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ORIGINAL ARTICLE

Impact of *LIMK1*, *MMP2* and *TNF-α* variations for intracranial aneurysm in Japanese population

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Genetic factors are known to have an important role in intracranial aneurysm (IA) pathogenesis. The purpose of this study is to identify single-nucleotide polymorphisms (SNPs) that are associated with IA in Japanese population. A total of 2050 IA patients and 1835 controls recruited in Biobank Japan, The University of Tokyo were used in this study. In all, 45 SNPs in 24 genes encoding proteins, which have been considered to be possible risk factors to IA pathogenesis, were genotyped using multiplex PCR-invader assay. Association analysis was evaluated by logistic regression analysis before and after adjustment of age, smoking and hypertension status. This case–control association study revealed a SNP, rs6460071 located on *LIMK1* gene (*P*=0.00069) to be significantly associated with increased risk of IA. In addition, two SNPs, rs243847 (*P*=0.00086) and rs243865 (*P*=0.00090), on matrix metallopeptidase 2 (*MMP2*) gene and one SNP rs1799724 (*P*=0.0026) on tumor necrosis factor-α (*TNF-α*) gene, are marginally associated with IA in male- and female-specific manner, respectively. In conclusion, a large-scale case–control association study was conducted to verify genetic variations associated with IA in Japanese population. This study gave insights on the importance of stratified analysis between genders, and suggested that the underlying mechanism of IA pathogenesis might differ between females and males.
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Keywords: candidate gene approach; case–control association study; intracranial aneurysm; Japanese; SNP

INTRODUCTION

Intracranial aneurysms (IAs) are balloon-like dilations of the intracranial arterial wall in the brain. Rupture of IA leads to subarachnoid hemorrhage (SAH), which causes fatality in ~50% of the cases and significant disability in 30% of cases.[1] The age- and sex-adjusted annual incidence rates and mortality rates for SAH were 23 and 9 per 100 000 population for all ages, respectively, in Japan.[1] The annual rupture rate is ~2.7%.[2]

IA is a multifactorial disease, in which both environmental and genetic factors have equally important roles in its etiology. Environmental factors, such as cigarette smoking, hypertension and female gender, are known to be associated with IA.[3–5] Furthermore, several studies suggested an increased occurrence of IA and SAH in first- and second-degree relatives of SAH, with the incidence rate of 6–10%.[6–9] Several Mendelian disorders, such as autosomal-dominant polycystic kidney disease and Ehlers–Danlos syndrome type IV are associated with an increased risk of IA formation.[10,11] All these evidence fortified the roles of genetic factors in the pathogenesis of IA.

In the last decade, the understanding of the hypothesis 'common variants, common disease' have greatly aided in the identification of common variants associated to polygenic diseases. Although several common variants were identified to be associated with the increased risk of IA development through candidate gene approaches[12–16] and genome-wide association studies,[17,18] only few associations were consistently replicated.[19,20] These might be because of the lack of statistical power of the study or differences in the allele frequencies across different populations. With the rationale of limited reports on the susceptibility loci for IA among Asia populations, we conducted a case–control association study using a total of 2050 IA patients and 1835 control samples, and screened a total of 45 single-nucleotide polymorphisms (SNPs) in 24 genes, which have been considered as potential genetics risk factors to IA pathogenesis.

MATERIALS AND METHODS

Study population

All DNA samples were recruited from Biobank Japan (http://biobankjp.org), which has a collaborative network of 66 hospitals throughout Japan. The identification of IA in the case samples were made by computerized tomography angiogram, magnetic resonance angiogram or cerebral digital subtraction angiogram. The demographic and clinical parameters of cases and controls were summarized in Table 1. This project is approved by the ethics committee at the Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

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Table 1 Demographic and clinical parameters in patients and controls groups

Characteristics	*Cases*	*Controls*	P-*value**
Total numbers	2050	1835	
Female (%)	1328 (64.5)	1188 (64.7)	0.953
Age			
Mean (s.d.)	61.8 (10.9)	51.0 (14.9)	<0.001
Range	23–88	18–96	
Hypertension	819 (40.5)	413 (22.5)	<0.001
Smoking	1001 (48.8)	776 (42.3)	<0.001

**P*-value showing differences between cases and control individuals.

Selection of SNPs and genotyping

We selected a total of 45 SNPs in 24 genes that were previously reported to have association with sporadic or familial IA from various case–control and linkage studies. These included a gene in a region on chromosome 14q23 as well as *COL1A2*, *COL3A1*, *COL4A1*, *VCAN, ELN*, *endoglin*, *eNOS3*, *FBN2*, *HSPG2*, *IL-6*, *KLK-8*, *LIMK1*, *LOXL2*, *LOXL3*, matrix metallopeptidase 2 (*MMP2*), *MMP9*, *MMP12*, *MTHFR*, *ATT*, *TIMP1*, *TIMP2*, *TIMP3* and tumor necrosis factor-α (*TNF-α*) genes. We emphasized on genes encoding extracellular matrix proteins, cytokines and angiogenic factors. SNPs that were shown to be not polymorphic in Japanese population in previous reports were excluded from the study. Supplementary Table 1 summarizes the list of the selected SNPs and its respective references. For fine mapping, we set the selection criteria of tag SNPs in the *MMP2* gene based on the measurement of linkage disequilibrium with r^2 value of >0.8 and minor allele frequency of >10% from the HapMap database (http://www.hapmap.org/). All the selected SNPs were genotyped using multiplex PCR-invader assay.[21]

Statistical analysis

The association study between the case and control groups of each SNP was estimated by logistic regression analysis. In addition, we included age (10-year interval), hypertension status (either systolic pressure of ⩾140 mm Hg or diastolic pressure of ⩾90 mm Hg) and smoking status (current/former, never) as covariates in this analysis. To identify gender-specific associated variants to IA development, stratified analysis between gender was performed, which compared the odds ratio (OR) of the associated SNP between the two gender strata by means of *P*-heterogeneity derived from Woolf's test (Rmeta package of R-program). *P*-values and OR with 95% confidence interval (CI) were calculated for allelic, dominant and recessive models, and OR were calculated with respect to the risk allele. SNPs that showed *P*-value of <0.05 in the Hardy–Weinberg equilibrium were excluded from further evaluation. Bonferroni's correction was used to assess the significance level of the association. When the association was carried out using all cases and controls, we applied Bonferroni's correction on the basis of 38 independent effective tests (α=0.05/38=0.0013) after exclusion of SNPs failed to be genotyped by invader assay and SNPs that deviated from Hardy–Weinberg equilibrium, whereas the evaluation of gender-specific association was based on 76 independent effective tests (α=0.05/(38×2)=0.00066). Power calculation showed that our study would have >95% power to detect a per-allele OR ⩾1.3 for an allele with 30% frequency at the Bonferroni threshold significance level (α=0.0013).

After identification of variants that are significantly associated with IA, we performed scoring analysis to evaluate the combined effects of the variants on the risk of IA. We assigned a score of 2 to individuals who are homozygous of the risk allele; a score of 1 to those with one risk allele and score of 0 to those without the risk allele. After adding up the scores, individuals were categorized into four different score groups as shown in Supplementary Table 3.

Logistic regression and association analysis were carried out using PLINK 1.06 (http://pngu.mgh.harvard.edu/~purcell/plink/). Rmeta package from R-program (http://www.r-project.org/) was used to perform stratified analysis between genders. Association analysis among the score groups was performed

Table 2 Association study with *P*-value of <0.01 by using logistic regression analysis before and after adjustment of age, hypertension and smoking status

							RAF		*Logistic regression before adjustment*				*Logistic regression after adjustment*					
CHR	*SNP*	*BP*	*A1*	*A2*	*Category*		*Case*	*Control*	*ADD_P**	*ADD_OR*	*DOM_P†*	*DOM_OR*	*REC_P‡*	*REC_OR*	*ADD_P**	*ADD_OR*	*DOM_P†*	*DOM_OR*	*REC_P‡*	*REC_OR*	*Gene*
2	rs2241028	74758934	A	G	All		0.22	0.21	0.62	1.03 (0.92–1.15)	0.58	1.04 (0.91–1.18)	0.93	1.01 (0.75–1.38)	0.13	1.1 (0.97–1.24)	0.11	1.13 (0.97–1.3)	0.61	1.09 (0.78–1.52)	*LOXL3*
					Female		0.21	0.22	0.22	0.92 (0.80–1.05)	0.20	0.90 (0.76–1.05)	0.66	0.92 (0.63–1.33)	0.92	0.99 (0.85–1.16)	0.85	0.98 (0.85–1.23)	0.88	1.03 (0.68–1.56)	
					Males		0.23	0.19	0.011	1.27 (1.06–1.54)	0.0078	1.35 (1.08–1.68)	0.44	1.24 (0.72–2.12)	0.0047	1.33 (1.09–1.63)	0.0028	1.43 (1.13–1.8)	0.41	1.27 (0.72–2.24)	
6	rs1799724	31542482	T	C	All		0.20	0.17	0.0079	1.17 (1.04–1.31)	0.016	1.18 (1.03–1.35)	0.071	1.35 (0.97–1.86)	0.015	1.17 (1.03–1.32)	0.031	1.18 (1.02–1.37)	0.08	1.37 (0.96–1.94)	*TNF-α*
			T	C	Female		0.20	0.17	0.0026	1.25 (1.08–1.44)	0.0087	1.25 (1.06–1.48)	0.022	1.67 (1.08–2.59)	0.0048	1.27 (1.08–1.49)	0.014	1.27 (1.05–1.54)	0.035	1.7 (1.04–2.77)	
			T	C	Males		0.19	0.19	0.66	1.04 (0.87–1.25)	0.62	1.06 (0.84–1.33)	0.93	1.02 (0.63–1.67)	0.75	1.03 (0.85–1.25)	0.75	1.04 (0.82–1.32)	0.87	1.04 (0.63–1.74)	
7	rs6460071	73497196	G	A	All		0.90	0.88	0.0015	1.26 (1.09–1.45)	0.00069	1.31 (1.12–1.53)	0.77	1.09 (0.62–1.91)	0.0078	1.24 (1.06–1.45)	0.0042	1.29 (1.08–1.53)	0.84	1.07 (0.57–1.99)	*LIMK1*
			G	A	Female		0.90	0.88	0.0057	1.29 (1.08–1.54)	0.002	1.36 (1.12–1.66)	0.85	0.93 (0.46–1.9)	0.048	1.23 (1.00–1.51)	0.02	1.31 (1.04–1.64)	0.56	0.78 (0.35–1.78)	
			G	A	Males		0.90	0.88	0.11	1.21 (0.96–1.54)	0.12	1.23 (0.95–1.59)	0.47	1.42 (0.56–3.61)	0.089	1.24 (0.97–1.6)	0.1	1.26 (0.96–1.65)	0.39	1.54 (0.57–4.15)	
16	rs243865	55511806	T	C	All		0.07	0.07	0.12	1.15 (0.97–1.37)	0.085	1.18 (0.98–1.42)	0.77	0.88 (0.36–2.11)	0.035	1.23 (1.02–1.5)	0.035	1.25 (1.02–1.53)	0.56	1.34 (0.51–3.53)	*MMP2*
			T	C	Female		0.07	0.07	0.79	0.97 (0.78–1.20)	0.77	1.03 (0.77–1.22)	0.94	1.05 (0.32–3.45)	0.95	1.01 (0.78–1.30)	0.95	0.99 (0.76–1.28)	0.52	1.56 (0.41–5.88)	
			T	C	Males		0.08	0.05	0.003	1.59 (1.17–2.16)	0.00090	1.75 (1.26–2.43)	0.61	0.71 (0.19–2.65)	0.0014	1.7 (1.23–2.35)	0.00057	1.85 (1.3–2.62)	0.98	0.98 (0.24–4.07)	
16	rs243847	55523998	T	C	All		0.62	0.59	0.0054	1.14 (1.04–1.25)	0.036	1.15 (1.01–1.31)	0.011	1.25 (1.05–1.48)	0.0042	1.16 (1.05–1.28)	0.05	1.16 (1.00–1.34)	0.0042	1.32 (1.09–1.59)	*MMP2*
			T	C	Female		0.61	0.60	0.34	1.06 (0.94–1.18)	0.61	1.04 (0.89–1.23)	0.25	1.14 (0.91–1.41)	0.27	1.08 (0.94–1.23)	0.47	1.07 (0.89–1.29)	0.26	1.15 (0.9–1.48)	
			T	C	Males		0.63	0.56	0.0009	1.29 (1.11–1.5)	0.0046	1.38 (1.10–1.72)	0.0083	1.46 (1.10–1.94)	0.0015	1.3 (1.10–1.52)	0.019	1.32 (1.05–1.67)	0.0029	1.58 (1.17–2.12)	

Abbreviations: ADD, additive; BP, base-pair location of the SNP; CHR, chromosome; CI, confidence interval; DOM, dominant; IA, intracranial aneurysm; OR, odds ratio; RAF, risk allele frequency; REC, recessive; SNP, single-nucleotide polymorphism.
P-values and OR with 95% CI were calculated for ADD (*), DOM (†) and REC (‡) models, and OR were reported with respect to the risk allele (allele A1).

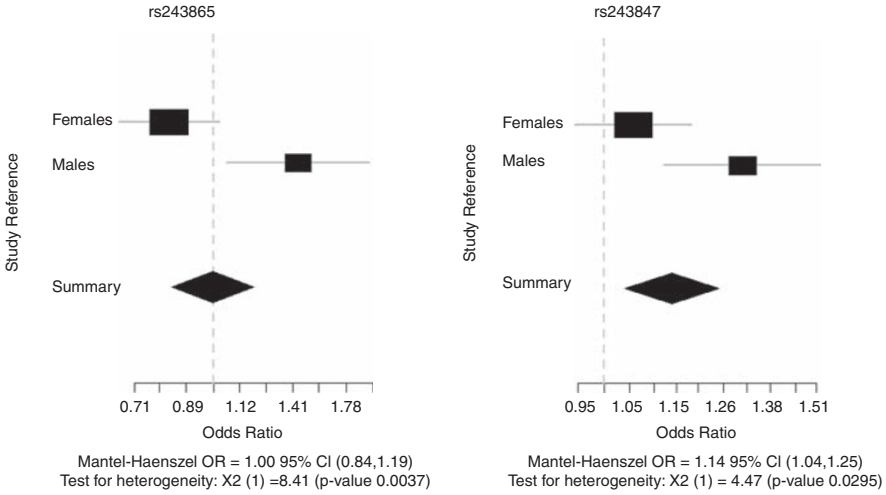

Figure 1 Stratified analysis of rs243865 and rs243847 on *MMP2* gene based on gender. *P*-heterogeneity from this analysis was utilized to evaluate the statistical differences in between the association of males and females patients.

using 2×2 contingency table Fisher's exact test by considering score group 0 as a reference group (who did not carry any risk allele).

RESULTS

A total of 45 SNPs in 24 candidate genes were genotyped by using a total of 2050 cases and 1835 controls. Among them, four SNPs that were unsuccessfully genotyped by invader assay, and three SNPs that were deviated from Hardy–Weinberg equilibrium, were excluded for further analysis. Supplementary Table 2 summarized the association analysis of all the SNPs by logistic regression analysis.

Table 2 reveals a summary of the association analysis for SNPs that showed *P*-value of <0.01 by logistic regression analysis before and after adjustment with the covariates including age, smoking and hypertension status. We observed one SNP, rs6460071, in LIM domain kinase 1 (*LIMK1)* gene to be significantly associated with IA after applying strict Bonferroni's correction (*P*-dominant=0.00069; Bonferroni-adjusted *P*-value=0.026; OR=1.31; 95% CI=1.12–1.53) as shown in Table 2. Two other SNPs (rs710968 and rs62476409), which are in strong linkage disequilibrium with this SNP, were included for genotyping, but we failed to genotype them.

We also found additional two SNPs, rs243847 and rs243865, in the *MMP2* gene to be marginally associated with male IA patients (*P*-additive=0.00087 and *P*-dominant=0.00090; Bonferroni-adjusted *P*-value=0.067 and 0.068; OR=1.29 and 1.75; 95% CI=1.11–1.50 and 1.26–2.43, respectively) but not in female IA patients (*P*-additive=0.34 and *P*-dominant=0.77; OR=1.06 and 1.03; 95% CI=0.94–1.18 and 0.82–1.30, respectively). Stratified analysis indicated the association of these two SNPs (rs243847 and rs243865) on the *MMP2* gene showed significantly different between female and male IA patients with *P*-heterogeneity of 0.029 and 0.0037 (Figure 1). The SNP rs243865 was statically significance after adjustment of age, hypertension status and smoking status in male IA patients.

As the SNPs in the *MMP2* gene showed significant association with IA in males, we further genotyped the tag SNPs and SNPs in the coding region of the *MMP2* gene. Although we were unable to find another SNPs that showed significant association with the male IA patients, we found a synonymous coding SNP, rs2287074, showing moderate association (*P*-additive=0.0061; OR=1.25; 95% CI=1.07–1.47) as shown in Table 3.

On the other hand, the r^2-value between rs243847 and rs243865 is 0.01, indicating independent association of these two SNPs with the increased risk of IA. Scoring analysis of a combination of the two SNPs revealed that individuals with scores 3 or 4 have three times higher risk of developing IA (Supplementary Table 3). These phenomena implied the importance of *MMP2* gene in IA development, especially in male patients.

In addition, we observed suggestive association of a SNP rs1799724 in the *TNF-α* gene with the risk in female IA (*P*-additive=0.0026; OR=1.25; 95% CI=1.08–144).

DISCUSSION

Many case–control association studies and linkage studies have been performed to identify common genetic variations associated with IA. However, many of the reported variations were not successfully replicated. To our knowledge, this is the largest case–control association study of IA in Japanese population. With >95% statistical power of this study, we attempted to verify the association of genetic variants that were previously reported to be associated with IA.

We verified a SNP rs6460071 in the *LIMK1* gene to be significantly associated with the increased risk of IA. LIMK1 is a protein kinase, which is involved in actin cytoskeleton reorganization through phosphorylation and inactivation of cofilin and thereby stabilizes cytoskeleton structure. The *LIMK1* gene is located on chromosome 7q11, in which the presence of a susceptible gene for IA was indicated by linkage studies of two different ethnic groups.[22,23] Akagawa *et al.*[24] illustrated that SNPs in *ELN* and *LIMK1* at chromosome 7q11 might exert the synergistic effect on development of IA by affecting the stability and synthesis of vascular walls by sharing elastin signaling pathway. However, our result failed to find association with genetic variations in the *ELN* gene with IA, in agreement with several other studies,[25,26] suggesting that the *LIMK1* gene is more likely candidate for the IA susceptibility gene at this chromosomal region. Nevertheless, additional study is needed to further verify the current finding.

Several epidemiological studies of SAH indicated that the incidence in women is higher than men, and the risk of IA and its rupture in women rises during and after menopause, suggesting the involvement of hormonal factors.[27] Harrod *et al.*[28] hypothesized that decreases in both circulating estrogen levels and cerebrovascular estrogen receptor density may contribute to the increased risk of IA and SAH in women during and after menopause. In our stratified association studies, we observed gender-specific associations of some SNPs with IA.

Table 3 Fine-mapping of *MMP2* with males IA patients

					RAF		*Logistic regression*												
CHR	*SNP*	*BP*	*A1*	*A2*	*Case*	*Control*	*ADD_P*	*ADD_OR*	*ADD_L95*	*ADD_U95*	*DOM_P*	*DOM_OR*	*DOM_L95*	*DOM_U95*	*REC_P*	*REC_OR*	*REC_L95*	*REC_U95*	*Designation*
16	rs243867	55509900	G	A	0.601	0.583	0.34	1.08	0.92	1.26	0.39	1.10	0.88	1.38	0.5	1.10	0.83	1.47	Upstream
16	rs11643630	55510459	G	T	0.578	0.559	0.31	1.08	0.93	1.26	0.12	1.20	0.95	1.50	0.97	1.00	0.76	1.31	Upstream
16	rs243865	55511806	T	C	0.082	0.053	0.003	1.59	1.17	2.16	0.0009	1.75	1.26	2.43	0.61	0.71	0.19	2.65	Upstream
16	rs17859859	55516395	T	C	0.164	0.143	0.14	1.17	0.95	1.44	0.22	1.16	0.92	1.47	0.21	1.58	0.77	3.23	Intronic
16	rs857403	55516708	T	A	0.260	0.255	0.75	1.03	0.87	1.22	0.84	1.02	0.83	1.27	0.7	1.09	0.72	1.64	Intronic
16	rs1477017	55517162	C	T	0.312	0.278	0.05	1.18	1.00	1.38	0.1	1.20	0.97	1.48	0.12	1.34	0.93	1.93	Intronic
16	rs11076101	55518258	T	C	0.150	0.140	0.45	1.09	0.87	1.35	0.55	1.08	0.85	1.37	0.44	1.40	0.60	3.25	Intronic
16	rs17301608	55518610	T	C	0.395	0.364	0.1	1.14	0.98	1.33	0.13	1.18	0.95	1.47	0.24	1.20	0.89	1.62	Intronic
16	rs1132896	55519535	C	G	0.164	0.145	0.19	1.15	0.93	1.42	0.28	1.14	0.90	1.44	0.22	1.57	0.77	3.22	SYN
16	rs1053605	55519607	A	G	0.151	0.142	0.48	1.08	0.87	1.35	0.55	1.08	0.85	1.37	0.54	1.31	0.56	3.09	SYN
16	rs866770	55520060	G	A	0.172	0.164	0.55	1.06	0.87	1.30	0.56	1.07	0.85	1.35	0.76	1.10	0.59	2.04	Intronic
16	rs2241146	55522234	T	C	0.191	0.180	0.45	1.08	0.89	1.31	0.5	1.08	0.86	1.36	0.61	1.17	0.63	2.18	Intronic
16	rs9928731	55523011	A	G	0.554	0.511	0.026	1.18	1.02	1.37	0.049	1.27	1.00	1.60	0.09	1.24	0.96	1.60	Intronic
16	rs243849	55523705	A	G	0.169	0.160	0.56	1.06	0.87	1.30	0.54	1.08	0.85	1.36	0.87	1.05	0.56	1.96	SYN
16	rs243847	55523998	C	T	0.629	0.564	0.00086	1.29	1.11	1.50	0.0046	1.38	1.10	1.72	0.0083	1.46	1.10	1.94	Intronic
16	rs12923011	55525160	T	C	0.165	0.143	0.11	1.18	0.96	1.46	0.18	1.18	0.93	1.49	0.2	1.59	0.78	3.26	Intronic
16	rs243844	55526876	A	G	0.724	0.673	0.0052	1.26	1.07	1.48	0.0088	1.33	1.07	1.65	0.07	1.39	0.97	2.00	Intronic
16	rs17859943	55527073	T	C	0.996	0.991	0.1	2.45	0.85	7.09	0.1	2.45	0.85	7.09	NA	NA	NA	NA	Non-SYN
16	rs2287074	55527113	A	G	0.353	0.303	0.0061	1.25	1.07	1.47	0.02	1.29	1.04	1.60	0.031	1.45	1.03	2.04	SYN
16	rs243839	55529411	G	A	0.365	0.348	0.36	1.08	0.92	1.26	0.41	1.10	0.88	1.36	0.52	1.11	0.81	1.52	Intronic
16	rs9923304	55530301	T	C	0.350	0.300	0.0072	1.24	1.06	1.46	0.02	1.29	1.04	1.60	0.039	1.43	1.02	2.00	Intronic
16	rs2287075	55532120	A	G	0.105	0.094	0.33	1.13	0.88	1.46	0.27	1.17	0.89	1.53	0.85	0.90	0.31	2.59	Intronic
16	rs11639960	55533270	G	A	0.299	0.265	0.047	1.19	1.00	1.40	0.028	1.27	1.03	1.57	0.54	1.13	0.76	1.68	Intronic
16	rs243835	55536622	C	T	0.389	0.379	0.55	1.05	0.90	1.23	0.3	1.12	0.90	1.40	0.79	0.96	0.71	1.30	Intronic
16	rs14070	55536727	A	G	0.290	0.278	0.47	1.06	0.90	1.26	0.54	1.07	0.86	1.32	0.56	1.12	0.76	1.67	SYN

Abbreviations: ADD, additive; BP, base-pair location of the SNP; CHR, chromosome; CI, confidence interval; DOM, dominant; IA, intracranial aneurysm; OR, odds ratio; RAF, risk allele frequency; REC, recessive; SNP, single-nucleotide polymorphism; SYN, synonymous.
P-values and OR with 95% CI were calculated for ADD, DOM and REC models, and OR were reported with respect to the risk allele (allele A1).

Abnormal arterial wall remodeling and a consequent weakening of the arterial wall is a possible pathway contributing to IA development. MMP2, a member of the matrix metalloproteinase family, is produced by the vascular smooth muscle cells and has an important role in extracellular matrix remodeling in blood vessels.[29,30] Importantly, MMP2 is upregulated in tissue affected by IA, compared with the normal vessel wall, and increased plasma MMP2 has been observed in patients with IA.[30,31] A previous study reported that the expression and activity of MMP2 on rat aortic smooth muscle cells were different between male and female mice, and implied that gender differences in MMP2 might be associated with the phenotypic differences in human abdominal aortic aneurysm formation.[32] Our study also suggest that two genetic variants (rs243847 and rs243865) on *MMP2* gene might confer an increased risk of IA in male patients, but this finding should require confirmation by using an independent study.

TNF-α is a pro-inflammatory cytokines that has as a key role in initiating and regulating the cascade event leading to inflammatory response. A recent report showed that TNF-α mRNA is significantly increased in human IA, suggesting that the role of this cytokines in promoting inflammation and subsequent apoptosis of cerebral vascular cells.[33] We observed a SNP, rs1799724, in the *TNF-α* gene showing suggestive association only with female IA patients, suggesting that this variant might be a female gender-specific risk factor for IA. Our result was supported by a recent report, which found that the genotype frequency of this SNP was significantly differing between females and males.[34] In addition, several reports showed that estrogen is known to inhibit TNF-α activity and reduced estrogen levels in post-menopausal women predispose to a higher incidence of aneurysm development.[35–38] Interestingly, there were reports showing that significant differences in the levels of TNF-α production when females and males stimulated under certain physiological condition, such as psychological stress and early alcohol-induced liver injury.[39,40] In our study, the risk allele of rs1799724 (−857T) are known to have a significantly higher level of TNF-α production from concanavalin A-activated peripheral blood mononuclear cells,[41] implying the importance of this variant in IA development, particularly in female IA.

In conclusion, we performed a large-scale case-control association study and verified genetic variations associated with IA in Japanese population. To our knowledge, this study is the first report that emphasized the importance of stratified analysis between genders and suggested the underlying mechanism of IA pathogenesis might differ between females and males.

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