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REGULAR ARTICLE

A rare Asian founder polymorphism of *Raptor* may explain the high prevalence of Moyamoya disease among East Asians and its low prevalence among Caucasians

Wanyang Liu · Hirokuni Hashikata · Kayoko Inoue · Norio Matsuura · Yohei Mineharu · Hatasu Kobayashi · Ken-ichiro Kikuta · Yasushi Takagi · Toshiaki Hitomi · Boris Krischek · Li-Ping Zou · Fang Fang · Roman Herzig · Jeong-Eun Kim · Hyun-Seung Kang · Chang-Wan Oh · David-Alexandre Tregouet · Nobuo Hashimoto · Akio Koizumi

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Abstract
Background In an earlier study, we identified a locus for Moyamoya disease (MMD) on 17q25.3.
Methods Linkage analysis and fine mapping were conducted for two new families in additional to the previously studied 15 families. Three genes, *CARD14*, *Raptor*, and *AATK*, were selected based on key words, namely, "inflammation", "apoptosis", "proliferation", and "vascular system", for further sequencing. A segregation analysis of 34 pedigrees was performed, followed by a case–control study in Japanese (90 cases vs. 384 controls), Korean (41 cases vs. 223 controls), Chinese (23 cases and 100 controls), and Caucasian (25 cases and 164 controls) populations.
Results Linkage analysis increased the LOD score from 8.07 to 9.67 on 17q25.3. Fine mapping narrowed the linkage signal to a 2.1-Mb region. Sequencing revealed that only one newly identified polymorphism, ss161110142, which was located at position −1480 from the transcription site of the *Raptor* gene, was common to all four unrelated sequenced familial affected individuals. ss161110142 was then shown to segregate in the 34 pedigrees studied, resulting in a two-point LOD score of 14.2 ($P = 3.89 \times 10^{-8}$). Its penetrance was estimated to be 74.0%. Among the Asian populations tested (Japanese, Korean, and Chinese), the rare allele was much more frequent in cases (26, 33, and 4%, respectively) than in controls (1, 1, and 0%, respectively) and was associated with an increased odds ratio of 52.2 (95% confidence interval 27.2–100.2) ($P = 2.5 \times 10^{-49}$). This allele was, however, not detected

W. Liu, H. Hashikata and K. Inoue have contributed equally to this study.

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W. Liu · H. Hashikata · K. Inoue · N. Matsuura · Y. Mineharu · H. Kobayashi · T. Hitomi · A. Koizumi (✉)
Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Konoe-cho, Yoshida, Sakyo-ku, Kyoto 606-8501, Japan
e-mail: koizumi@pbh.med.kyoto-u.ac.jp

H. Hashikata · Y. Mineharu · K. Kikuta · Y. Takagi · N. Hashimoto
Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto, Japan

B. Krischek
Department of Neurosurgery, University of Tübingen, Tübingen, Germany

L.-P. Zou
Department of Pediatrics, Chinese People's Liberation Army General Hospital, Beijing, China

F. Fang
Beijing Children's Hospital, Capital Medical University, Beijing, China

R. Herzig
Department of Neurology, Stroke Center, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic

J.-E. Kim · H.-S. Kang · C.-W. Oh
Department of Neurosurgery, Seoul National University College of Medicine, Seoul, Korea

D.-A. Tregouet
Institut National de la Santé et de la Recherche Médicale (INSERM), UMRS_937, Université Pierre et Marie Curie, Paris 6, Paris, France

in the Caucasian samples. Its population attributable risk was estimated to be 49% in the Japanese population, 66% in the Korean population, and 9% in the Chinese population.
Conclusion ss161110142 may confer susceptibility to MMD among East Asian populations.

Keywords Association studies in genetics · Cerebral stroke · Childhood stroke · Genetic linkage · Moyamoya disease

Introduction

Moyamoya disease (MMD: MIM%607151) is an idiopathic disorder characterized by steno-occlusive lesions around the terminal portions of the internal carotid arteries accompanied by collateral vessels (moyamoya vessels) [1].

While the incidence of MMD is worldwide [2], it is particularly high in East Asian countries, such as Japan, Korea, and China [3, 4]. In Japan, the most recent prevalence and annual incidence statistics were reported to be 10.5 and 0.94 per 100,000 persons, respectively [3]. In comparison, the incidence in Europe is estimated to be about one-tenth of that in Japan [3, 4], while in the USA, the incidence is about 0.086 per 100,000 persons and is higher among Asian Americans and African Americans than among Caucasian Americans [3, 4]. MMD has been attracting increasing attention as an important cause of cerebral stroke in children [5].

There is epidemiological evidence that about 15% of MMD patients have familial occurrence [6, 7]. A recent genome-wide linkage analysis identified a susceptibility locus for MMD at 17q25.3 [8]. The primary aim of the study reported here was to carry out positional cloning for MMD at the 17q25.3 locus. Based on our results, we report here the identification of a rare variant within the promoter of the *Raptor* gene that appears to be a strong candidate for MMD among East Asians.

Methods

Study population

The study was approved by the Ethics Committee of the Kyoto University Institutional Review Board, and written informed consent was obtained from all subjects. Two groups of case participants were enrolled in this study. The first group comprised familial participants selected to join this study because of the presence of more than one case among blood relatives. Specifically, 194 family members with 36 Japanese probands and five family members with one Korean proband were enrolled in this study. Medical records pertaining to vascular diseases and risk factors were collected from all of the family members for verification of the diagnosis. The two new families joined this study after the first linkage study had been completed.

Members of the probands' families admitted to Kyoto University Hospital or any of the other hospitals collaborating in this study were recruited. With the patients' consent, samples and clinical data were collected, de-identified and banked.

The second group comprised singular participants who joined this study as single cases without affected blood relatives. Irrespective of the family histories, single patients who joined without affected blood relatives were classified as singular participants. These cases were recruited from Kyoto University ($n = 90$), Seoul National University in Korea ($n = 41$), the Chinese People's Liberation Army General Hospital and Capital Medical University in China ($n = 23$), Tubingen University in Germany ($n = 21$), and the Stroke Center, Department of Neurology, Palacky University and University Hospital Olomouc in the Czech Republic ($n = 4$). Panels of 384 Japanese, 223 Korean, 100 Chinese, and 164 Caucasian (mostly German) participants were selected from the same respective centers as for singular cases to serve as controls for this study. Magnetic resonance angiography (MRA) screening was carried out for all Japanese controls, but not for all of the Korean, Chinese, and Caucasian controls. The small incidence of MMD in the general population was assumed not to affect the results of the association study.

The diagnosis of MMD for the probands of the families or singular participants was rigorously based on the Japanese criteria, the so-called RCMJ (Research Committee on Moyamoya Disease of the Ministry of Health, Welfare and Labor, Japan) criteria (Table 1) [9]. A number of the familial participants other than the probands who did not satisfy the RCMJ criteria were nevertheless classified as MMD cases because they met the "broad" classification (Table 1) [8].

Linkage analysis and haplotype estimation

Two additional families (pedigrees 19 and 20) were genotyped (Fig. 1). It should be noted that family number 12 was vacant for the previously reported linked locus [8]. Genotyping, mapping, and haplotype estimation were conducted as previously reported [8]. Briefly, genomic DNA was extracted from blood samples from living patients using a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany).

A total of 13 markers (D17S2195, D17S1847, D17S1806, D17S784, rs2071148, rs2280147, rs2293099, D17S704, D17S668, D17S928, rs2291395, rs2279395, and

Table 1 Diagnostic criteria of Moyamoya disease

RCMJ criteria: all of the following findings:
Steno-occlusive lesions around the terminal portions of the internal carotid arteries (including the proximal portions of the anterior cerebral arteries and middle cerebral arteries)
Moyamoya vessels at the base of the brain illustrated by abnormal vascular networks on conventional angiography or more than two flow voids in the basal ganglia on MRI
Findings 1 and 2 are present bilaterally
Known diseases with similar angiographic findings (i.e., arteriosclerosis, autoimmune disease, meningitis, brain neoplasm, Down syndrome, neurofibromatosis type 1, head trauma, irradiation to the head, protein C deficiency, protein S deficiency, and other diseases) should be ruled out
Broad classification: any steno-occlusive lesions that fulfill the following findings:
Steno-occlusive lesions around the terminal portions of the internal carotid arteries
Findings of moyamoya vessels may be absent
Bilateral involvement is not essential
Known diseases with similar angiographic findings should be ruled out

RCMJ, Research Committee on Moyamoya Disease of the Ministry of Health, Welfare and Labor, Japan in 1997; MRI, magnetic resonance imaging

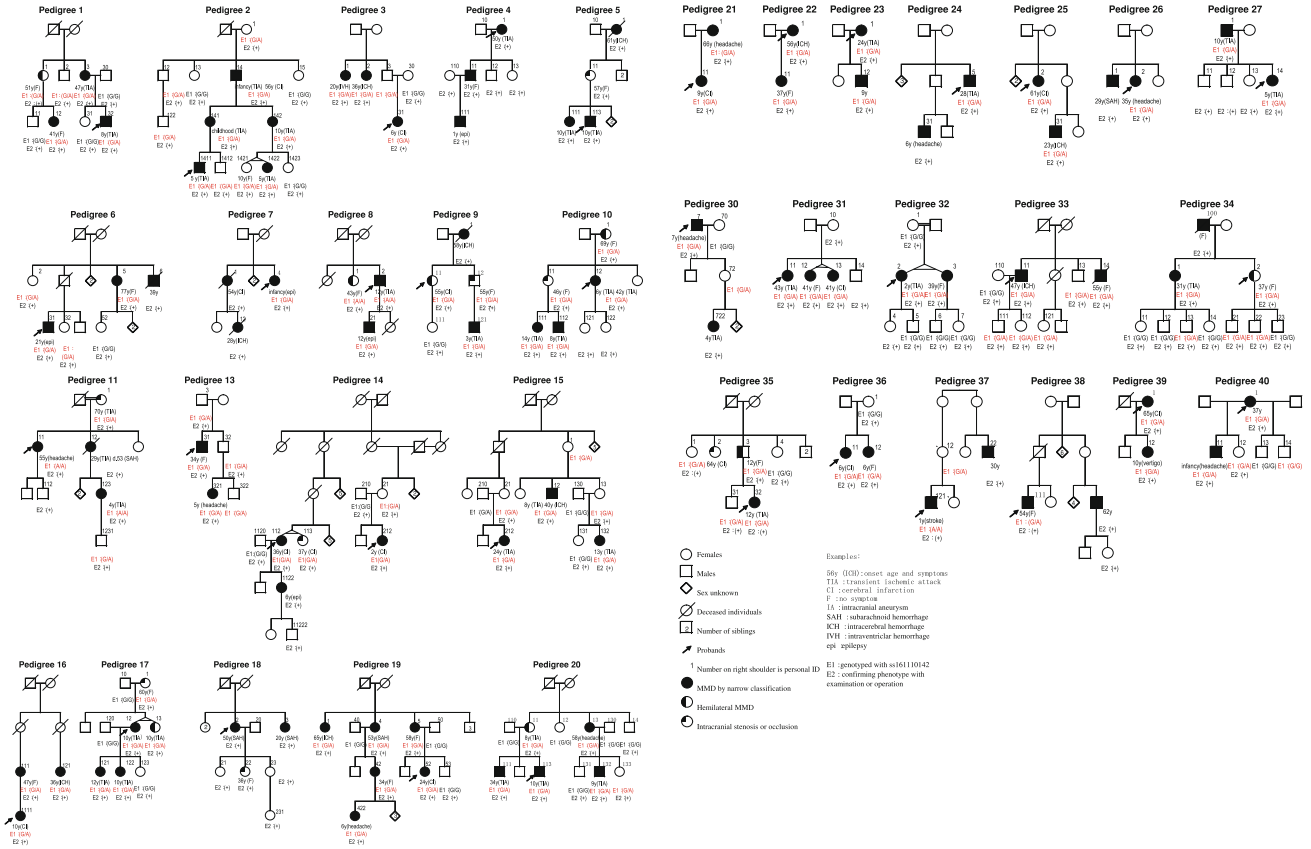

Fig. 1 Segregation of the ss161110142 A allele in *Raptor* in patients with the Moyamoya disease (MMD) phenotype. All affected individuals tested showed complete segregation for the 34 pedigrees. Genomic DNA samples were not available for pedigrees 4, 5, and 18 and for individuals 1122 and 11222 of pedigree 14

rs2292971) were genotyped at 5.1-Mb intervals in the 17q25-qter linkage region [8]. The marker locations were obtained from NCBI Map Viewer (http://www.ncbi.nlm.nih.gov/mapview/). The linkage analysis was performed using a multipoint parametric linkage method, assuming a dominant model [7, 8]. We assumed that obligatory carriers should be treated as affected, as previously reported [8]. The phenotype of unaffected related individuals in the

pedigrees was classified as "unknown", while "non-founder" spouses were classified as "unaffected". On the basis of the observed prevalence of 6.03 per 100,000 persons [10], the disease allele frequency should be set at 0.00003015, but we set it more conservatively at 0.0001 owing to the increasing number of asymptomatic patients that have recently been diagnosed by magnetic resonance imaging (MRI) and MRA [11]. We therefore assumed a phenocopy frequency of 0.00001. The allele frequencies for each microsatellite marker were estimated from all unrelated founders using the Merlin software [12]. Analyses were carried out using GENEHUNTER ver. 2.0 (http://www.broad.mit.edu/ftp/distribution/software/genehunter/) [13].

Since individual 132 of Pedigree 20 had recombination between rs2280147 and rs2293099, we determined the 3′ flanking region by further additional markers, namely rs71166116 (*CHMP6*), rs9896314 (*AZI1*), rs62075318 (*AZI1*), and P1026P (*BAHCC1*). The haplotype for Pedigree 20 was constructed using GENEHUNTER [13], resulting in the allele frequencies of these four markers being set at 0.50.

Direct sequencing

All coding exons, intron–exon boundaries, putative promoter sequences, and 3′-untranslated regions (UTRs) of *CARD14*, *Raptor* (*KIAA1303*), *AATK*, and *BAHCC1* were analyzed by direct sequencing of the four affected individuals (individuals 12, 1411, 112, and 12 in pedigrees 1, 2, 14 and 15, respectively). The *CARD14*, *Raptor* (*KIAA1303*), and *AATK* genes were candidate genes, while the *BAHCC1* gene was used to search for possible flanking markers. Primers for the coding exons were designed from intron sequences at distances >100 bp from the intron–exon boundary and commercially synthesized by PROLIGO Primers & Probes (Kyoto, Japan; http://www.proligo.com) [8]. For *Raptor*, a regulatory region at about 2 kbp upstream of the first exon was sequenced. We checked the single nucleotide polymorphism database (dbSNP) as a reference (http://www.ncbi.nlm.nih.gov/SNP/index.html). The primers for each gene are shown in Supplementary Table 1 in the Electronic Supplementary Material (ESM).

Confirmation of segregation and linkage in familial cases

A variant (ss161110142) located at position −1480 from the transcription site of the *Raptor* gene was tested for segregation and linkage in the entire families. For three of the 37 families (pedigrees 4, 5, and 18, respectively), we were unable to test the segregation and linkage owing to the lack of availability of current DNA samples (Fig. 1). In pedigree 14, genomic DNA samples were not available for individuals 1122 and 11222. The two-point logarithm of the odds ratio (LOD) score was calculated for the 34 genotyped pedigrees using GENHUNTER with the same parameter set as that used for the mapping described above. The penetrance was estimated as the proportion of subjects with MMD according to the broad classification among the genotyped subjects with the A allele of the ss161110142 G/A SNP.

Association study and statistical analysis

Six other *Raptor* SNPs, namely, rs9911978, rs12950635, rs4890047, rs4889863, rs11655474, and rs8080957, were then tested for association with MMD in the case–control study. These SNPs were selected to capture the variability of the first 65 kb of the *Raptor* gene by using the Tagger program [14] with criteria of $r^2 > 0.65$ and a minor allele frequency of >0.05 in Japanese and Europeans. A third rationale by which to select these SNPs was to test whether a haplotype harboring the ss161110142 A allele could be a founder haplotype. Typing was conducted using the TaqMan probe (TaqMan SNP Genotyping Assays; Applied Biosystems, Foster City, CA).

The association with MMD was tested using the Cochran–Armitage test, and deviation from Hardy–Weinberg equilibrium (HWE) was investigated using Fisher's exact test. The association between *Raptor* haplotypes and MMD was tested using the THESIAS software [15]. In order to correct for the number of tested SNPs, two-sided *P* values <0.0071 (i.e., 0.05 divided by 7) were considered to indicate statistical significance. The population attributable risk (PAR) is defined by: $\mathrm{PAR} = 100 \times (K - y)/K$, where K is the population prevalence and y is the phenocopy proportion [16]. PAR can be estimated by the proportion of the number of cases with the ss161110142 A allele.

Results

Clinical and demographic features of the participants

A total of 37 families participated in this study (Fig. 1). Specifically, 194 Japanese and five Korean family participants joined the study (Table 2). There were three peaks for clinical onset among Japanese patients: the first peak occurred at <10 years of age, the second small peak at 30–40 years, and the third peak at 50–60 years. Approximately, 42% of the Japanese patients were diagnosed before the age of 15 years. Thee major symptom of these pediatric patients was transient ischemic attacks (TIA). In contrast, the absence of symptoms or hemorrhage were the major symptoms in adult cases. These observations are consistent with a recent nationwide study [10].

Table 2 Summary of demographic and clinical profiles of cases and controls

Study cohort	Familial participant		Single subject participant			
Cases	Japanese	Korean	Japanese	Korean	Chinese	Caucasian
Number of participants	194	5	90	41	23	25
Age (mean ± SD)	39.2 ± 20.6	23.1 ± 12.8	47.7 ± 18.8	38.3 ± 13.4	25.3 ± 13.3	26.4 ± 15.2
Female:male	119:75	3:2	68:22	28:13	14:9	17:8
Number of pedigrees	36	1	0	–	0	0
Number of patients	109	2	–	–	–	–
Female:male	76:33	1:1	–	–	–	–
Family history	–	–	0	3	0	0
Characterization of patients						
Age of onset (years)						
<10	29	1	19	0	7	14
10–20	17	0	8	5	6	0
20–30	11	0	7	7	4	3
30–40	16	1	9	9	3	5
40–50	9	0	25	12	3	2
50–60	16	0	10	5	0	1
60+	11	0	8	3	0	0
Unknown	0	0	4	0	0	0
Young onset (<15)	46	1	25	1	10	14
Adult onset (15≤)	63	1	61	40	13	11
Clinical symptoms						
Cerebral infarction	13	0	8	14	3	11
TIA	39	1	33	8	1	2
Hemorrhage	14	0	19	0	3	1
Unknown stroke	1	1	1	16	6	3
Seizure	5	0	1	0	0	2
Headache	9	0	8	2	2	3
Asymptomatic	23	0	4	0	0	1
Other	5	0	16	1	8	2
Total	109	2	90	41	23	25
Clinical symptoms: young onset (<15 years)						
Cerebral infarction	4	0	0	0	1	2
TIA	29	1	16	0	1	2
Hemorrhage	0	0	2	0	0	0
Unknown stroke	1	0	0	1	2	3
Seizure	4	0	0	0	0	2
Headache	4	0	5	0	0	3
Asymptomatic	2	0	0	0	0	1
Other	2	0	2	0	6	1
Total	46	1	25	1	10	14
Female:male	28:18	0:1	22:3	1:0	6:4	9:5
Clinical symptoms: adult onset (≥15 years)						
Cerebral infarction	9	0	8	14	2	9
TIA	10	0	17	8	0	0
Hemorrhage	14	0	17	0	3	1
Unknown stroke	0	1	1	15	4	0
Seizure	1	0	1	0	0	0
Headache	5	0	3	2	2	0

Table 2 continued

Study cohort	Familial participant		Single subject participant			
Cases	Japanese	Korean	Japanese	Korean	Chinese	Caucasian
Asymptomatic	21	0	4	0	0	0
Other	3	0	10	1	2	1
Total	63	1	61	40	13	11
Female:male	48:15	1:0	43:18	27:13	8:5	8:3

Controls	Japanese	Korean	Chinese	Caucasian
Number of participants	384	223	100	164
Age (mean ± SD)	60.8 ± 9.6	40.0 ± 8.4	38.2 ± 10.3	48.0 ± 19.6
Female:male	205:179	198:25	100:0	71:93
Angiography[a]	384	46	0	68
Screening by angiography (%)	100.0	20.6	0	41.5

TIA, Transient ischemic attack; other, including unknown symptoms or other types; SD, standard deviation

[a] Angiography: conventional angiography, magnetic resonance angiography (MRA), computed tomography (CT), among others

The study also included 90 Japanese, 41 Korean, 23 Chinese, and 25 Caucasian singular participants (Table 2). With the exception of three cases, none of these patients had family histories.

A brief description of the 384 Japanese, 223 Korean, 100 Chinese, and 164 Caucasian controls is given in Table 2.

Fine mapping of the 17q25.3

The addition of the two newly recruited families (pedigrees 19 and 20) increased the LOD score from 8.07 [8] to 9.67. One patient (individual 132 in pedigree 20) had recombination between rs2280147 and rs2293099 (Supplementary Fig. 1 in ESM). Fine mapping using the four additional SNPs, namely, rs71166116 (*CHMP6*), rs9896314 (*AZI1*), rs62075318 (*AZI1*), and P1026P (C>T) (*BAHCC1*), revealed recombination between rs62075318 and a variant in *BAHCC1* (P1026P) in pedigree 20, indicating that the core region was a 2.1-Mb region between D17S1806 and *BAHCC1* (P1026P).

This region contained 40 genes, including *BAIAP2*, whose sequence was reported in our earlier publication [8]. To select candidate genes, we chose the key words "inflammation" [17], "apoptosis" [18] or "proliferation" and "vascular system" [3, 4], all of which have been reported to be associated with pathological conditions of MMD. The selected genes were *CARD14*, which plays key roles in immune reactions [19], *AATK*, which is reported to be involved in apoptosis [20], and *Raptor* [regulatory associated protein of mammalian target of rapamycin (mTOR)] [21], which is associated with tissue hypertrophy [22], is a regulator of hypoxia-inducible factor [23], and is involved in HLA class I antibody-mediated endothelial cell proliferation [24].

Sequencing

We sequenced *CARD14*, *Raptor*, and *AATK* in four unrelated affected individuals. The results are shown in Supplementary Table 2 in ESM. Among these SNPs, the *Raptor* ss161110142 G/A polymorphism appeared to be very interesting because all four sequenced affected individuals were found to be heterozygous.

Confirmation of segregation and linkage with the ss161110142

We then genotyped the 34 families for the ss161110142 G/A SNP. As shown in Fig. 1, all affected individuals in the tested families were found to carry the ss161110142 A allele and showed complete segregation. The two-point LOD score was calculated to be 14.2 ($P = 3.89 \times 10^{-8}$). The estimated penetrance of the A allele was 74.0% (88/119) [95% confidence interval (CI) 66–82%].

Association study

The last stage of our analysis consisted of a case–control association study for MMD performed in different ethnicities. The ss161110142 A allele was common in the Asian cases (from 4% in Chinese cases to 33% in Korean cases), while its frequency was about 1% in Japanese and Korean controls (Table 3). The ss161110142 A allele was not

Table 3 Association between seven SNPs in *Raptor* and Moyamoya disease among the different populations

rs ID[a]	Allele	Populations	Controls					Cases					OR[d] (95% CI)	*P*[e]
	½		11	12	22	Frequency[b]	*P*, HWE[c]	11	12	22	Frequency[b]	*P*, HWE[c]		
ss161110142	G/A	Japanese	377	6	1	0.01	0.072	46	42	2	0.26	0.056	51.52 (21.92–121.05)	2.08×10^{-29}
			98.18%	1.56%	0.26%			51.11%	46.67%	2.22%				
		Korean	218	5	0	0.01	1.000	14	27	0	0.33	0.003	84.09 (28.08–251.76)	9.42×10^{-22}
			97.76%	2.24%	0.00%			34.15%	65.85%	0.00%				
		Chinese	100	0	0	0.00	1.000	21	2	0	0.04	1.000	NA	3.37×10^{-2}
			100.00%	0.00%	0.00%			91.30%	8.70%	0.00%				
		All Asian	695	11	1	0.01	0.108	81	71	2	0.24	0.002	52.20 (27.18–100.23)	2.48×10^{-49}
			98.30%	1.56%	0.14%			52.60%	46.10%	1.30%				
		Caucasian	164	0	0	0.00	1.000	25	0	0	0.00	1.000	NA	NA
			100.00%	0.00%	0.00%			100.00%	0.00%	0.00%				
rs9911978	A/G	Japanese	149	177	58	0.38	0.707	16	53	21	0.53	0.144	1.81 (1.31–2.51)	3.00×10^{-4}
			38.80%	46.09%	15.10%			17.78%	58.89%	23.33%				
		Korean	82	101	40	0.41	0.421	8	21	12	0.55	1.000	1.78 (1.11–2.86)	0.016
			36.77%	45.29%	17.94%			19.51%	51.22%	29.27%				
		Chinese	29	51	20	0.46	0.975	10	13	0	0.28	0.184	0.47 (0.23–0.95)	0.033
			29.00%	51.00%	20.00%			43.48%	56.52%	0.00%				
		All Asian	260	329	118	0.40	0.457	34	87	33	0.50	0.158	1.48 (1.16–1.90)	1.70×10^{-3}
			36.78%	46.53%	16.69%			22.08%	56.49%	21.43%				
		Caucasian	88	61	15	0.28	0.437	13	8	4	0.32	0.326	1.23 (0.65–2.33)	0.534
			53.66%	37.20%	9.15%			52.00%	32.00%	16.00%				
rs12950635	T/C	Japanese	48	164	172	0.66	0.411	5	40	45	0.72	0.484	1.33 (0.93–1.91)	0.118
			12.50%	42.71%	44.79%			5.56%	44.44%	50.00%				
		Korean	25	82	116	0.70	0.105	5	12	24	0.73	0.189	1.15 (0.68–1.95)	0.612
			11.21%	36.77%	52.02%			12.20%	29.27%	58.54%				
		Chinese	6	34	60	0.77	0.844	3	10	10	0.65	1.000	0.56 (0.28–1.12)	0.097
			6.00%	34.00%	60.00%			13.04%	43.48%	43.48%				
		All Asian	79	280	348	0.69	0.059	13	62	79	0.71	0.980	1.12 (0.85–1.47)	0.406
			11.17%	39.60%	49.22%			8.44%	40.26%	51.30%				
		Caucasian	4	62	98	0.79	0.169	1	10	14	0.76	1.000	0.86 (0.43–1.73)	0.671
			2.44%	37.80%	59.76%			4.00%	40.00%	56.00%				

Table 3 continued

rs ID[a]	Allele	Populations	Controls					Cases					OR[d] (95% CI)	P^e
	½		11	12	22	Frequency[b]	P, HWE[c]	11	12	22	Frequency[b]	P, HWE[c]		
rs4890047	C/T	Japanese	147	176	61	0.39	0.546	16	52	22	0.53	0.206	1.80 (1.30–2.50)	4.00×10^{-4}
			38.28%	45.83%	15.89%			17.78%	57.78%	24.44%				
		Korean	78	104	41	0.42	0.612	8	20	13	0.56	1.000	1.79 (1.11–2.87)	0.016
			34.98%	46.64%	18.39%			19.51%	48.78%	31.71%				
		Chinese	31	49	20	0.45	1.000	8	15	0	0.33	0.063	0.60 (0.31–1.19)	0.141
			31.00%	49.00%	20.00%			34.78%	65.22%	0.00%				
		All Asian	256	329	122	0.41	0.389	32	87	35	0.51	0.156	1.53 (1.19–1.95)	8.00×10^{-4}
			36.21%	46.53%	17.26%			20.78%	56.49%	22.73%				
		Caucasian	91	60	13	0.26	0.585	15	8	2	0.24	0.822	0.89 (0.44–1.78)	0.739
			55.49%	36.59%	7.93%			60.00%	32.00%	8.00%				
rs4889863	A/G	Japanese	149	174	61	0.39	0.440	16	52	22	0.53	0.206	1.82 (1.31–2.53)	3.00×10^{-4}
			38.80%	45.31%	15.89%			17.78%	57.78%	24.44%				
		Korean	76	106	41	0.42	0.783	8	20	13	0.56	1.000	1.75 (1.09–2.82)	0.020
			34.08%	47.53%	18.39%			19.51%	48.78%	31.71%				
		Chinese	30	50	20	0.45	1.000	8	15	0	0.33	0.063	0.59 (0.30–1.16)	0.126
			30.00%	50.00%	20.00%			34.78%	65.22%	0.00%				
		All Asian	255	330	122	0.41	0.425	32	87	35	0.51	0.156	1.52 (1.19–1.95)	8.00×10^{-4}
			36.07%	46.68%	17.26%			20.78%	56.49%	22.73%				
		Caucasian	91	60	13	0.26	0.585	15	8	2	0.24	0.822	0.89 (0.44–1.78)	0.739
			55.49%	36.59%	7.93%			60.00%	32.00%	8.00%				
rs11655474	T/C	Japanese	24	148	212	0.74	0.920	2	30	58	0.81	0.691	1.47 (0.98–2.21)	0.062
			6.25%	38.54%	55.21%			2.22%	33.33%	64.44%				
		Korean	16	86	121	0.74	0.993	0	12	29	0.85	0.781	2.10 (1.10–4.01)	0.022
			7.17%	38.57%	54.26%			0.00%	29.27%	70.73%				
		Chinese	8	39	53	0.73	0.971	3	7	13	0.72	0.415	0.96 (0.47–1.96)	0.917
			8.00%	39.00%	53.00%			13.04%	30.43%	56.52%				
		All Asian	48	273	386	0.74	1.000	5	49	100	0.81	0.997	1.49 (1.10–2.03)	0.011
			6.79%	38.61%	54.60%			3.25%	31.82%	64.94%				
		Caucasian	19	61	84	0.70	0.175	2	10	13	0.72	1.000	1.11 (0.57–2.15)	0.754
			11.59%	37.20%	51.22%			8.00%	40.00%	52.00%				

Table 3 continued

rs ID[a]	Allele	Populations	Controls					Cases					OR[d] (95% CI)	P^e
	1/2		11	12	22	Frequency[b]	P, HWE[c]	11	12	22	Frequency[b]	P, HWE[c]		
rs8080957	A/G	Japanese	28	157	199	0.72	0.818	2	31	57	0.81	0.600	1.59 (1.06–2.38)	0.023
			7.29%	40.89%	51.82%			2.22%	34.44%	63.33%				
		Korean	16	98	109	0.71	0.449	0	14	27	0.83	0.525	2.00 (1.09–3.68)	0.024
			7.17%	43.95%	48.88%			0.00%	34.15%	65.85%				
		Chinese	9	45	46	0.69	0.893	4	8	11	0.65	0.434	0.86 (0.44–1.70)	0.667
			9.00%	45.00%	46.00%			17.39%	34.78%	47.83%				
		All Asian	53	300	354	0.71	0.390	6	53	95	0.79	0.916	1.51 (1.12–2.03)	0.007
			7.50%	42.43%	50.07%			3.90%	34.42%	61.69%				
		Caucasian	26	77	61	0.61	0.932	3	12	10	0.64	1.000	1.15 (0.62–2.14)	0.653
			15.85%	46.95%	37.20%			12.00%	48.00%	40.00%				

NA, Data not available

[a] rs ID, Reference single nucleotide polymorphism (SNP) accession identity number

[b] Frequency of risk allele 2

[c] P, Hardy–Weinberg Equilibrium (HWE) two-sided probability value from the test for deviation from the HWE

[d] OR, Allelic odds ratio with its 95% confidence interval (CI) except for ss161110142, which was calculated under the assumption of dominant effect because of very rare allele frequency

[e] P Cochran–Armitage trend test's P value

present at all in Chinese controls. Consequently, the A allele was associated with a very high odds ratio (OR) for MMD: 51.5 (95% CI 21.9–121.1) ($P = 2.08 \times 10^{-29}$) in Japanese and 84.1 (95% CI 28.1–251.8) ($P = 9.42 \times 10^{-22}$) in Koreans. Note that the genotype distribution of the ss161110142 SNP followed HWE in Asian controls but showed some deviation from HWE in Korean cases, which would not be unexpected if this SNP is truly associated with MMD. In contrast, the ss161110142 A allele was not detected in either the patients or the controls among the Caucasian participants in the study.

Haplotype frequencies derived from the seven studied *Raptor* SNPs are shown in Table 4. Due to the moderate size of the Chinese samples, haplotypes were inferred only in Japanese and Korean populations. The same *Raptor* haplotype structure was observed in Japanese and Koreans. Interestingly, the ss161110142 A allele was carried by only one haplotype that was very rare in controls but very frequent in cases, suggesting the existence of a founder haplotype spanning 65 kb common to Japanese and Korean subjects with MMD.

Population attributable risks were estimated to be 49% (44/90) for Japanese, 66% (27/41) for Koreans, and 9% (2/23) for Chinese.

Discussion

Here, we report the results of positional cloning based on tried and true tactics. We identified ss161110142 as a sensitivity SNP for MMD. Although the *Raptor* ss161110142 A allele was found to be a rare variant in East Asian general populations, it was very common in East Asian MMD patients, with approximately 50% of the Japanese and 66% Korean MMD patients being heterozygous for this allele. Intensive sequencing of coding regions and a promoter of *Raptor* did not reveal any variants specific for cases with MMD, with the exception of the ss161110142. The presence of this rare allele was found to elevate susceptibility to MMD by 52.2-fold. We thus tentatively conclude that ss161110142 of *Raptor* is very likely to confer susceptibility to MMD.

There might be a caveat, however, with respect to a causal role of ss161110142 in MMD. Sequencing was not conducted in genes around *Raptor*, and we also did not further explore the founder haplotype around *Raptor*. More importantly, the functional link was not explained pathologically. Those limitations to our study contribute to an uncertainty regarding the causal role of *Raptor*, leaving open the possibility that this variant of *Raptor* may simply be a marker having a very strong linkage disequilibrium with unknown causative mutations of unsequenced parts of *Raptor* or a nearby gene.

Table 4 Main *Raptor* haplotypes derived from the study of ss161110142, rs9911978, rs12950635, rs4890047, rs4889863, rs11655474, and rs8080957 in the Japanese and Korean case–control studies

Polymorphisms							Haplotype frequencies			
							Japanese		Korean	
ss161110142	rs9911978	rs12950635	rs4890047	rs4889863	rs11655474	rs8080957	Controls	Cases	Controls	Cases
G	G	C	T	G	C	G	0.366	0.274	0.395	0.219
G	A	C	C	A	C	A	0.021	0	0.025	0.024
G	A	C	C	A	T	A	0.248	0.181	0.261	0.146
G	A	T	C	A	C	G	0.336	0.272	0.291	0.268
A	G	C	T	G	C	G	0.009	0.253	0.011	0.329

Based on signaling by the mammalian target of the mTOR complex, it has been reported that *Raptor* is associated with vascular smooth muscle cell proliferation and intimal expansion mediated by interferon-γ [25] and with HLA class I antibody-mediated endothelial cell proliferation [24]. The ss161110142 variant is predicted to be located one base upstream of the GATA-1 site (http://www.cbs.dtu.dk/services/Promoter/) [26]. As such, this variant may modify signals mediated by mTOR complexes by changing gene expression levels in tissue-specific manners. The involvement of *Raptor* as a key molecule can potentially explain the smooth muscle cell proliferation observed in steno-occlusive lesions [27, 28] provide the basis of several immunological hypotheses [28, 29]. In fact, elevations of hypoxia-inducible factor [30] and basic fibroblast growth factor (bFGF) [31] have been reported in MMD. Therefore, it is possible that the mTOR signaling pathway may bridge the missing link between genetic factors and pathological consequences.

The high prevalence of the ss161110142 variant can explain the large prevalence of MMD among East Asian populations compared with Caucasian populations. In particular, the high PARs can explain the greater prevalence of MMD in Japanese (49%) and Koreans (66%) compared with other ethnicities. However, the issue of whether this variant is a single risk factor for MMD should be addressed because of the large gap between the prevalence of carriers of the ss161110142 A allele (2%) and the prevalence of MMD (as low as 6.03 per 100,000 persons) [10]. The large gap in the estimated frequencies (330-fold) strongly indicates that other factors contribute to the development of MMD. Although we were unable to demonstrate direct evidence for the involvement of ss161110142 in MMD, it may be that this allele elevates the risk of MMD synergistically with unknown factors. Pathologic clues are expected to be identified by functional characterization of *Raptor*.

Kraemer et al. [32] recently reported the clinical features and course of MMD in Caucasians. MMD in Caucasian differs from Asian MMD in the timing of onset of vasculopathy and lower rate of hemorrhage. Although we could not confirm such differences in this study—mainly due to limitations in the size of the study population—it is plausible to assume that the *Raptor* gene may modify the clinical features and course of MMD.

The present study clearly demonstrated a founder haplotype harboring *Raptor* ss161110142 variant in East Asian patients with MMD, giving an explanation for high prevalence in Asian. Further studies including functional analysis are required to envisage the gene–environment interactions in the process of MMD.

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