurysm, with the odds ratios (MAF, %) of rs1465567 of *EGFLAM*, rs113710653 of *SPATC1L*, and rs143881017 of *RNASE13* being 1.63 (25.1%), 5.32 (1.9%), and 5.77 (0.5%), respectively. Although rs1465567 of *EGFLAM* was a common variant with a small effect size, rs113710653 of *SPATC1L* and rs143881017 of *RNASE13* were low-frequency variants with moderate to large effect sizes.

There are some limitations to the present study: i) Given that the number of subjects with aortic aneurysm was relatively small and the results of the study were not replicated, our findings will require validation with other independent subject panels or in other ethnic groups. ii) It is possible that rs1465567 of *EGFLAM*, rs113710653 of *SPATC1L*, or rs143881017 of *RNASE13* is in linkage disequilibrium with other polymorphisms in the same gene or in other nearby genes that are actually responsible for the development of true or dissecting aneurysm. iii) The functional relevance of these SNPs to the pathogenesis of true or dissecting aneurysm remains to be elucidated.

In conclusion, rs1465567 of *EGFLAM* and rs113710653 of *SPATC1L* may be susceptibility loci for true aortic aneurysm and rs143881017 of *RNASE13* may be such a locus for dissecting aortic aneurysm in Japanese individuals. Determination of genotypes for these SNPs may prove informative for assessment of the genetic risk for these conditions in Japanese individuals.

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