Distribution of Moyamoya Disease Susceptibility Polymorphism p.R4810K in RNF213 in East and Southeast Asian Populations

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Abstract

Moyamoya disease is an idiopathic vascular disorder of the intracranial arteries. Ring finger 213 (*RNF213*) was previously identified as the strongest susceptibility gene for moyamoya disease in East Asian people by a genome-wide linkage analysis and exome analysis. The coding variant p.R4810K in *RNF213* was strongly associated with moyamoya disease in the Japanese (odds ratio: 338.94, $p = 1.05 \times 10^{-100}$) and Korean (odds ratio: 135.63, $p = 7.59 \times 10^{-27}$) populations, and much less strongly associated in the Chinese population (odds ratio: 14.70, $p = 2.63 \times 10^{-5}$). The present study investigated the distribution of variant p.R4810K in *RNF213* in 2,508 participants from East and Southeast Asian countries using a TaqMan probe. p.R4810K was detected at an allele frequency of about 1.00% in 4 of 11 investigated locations in China. In contrast, p.R4810K was detected homogeneously at relatively high frequencies of 1.00–1.72% in all investigated locations in Korea and Japan, including Okinawa. p.R4810K was not detected in Southeast Asian populations. The population susceptible to moyamoya disease was estimated to be 16.16 million people in East Asian countries, including 11.41 million Chinese, 1.36 million Korean, and 3.39 million Japanese people. The number of patients with moyamoya disease, which was estimated at approximately one per 300 carriers of p.R4810K, was considered to be 53,800 in East Asian populations.

Key words: moyamoya disease, p.R4810K, *RNF213*, East Asian, Southeast Asian

Introduction

Moyamoya disease (Mendelian Inheritance in Man [MIM] number 607151) is a rare idiopathic progressive disorder characterized by occlusive lesions in the supraclinoid internal carotid artery and its main branches in the circle of Willis. The reduction in blood flow in the regions affected by the occlusive lesions is compensated by the development of a fine vascular network that resembles “puffs of smoke” (“moyamoya” in Japanese).^{12,13} Moyamoya disease occurs with the highest prevalences in East Asian countries such as Japan, Korea, and China, especially compared with other countries worldwide.^{2,7,10}

We previously identified a susceptibility locus for familial moyamoya disease on 17q25.3.¹¹ Significant associations with genes in this region had been reported,^{4,8} but rigorous identification had not been

determined. The ring finger 213 (*RNF213*) gene was finally identified as a susceptibility gene for moyamoya disease by the conventional rigorous positional cloning approach, exome analysis, and functional analysis using zebrafish.⁹ Overall, 74.5% of East Asian patients with moyamoya disease carry the rare founder variant p.R4810K of *RNF213*. However, a gradient of prevalence was observed in patients with moyamoya disease: 90% in Japanese, 79% in Korean, and 23% in Chinese patient populations. On the other hand, no gradient of prevalence was observed in these three general populations. In total, 2.4% of the East Asian general population carries p.R4810K. The minor allele frequencies of p.R4810K were 1.4%, 1.3%, and 1.0% for the Japanese, Korean, and Chinese in general populations, respectively.⁹ These similar allele frequencies of p.R4810K in these three East Asian general populations do not explain the lower frequency of Chinese patients with moyamoya disease compared with Japanese and Korean patients with moyamoya

disease. Moyamoya disease occurs in approximately one of 300 carriers in Japan.⁸⁾ This discrepancy in the allele frequency between cases and controls in Chinese people might be attributable to selection bias in the general populations in the previous study.⁹⁾

The primary aim of the present study was to estimate the population susceptible to moyamoya disease by conducting large scale screening for p.R4810K in general populations in East and Southeast Asia. The secondary aim was to estimate association of the p.R4810K variant of *RNF213* in patients with moyamoya disease.

Materials and Methods

A total of 2,508 unrelated participants from East Asian and Southeast Asian countries were recruited, comprising 587 Chinese, 294 Korean, 1474 Japanese, 103 Vietnamese, and 50 Filipino people (Table 1). The Japanese participants were recruited from mainland Japan, including Hokkaido, Honshu, Shikoku, and Kyushu, and Okinawa. The Korean participants were recruited from five locations. The Chinese participants were recruited from 11 loca-

tions. Blood samples were obtained from two sources. One source was the Kyoto University Human Specimen Bank, which collected blood samples from the 1990s and 2000s as previously described.^{5,6)} These blood samples included Chinese, Korean, Japanese, and Philippine samples. The other source involved samples collected in an international collaboration that included Vietnam and Seoul, South Korea.

Ethical approval for this study was given by the Institutional Review Board and Ethics Committee of Kyoto University School of Medicine, Kyoto University, Japan (approval number: G140; approval date: Oct. 18, 2004), by the Institutional Review Board of Seoul National University Hospital, Seoul, South Korea (approval number: H0507-509-153; approval date: Aug. 24, 2005). The study participants were recruited in these institutes. Subjects who participated in this study after 2000 were recruited by School of Medicine, Kyoto University. All subjects gave written informed consent. Subjects who donated blood samples before 2000 were recruited by Tohoku University School of Medicine (Sendai, Miyagi), or Akita University School of Medicine (Akita, Akita), or Kyoto University School of Medicine, and

Table 1 Demographic features of the participants in the East and Southeast Asian populations

Area	Country	Location	Size of population (million)	Sex (male:female)	Age (yrs)*	Angiography**
East Asia	China	Dehui	1.00	0:50	NA	0
		Huludao	2.82	0:50	NA	0
		Beijing	19.61	0:50	38.4 ± 10.6	0
		Jinan	6.81	0:50	NA	0
		Xian	8.47	0:94	NA	0
		Baoji	3.72	0:48	NA	0
		Shanghai	23.02	0:50	NA	0
		Changsha	7.04	0:50	NA	0
		Heping	4.60	0:46	NA	0
		Nanning	6.66	0:50	NA	0
		Tainan	1.88	0:49	36.4 ± 11.5	0
		Total	85.63	0:587	37.4 ± 11.1	0
	Korea	Seoul	10.46	80:25	42.2 ± 13.3	46
		Chonan	0.58	0:29	34.6 ± 8.8	0
		Haman	0.06	0:46	43.6 ± 6.8	0
		Pusan	3.60	0:49	39.4 ± 7.8	0
		Jeju-do	0.53	0:65	NA	0
		Total	15.23	80:214	40.9 ± 10.9	46
	Japan	Mainland	123.62	608:766	57.7 ± 14.1	384
		Okinawa	1.38	0:100	47.5 ± 7.2	0
		Total	125.00	608:866	57.0 ± 14.0	384
Southeast Asia	Vietnam	Hanoi	6.50	0:103	22.8 ± 10.2	0
	Philippines	Manila	1.66	0:50	NA	0

*Values are means ± standard deviation. **Conventional digital subtraction angiography, magnetic resonance angiography, computed tomography angiography, and others. NA: not applicable.

gave verbal informed consent. The use of blood samples donated before 2000 for genetic analysis was also approved by the Institutional Review Board and Ethics Committee of Kyoto University School of Medicine (approval number: G140; approval date: Oct. 18, 2004).

Peripheral blood (10 ml) was collected from all participants. Genomic deoxyribonucleic acid (DNA) was extracted from the blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Germantown, Maryland, USA) according to the manufacturer's protocol. The quality and concentration of the extracted DNA were assessed using an Infinite® M200 PRO (TECAN, Kawasaki, Kanagawa). The DNA was stored in a freezer at -20°C until analysis.

Genotyping of p.R4810K in all participants was conducted using a TaqMan® probe (Custom TaqMan® SNP Genotyping Assays; Applied Biosystems, Foster City, CA, USA) and a 7300/7500 Real-Time PCR System (Applied Biosystems) according to the manufacturer's protocols. Briefly, the polymerase chain reaction (PCR) amplifications were performed with 1–20 ng of purified genomic DNA, 0.1 μl of $40\times$ SNP Genotyping Assay, 6.25 μl of $2\times$ TaqMan® Universal PCR Master Mix, No

AmpErase® UNG, and 5.15 μl of deoxyribonuclease-free water. The final reaction volume was 12.5 μl /well in 96-well plates. The standard protocol for the cycling conditions was maintenance for 10 minutes at 95°C for AmpliTaq Gold® (Applied Biosystems) enzyme activation, followed by 40 cycles of 15 seconds at 92°C for denaturation, and 1 minute at 60°C for annealing/extension. After each PCR amplification, an endpoint plate read was performed using the Real-Time PCR System (Applied Biosystems). The associated sequence detection system software uses the fluorescence measurements taken during the plate read to plot fluorescence (normalized reporter signal: Rn) values based on the signals from each well. The plotted fluorescence signals indicate which alleles are present in each sample.

Results

The demographic features of all participants in this study are shown in Table 1. The sampled cities covered 234.02 million people. A total of 2,508 participants from three East Asian countries and two Southeast Asian countries were recruited from 1987

Table 2 Geographic distribution of p.R4810K in RNF213 in East and Southeast Asian populations

Area	Country	Location	Genotype of p.R4810K			Sample size	Minor allele frequency (%)	Total population (million)	Estimated population with p.R4810K in a country (million)
			GG	GA	AA				
East Asia	China	Dehui	50	0	0	50	0.00	1340.00	11.41
		Huludao	50	0	0	50	0.00		
		Beijing	49	1	0	50	1.00		
		Jinan	50	0	0	50	0.00		
		Xian	92	2	0	94	1.06		
		Baoji	48	0	0	48	0.00		
		Shanghai	49	1	0	50	1.00		
		Changsha	50	0	0	50	0.00		
		Heping	46	0	0	46	0.00		
		Nanning	49	1	0	50	1.00		
		Tainan	49	0	0	49	0.00		
		Total	582	5	0	587	0.43		
	Korea	Seoul	102	3	0	105	1.43	50.00	1.36
		Chonan	28	1	0	29	1.72		
		Haman	45	1	0	46	1.09		
		Pusan	48	1	0	49	1.02		
		Jeju-do	63	2	0	65	1.54		
		Total	286	8	0	294	1.36		
	Japan	Mainland	1339	32	3	1374	1.38	125.00	3.39
		Okinawa	98	2	0	100	1.00		
		Total	1437	34	3	1474	1.36		
Southeast Asia	Vietnam	Hanoi	103	0	0	103	0.00	90.50	0.00
	Philippines	Manila	50	0	0	50	0.00	94.00	0.00

Fig. 1 Locations of the sampling sites in East and Southeast Asian countries.

to 2009. Briefly, the recruited subjects comprised 587 participants from 11 Chinese locations, 294 from five Korean locations, 1474 from five Japanese locations, 103 from one Vietnamese location, and 50 from one Philippines location. Most of the participants were females, with the exception of 608 Japanese males and 80 Korean males. Information on age was only available for the participants from certain locations. Angiography was only conducted for 384 Japanese and 46 Korean participants.

The geographic distributions of p.R4810K in the different ethnicities are shown in Table 2 and Fig. 1. p.R4810K was detected in East Asian populations, but not in Southeast Asian populations. p.R4810K was detected at allele frequencies of 0.43%, 1.36%, and 1.36% in the Chinese, Korean, and Japanese populations, respectively. p.R4810K was detected at an allele frequency of about 1.00% in 4 of 11 investigated locations in China, suggesting that the distribution of p.R4810K was heterogeneous and limited to certain specific locations in China. In contrast, p.R4810K was homogeneously located throughout Korea and Japan at relatively high allele frequencies of 1.00–1.72%. p.R4810K was not detected in 49 Taiwanese, 103 Vietnamese, and 50 Filipino participants.

Discussion

The present study confirmed the presence of similar allele frequencies of the variant p.R4810K of *RNF213* in the Japanese and Korean general populations. However, expansion of the examined population revealed that the allele frequency of p.R4810K in the Chinese population was 0.43%, only one-third of the

frequency in the Japanese or Korean populations. Therefore, our previous observation was considered to be attributable to selection bias.⁹⁾ The lower prevalence of p.R4810K in the Chinese general population might be proportional to the lower carrier rate (i.e., 23%) in Chinese patients. In accordance with this observation, a single dominant polymorphism may be associated with Japanese or Korean patients, whereas various polymorphisms in *RNF213* may be associated with Chinese patients. In fact, we observed five additional mutations in Chinese patients but no additional mutations in Korean or Japanese patients.⁹⁾

In the present study, magnetic resonance angiography was only conducted for limited numbers of participants. This limitation did not affect our results because of the very low prevalences of moyamoya disease in the general populations. The estimated total numbers of carriers were 11.41 million for the Chinese, 1.36 million for the Korean, and 3.39 million for the Japanese populations. Assuming that moyamoya disease occurs in one of 300 carriers with p.R4810K,^{8,9)} the estimated numbers of patients with moyamoya disease attributable to p.R4810K are 38,000 in the Chinese, 4,500 in the Korean, and 11,300 in the Japanese populations. Although these numbers are very approximate and minimum estimates, because only the p.R4810K variant is considered, the large number of potential sufferers suggests that more attention should be paid to moyamoya disease in East Asian countries.

Differences in the clinical profiles of moyamoya disease have been recognized in China, Korea, and Japan.^{1,3)} However, the differences between the clinical profiles of Korean and Japanese patients are unlikely to be explained by genetic differences. Specific factor(s) other than the genetic factor are needed to explain the variations in the clinical phenotypes and low penetrance among carriers.^{8,9)} Therefore, unknown modifier factor(s) may also be contributory to the differences in the clinical phenotypes. Further research is needed to identify such factor(s), which may also be crucial for the development of moyamoya disease among carriers of variant p.R4810K in *RNF213*.

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