Rare variants of \textit{RNF213} and moyamoya/non-moyamoya intracranial artery stenosis/occlusion disease risk: a meta-analysis and systematic review

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Abstract

\textbf{Background:} The p.R4810K and other rare variants of ring finger protein 213 gene (\textit{RNF213}) were illustrated as susceptibility variants for moyamoya (MMD) and non-moyamoya intracranial artery stenosis/occlusion disease (ICASO) recently. However, the effect sizes of p.R4810K were in great discrepancy even in studies of the same ethnic population and firm conclusions of other rare variants have been elusive given the small sample sizes and lack of replication. Thus, we performed this study to quantitatively evaluate whether or to what extent the rare variants of \textit{RNF213} contribute to MMD and ICASO in different populations.

\textbf{Methods:} A systematic search of PubMed, EMBASE, ISI web of science, CNKI, and WANFANG DATA was conducted up to 5 September 2017. Pooled odds ratios (ORs) with 95\% confidence intervals (CIs) were calculated using random- or fixed-effect models based on the between-study heterogeneity. The subgroup analyses were performed by the ethnicity and family history. Sensitivity and publication bias analysis were performed to test the robustness of associations. All the statistical analyses were conduct using STATA 12.0.

\textbf{Results:} Twenty studies including 2353 MMD cases and 5488 controls and 11 studies including 1778 ICASO cases and 3140 controls were included in this study. Pooled ORs indicated that \textit{RNF213} p.R4810K significantly increased MMD and ICASO risk in East Asians with great effect sizes of discrepancy (dominant model: odds ratios 184.04, 109.77, and 31.53 and 10.07, 28.52, and 5.59 for MMD and ICASO, respectively, in Japan, Korea, and China). It significantly increased familial MMD risk in Japan, Korea, and China with 5 ~ 36 times larger effect sizes than that for sporadic ones in each country (dominant model ORs 1802.44, 512.42, 1109.02 and 134.35, 99.82, and 30.52, respectively, for familial and sporadic cases). The effect sizes of \textit{RNF213} p.R4810K to sporadic MMD were 3 ~ 4 times larger in Japan and Korea than those in China. \textit{RNF213} p.R4810K also increased the ICASO risk in Japan and Korea with 2 ~ 4 times larger effect sizes than that in China (dominant model ORs 10.71, 28.52, and 5.59, respectively). Another two rare variants- p.E4950D and p.A5021V significantly increased MMD risk in Chinese population (dominant model ORs 9.06 and 5.01, respectively). Various other rare variants in \textit{RNF213} were identified in Japanese, Chinese, European, and Hispanic American populations without association evidence available yet.

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Background
Moyamoya disease (MMD) is an idiopathic steno-occlusive disease of intracranial arteries characterized by progressive bilateral and occasionally unilateral stenosis and occlusion of the distal internal carotid artery, with frequent involvement of the middle cerebral artery and anterior cerebral artery, and by the abnormal development of a hazy network of basal collateral vessels [1, 2]. MMD occurs worldwide, but its prevalence is highest in East Asian countries, including Japan, Korea, and China [3–6]. There are two incidence peaks for MMD, one in children around 10 years of age and another in adults in their 30–40 years [7]. Affected individuals are at risk for intracranial hemorrhagic or ischemic stroke, seizures, cognitive impairment, and developmental delays [1]. Although much progress has been made in our understanding of MMD, the etiology is still not well understood, and no medication can inhibit or reverse its progression. At present, direct or indirect neurosurgical revascularization is the mainstay MMD treatment [8]. Pathological clues for early diagnosis and novel therapeutic approaches are needed.

Based on the existence of familial cases and the observation of a strong ethnicity effect of MMD, a genetic contribution is strongly suspected [9, 10]. In 2011, two research groups identified ring finger protein 213 gene (RNF213) on 17q25.3 as a novel susceptibility gene for MMD in Japan and East Asian population, respectively [11, 12]. The RNF213 rare variant p.R4810K [rs112735431, corresponding to c.14429G>A on the basis of the NCBI Reference sequence NM_001256071.2, theminor allele frequency (MAF) in the 1000 Genome is 0.0012] significantly increases MMD risk in Japan, Korea, and China (odds ratios (ORs) were 338.94, 135.63, and 14.70 in a dominant model, respectively) [12]. Further replication studies confirmed that RNF213 p.R4810K was a founder mutation in East Asian and absent from European, Hispanic, and African-descent MMD cases [13–20]. Recently, several studies further revealed that RNF213 p.R4810K was associated with intracranial artery stenosis/occlusion that did not meet the diagnostic criteria for MMD (ICASO) in Japan, Korea, and China [21–25]. They hypothesized that some cases of ICASO ascribed to unknown etiology or atherosclerosis might be an early onset MMD which was misdiagnosed by the traditional imaging diagnostic methods [21–23, 25]. Since the therapeutic strategies are different for these diseases, genetic testing or sequencing of RNF213 is proposed for MMD and ICASO diagnosis [21–23, 25]. However, the carrier rates of RNF213 p.R4810K in MMD and ICASO were greatly discrepant in different studies. It varied from 66.7 to 90.1% in Japanese and Korean MMD patients, and to a lesser degree in Chinese ones with a range from 9.4 to 31.4%, the effect sizes were significantly different even in studies of the same ethnic population [12, 13, 18, 19, 26, 27]. For ICASO, there were more than 20% of patients who carried RNF213 p.R4810K in Japan and Korea, while the rates were much lower in China [21–25]. The lack of consistency of these studies is probably due to population stratification or small sample sizes in individual studies with inadequate statistical power. In addition, many non-p.R4810K rare variants (MAF < 0.005 in 1000 Genome database) in RNF213 have been identified in both Asian and Caucasian MMD cases recently [11–17, 19, 28]. However, RNF213 is a large gene (encodes 5207 amino acids) and harbors a number of missense variants in the general population as well as the patients [29]. The false assignment of pathogenicity may lead to incorrect therapeutic or prognostic assessments of patients [30]. Thus, scientifically quantitative evaluation of the contributions of RNF213 rare variants to MMD and ICASO is urgently needed for the future applications and studies.

Previously, Sun et al. performed meta-analysis to investigate the associations between RNF213 variants (mainly p.R4810K) and MMD susceptibility with eight studies included [31]. They concluded that RNF213 p.R4810K is closely associated with MMD risk. Recently, some other studies were subsequently performed. Considering the discrepant results and only MMD was involved in the previous meta-analysis, we performed this study to quantitatively evaluate whether or to what extent the rare variants of RNF213 contribute to MMD and ICASO in different populations.

Methods
This meta-analysis was conducted according to the Human Genome Epidemiology Network guidelines and followed the published recommendations to improve the
quality of meta-analyses of genetic association studies [32]. We assessed the quality of reporting of genotyping on the basis of the Strengthening the Reporting of Genetic Association Studies statement [33].

**Literature search strategy**

Electronic databases PubMed, EMBASE, Web of Science, WANFANG DATA, and China National Knowledge Infrastructure (CNKI) were used to retrieve potentially relevant articles on human genetic studies of MMD and ICASO that had been published up to 5 September 2017. Search terms used were *RNF213*[tw] or *RNF 213*[tw] or ring finger protein 213*[tw]. Articles in all languages were searched and translated as necessary. After relevant articles were retrieved, references were also checked for other potentially relevant articles not found in the initial search.

**Selection criteria and data extraction**

We included related studies evaluating associations of *RNF213* rare variants with proven MMD or ICASO (using computed tomography angiography or magnetic resonance angiography or digital subtraction angiography) in all ethnicities. The detailed inclusion criteria were (1) well-designed case control studies to investigate the relationship between at least one variant of *RNF213* and MMD or ICASO, or case-only studies which investigated the carrier rate of *RNF213* variants in MMD or ICASO and the carrier rates of the target variants were available in the general population; (2) clear diagnostic criteria of MMD and ICASO; (3) original papers contained independent and sufficient genotype data to calculate ORs and 95% confidence intervals (CIs); (4) all variants included in the meta-analysis should be evaluated in at least two published studies. Where duplicate or overlapped datasets existed, only the largest study was included. The studies without essential information or with overlapped data, review articles, case reports, and animal models were excluded. For the variants identified just in one study or the sample number that was limited to perform association analysis, a qualitative systematic review was performed.

Data were extracted by two of the authors (XL and JD), and differences were resolved by consensus (JY). For each included study, the following information was extracted: first author, year of publication, study population (country), mean age, familial history of MMD, numbers of patients and controls, frequency of genotypes, and Hardy–Weinberg equilibrium (HWE) status. Where genotype frequencies for each variant were unavailable, we estimated the number of cases per genotype category by using published information on risk allele frequencies and ORs for MMD or ICASO. The HWE of controls was obtained either directly from the article or indirectly by calculating from genotype distributions using a web-based program (http://www.oegge.org/software/hwe-mr-calc.shtml). Quality assessment of primary studies was performed using Newcastle–Ottawa quality assessment scale (NOS) [34]. Each study with NOS scores ≥ 6 was regarded as a high-quality study.

**Statistical analysis**

Statistical analyses were conducted using STATA12.0 software (Stata Corporation, College Station, TX, USA). Frequency of the genotypes and alleles between MMD/ICASO group and control group were compared using Chi-square or Fisher exact test. For each genetic variant with more than one publication, meta-analysis was performed to determine a pooled OR and 95% CI according to dominant, recessive and allelic models by using a fixed- or random-effect model. The significance of the pooled OR was determined using Z test, and p < 0.05 was considered statistically significant.

Heterogeneity among studies was assessed using Cochran Q test and quantified by using Higgins I² statistic. CIs for I² were also calculated. For Q test, p < 0.05 was considered as having significant heterogeneity. For variant association showing significant inter-study heterogeneity (Q test, p values < 0.05, and I² > 50%), the random-effect model was used as the pooling method; otherwise, the fixed-effect model was used. To evaluate ethnic-specific effects, subgroup analysis was performed according to the nationality of the study population. Publication bias was assessed by using the Egger regression asymmetry test and visualization of funnel plots if more than seven studies were included, and the significance was set at the p < 0.05 level. Sensitivity analysis was performed by sequentially excluding individual study to calculate the pooled OR of the remaining studies and assess the stability of the results.

**Results**

**Main characteristics of all the available studies**

Five hundred sixty-four articles were identified through the database check, and no article was identified through the related references check. After screening for duplication and eligibility, data from 24 studies met the inclusion criteria and was included. A detailed workflow chart showing the study selection is presented in Fig. 1.

In total, twenty articles investigated the association between seven *RNF213* rare variants (p.R2092C, p.D4013N, p.R4062Q, p.A4399T, p.R4810K, p.E4950D, and p.A5021V) and MMD [11–19, 21–24, 27, 35–40]; eleven articles investigated the association between *RNF213* p.R4810K and ICASO [21–25, 36, 38, 40–43]. These studies encompassed mainly Japanese, Korean, and Chinese populations. Detailed characteristics of all eligible studies are shown in Table 1.
Quantitative synthesis and heterogeneity analysis

**RNF213 rare variants and MMD**

Association between *RNF213* p.R4810K and MMD

The most robust variant associated with MMD was *RNF213* p.R4810K. Nineteen articles representing 23 studies evaluated their associations, of which 8 were conducted in Japanese; 7, in Korean; and 8, in Chinese with a total of 2331 MMD cases and 5476 controls.

The pooled results suggested a significant association between p.R4810K and MMD in all genetic models (dominant model: OR 85.91, 95% CI 56.36–130.95, \( p < 0.0001 \)) (Table 2). Country-based subgroup analysis showed that p.R4810K robustly associated with MMD in Japanese, Korean, and Chinese populations with 3.5 ~ 5.8 times effect sizes difference (dominant model ORs 184.04, 109.77, and 31.53 in Japan, Korea, and China, respectively) (Table 2, Fig. 2a).

Further stratified analysis by family history of MMD (familial index cases or sporadic cases) in each ethnic population revealed that *RNF213* p.R4810K significantly increased familial MMD risk in Japanese, Korean, and Chinese population, with 5 ~ 36 times larger effect sizes than that in sporadic cases (Table 2). For the sporadic MMD, the effect sizes of *RNF213* p.R4810K were in great discrepancy in different countries. It was 3 ~ 4 times larger in Japanese and Korean than that in Chinese (dominant model ORs 134.35, 99.82, and 30.52, respectively) (Table 2, Fig. 2a, b).

Association between *RNF213* non-p.R4810K variants and MMD

Except *RNF213* p.R4810K, the associations between the other six rare variants (p.R2092C, p.D4013N, p.R4062Q, p.A4399T, p.E4950D, and p.A5021V) and MMD were evaluated in at least two published studies. The detailed information was presented in Tables 1 and 2.

There were two rare variants—p.E4950D and p.A5021V—significantly associated with MMD in Chinese population in the pooled analysis (pooled ORs 9.06 and 5.01, 95% CIs 1.49–55.27 and 1.57–15.98, respectively, in a dominant model) (Fig. 3a, b). No
| Diseases | Rare variants | Author and reference | Year | Country | Sample size | Mean age (years) | Family history of MMD (%) | Genotype<sup>a</sup> | Carrier rate (%) OR(95% CI)<sup>b</sup> | NOS | HWE |
|----------|---------------|----------------------|------|---------|-------------|-------------------|--------------------------|----------------|--------------------------|-----|------|
| MMD      | p.R4810K<sup>G>A</sup> | Kamada et al. [11]   | 2011 | Japan   | 105/457     | -                 | -                       | 1/0            | 17/8/4/1                 | 8   | 0.65 |
|          |               | Liu et al. [12]      | 2011 | Japan   | 161/384     | 29.9 ± 21.4      | 61.8 ± 10.2               | 0/0           | 16/135/10                | 7   | 0.01 |
|          |               | Liu et al. [12]      | 2011 | Korea   | 38/223      | 38.7 ± 14.1      | 40.7 ± 10.9               | 0/0           | 8/30/0                   | 8   | 0.84 |
|          |               | Liu et al. [12]      | 2011 | China   | 52/150      | 22.7 ± 17.9      | 39.4 ± 10.9               | 0/0           | 40/11/1                  | 7   | 0.93 |
|          |               | Miyatake et al. [35] | 2012 | Japan   | 204/283     | 22.7 ± 17.9      | -                        | 201/0         | 36/153/15                | 7   | 0.88 |
|          |               | Miyawaki et al. [21] | 2012 | Japan   | 48/25       | 48.4 ± 18.7      | 498 ± 16.1                | -             | 7/40/1                   | 7   | -   |
|          |               | Wu et al. [13]       | 2012 | China   | 170/507     | 35.8 ± 13.2      | 372 ± 16.9                | 29/0          | 148/21/1                 | 9   | 0.96 |
|          |               | Wang et al. [27]     | 2013 | China   | 96/96       | 43.0 ± 13.7      | 426 ± 9.4                 | -             | 87/8/1                   | 9   | 0.96 |
|          |               | Miyawaki et al. [22] | 2013 | Japan   | 30/110      | 46.7 ± 18.4      | 391 ± 14.1                | -             | 10/19/1                  | 7   | 0.92 |
|          |               | Bang et al. [23]     | 2015 | Korea   | 131/51      | 51.3 ± 13.7<sup>c</sup> | -                     | -             | 32/59/1                  | 7   | -   |
|          |               | Lee et al. [15]      | 2015 | China   | 36/500      | 500 ± 0         | -                        | 222/0         | 30/6/0                   | 7   | 0.96 |
|          |               | Moteki et al. [16]   | 2015 | Japan   | 103/95      | 279(9-42)<sup>c</sup> | 262/0                 | -             | 27/11/5                  | 7   | 0.92 |
|          |               | Huang et al. [35]    | 2016 | Korea   | 52/80       | 41.6 ± 11.2      | 40 ± 10.4                | -             | 45/6/1                   | 8   | 0.96 |
|          |               | Kim et al. [37]      | 2016 | Korea   | 165/294     | 21.3 ± 13.6      | 409 ± 10.9               | 279/0         | 40/112/13                | 7   | 0.81 |
|          |               | Shoemaker et al. [17] | 2016 | Korea   | 5/5         | -                        | -                     | -             | 2/3/0                   | 7   | -   |
|          |               | Shoemaker et al. [17] | 2016 | Korea   | 11/11       | -                        | -                     | -             | 3/8/0                   | 7   | -   |
|          |               | Shoemaker et al. [17] | 2016 | China   | 6/6         | -                        | -                     | -             | 5/0/0                   | 7   | -   |
|          |               | Huang et al. [18]    | 2016 | Korea   | 81/100      | 42.7 ± 12.2      | 402 ± 11.9               | -             | 69/10/2                  | 8   | 0.92 |
|          |               | Bang et al. [24]     | 2016 | Korea   | 288/83      | 45.9 ± 12.7      | 560 ± 12.2               | 101/0         | 89/199/0                 | 7   | 0.96 |
|          |               | Zhang et al. [19]    | 2016 | China   | 255/300     | 26.7 ± 14.7      | 280 ± 15.9               | 137/0         | 175/78/2                  | 9   | -   |
|          |               | Park et al. [38]     | 2017 | Korea   | 25/100      | 49 ± 7.1        | -                        | -             | 7/18/0                   | 9   | 0.92 |
|          |               | Jang et al. [39]     | 2017 | Korea   | 264/1516    | 44.4          | -                        | -             | 86/177/1                 | 7   | 0.63 |
|          |               | Shinya et al. [40]   | 2017 | Japan   | 5/100       | 46.4 ± 19.3      | 688 ± 15.8               | -             | 1/0/0                    | 9   | 0.92 |
|          |               | Shoemaker et al. [17] | 2015 | European | 74/74       | -                        | -                     | -             | 73/1/0                   | 7   | -   |
|          |               | Shoemaker et al. [17] | 2015 | Hispanic | 6/6         | -                        | -                     | -             | 5/1/0                    | 8   | 0.92 |
|          |               | Liu et al. [12]      | 2011 | Czech   | 8/120       | -                        | -                     | -             | 7/1/0                    | 7   | -   |
|          |               | Cecchi et al. [14]   | 2014 | European | 22/12       | -                        | -                     | -             | 19/3/0                   | 6   | -   |
|          |               | Zhang et al. [19]    | 2016 | China   | 255/300     | 26.7 ± 14.7      | 280 ± 15.9               | 137/0         | 254/1/0                  | 9   | -   |
|          |               | Liu et al. [12]      | 2011 | Germany | 42/164      | -                        | -                     | -             | 41/1/0                   | 7   | -   |
|          |               | Moteki et al. [16]   | 2015 | Japan   | 370/279     | -                        | -                     | -             | 369/1/0                  | 7   | -   |
| Diseases | Rare variants | Author and reference | Year | Country | Sample size | Mean age (years) | Family history of MMD (%) | Genotype<sup>a</sup> | Carrier rate (%) | OR(95% CI)<sup>b</sup> | NOS | HWE |
|----------|---------------|----------------------|------|---------|-------------|-----------------|------------------------|----------------|----------------|-----------------|-----|------|
|          | p.A4399T     | Zhang et al. [19]    | 2016 | China   | 255, 300   | 26.7 ± 14.7, 280 ± 15.9 | 13.7 –         | 254/1/0, 300/0/0 | 0.39, 0.00 | 3.54 (0.14–87.33)<sup>c</sup> | 9   | –    |
| MMD      | (rs148731719) | Kamada et al. [11]   | 2011 | Japan   | 63, 53     | – – –             | – – –         | 59/4/0, 51/2/0 | 6.35, 3.77 | 1.73 (0.30–9.83) | 8   | 0.89 |
|          | (G>A)        | Miyatake et al. [35] | 2012 | Japan   | 204, 188   | 22.7 ± 17.9, –     | 20.1 –        | 191/12/1, 172/16/0 | 6.37, 8.51 | 0.73 (0.34–1.56) | 7   | 0.54 |
|          | p.E4950D     | Wu et al. [13]       | 2012 | China   | 170, 507   | 48.4 ± 18.7, 498 ± 16.1 | – – –         | 142/27/1, 462/45/0 | 16.47, 8.88 | 2.60 (1.53–4.43) | 9   | 0.3  |
|          | (rs148731719)| (G>A)               |      |         |            |                  |               |                       |               |               |                 |     |      |
|          | p.A5021V     | Wang et al. [27]     | 2013 | China   | 96, 96     | 43.0 ± 13.7, 426 ± 9.4 | – – –         | 96/0/0, 85/11/0 | 0.00, 11.46 | 0.04 (0.00–0.66)<sup>c</sup> | 9   | 0.55 |
|          | (rs138130613)| (C>T)               |      |         |            |                  |               |                       |               |               |                 |     |      |
|          | p.R4810K     | Huang et al. [36]    | 2015 | China   | 52, 80     | 41.6 ± 11.2, 40 ± 104 | – – –         | 49/3/0, 80/0/0 | 5.77, 0.00 | 11.38 (0.58–225.08)<sup>c</sup> | 8   | –    |
| ICASO    | (rs112735431)| (G>C)               |      |         |            |                  |               |                       |               |               |                 |     |      |
|          | p.A5021V     | Liu et al. [12]      | 2011 | China   | 52, 150    | 22.7 ± 17.9, 394 ± 10.9 | – – –         | 50/2/0, 150/0/0 | 3.85, 0.00 | 14.90 (0.70–315.60)<sup>c</sup> | 7   | –    |
|          | (rs138130613)| (C>T)               |      |         |            |                  |               |                       |               |               |                 |     |      |
|          | p.R4810K     | Wu et al. [13]       | 2012 | China   | 170, 507   | 48.4 ± 18.7, 498 ± 16.1 | – – –         | 169/1/0, 507/0/0 | 0.59, 0.00 | 8.98 (0.36–221.54)<sup>c</sup> | 9   | –    |
|          | (rs112735431)| (G>C)               |      |         |            |                  |               |                       |               |               |                 |     |      |
|          | p.R4810K     | Wang et al. [27]     | 2013 | China   | 50, 90     | – – –             | – – –         | 47/3/0, 89/1/0 | 6.00, 1.11 | 2.39 (0.39–14.80) | 9   | 0.91 |
|          | (rs112735431)| (G>C)               |      |         |            |                  |               |                       |               |               |                 |     |      |
|          | p.R4810K     | Huang et al. [36]    | 2015 | China   | 52, 80     | 41.6 ± 11.2, 40 ± 104 | – – –         | 49/3/0, 78/2/0 | 5.77, 2.50 | 5.68 (1.57–15.98) | 8   | 0.96 |
|          | (rs112735431)| (G>C)               |      |         |            |                  |               |                       |               |               |                 |     |      |
|          | p.R4810K     | Miyawaki et al. [21] | 2012 | Japan   | 41, 25     | 62.3 ± 11.3, 498 ± 16.1 | – – –         | 32/8/1, 25/0/0 | 21.95, 0.00 | 14.91 (0.83–268.43)<sup>c</sup> | 7   | –    |
|          | (rs112735431)| (G>C)               |      |         |            |                  |               |                       |               |               |                 |     |      |
|          | p.R4810K     | Miyawaki et al. [22] | 2013 | Japan   | 84, 110    | 61.5 ± 12.6, 391 ± 14.1 | – – –         | 64/20/0, 108/2/0 | 23.81, 1.82 | 16.88 (3.82–74.58) | 7   | 0.92 |
|          | (rs112735431)| (G>C)               |      |         |            |                  |               |                       |               |               |                 |     |      |
|          | p.R4810K     | Bang et al. [23]     | 2015 | Korea   | 221, 51    | – – –             | – – –         | 144/77/0, 51/0/0 | 34.84, 0.00 | 55.24 (3.36–907.41)<sup>c</sup> | 7   | –    |
|          | (rs112735431)| (G>C)               |      |         |            |                  |               |                       |               |               |                 |     |      |
|          | p.R4810K     | Huang et al. [36]    | 2015 | Korea   | 64, 80     | 42.5 ± 12.2, 40 ± 104 | – – –         | 58/5/1, 79/1/0 | 9.38, 1.25 | 8.17 (0.96–69.74) | 8   | 0.96 |
|          | (rs112735431)| (G>C)               |      |         |            |                  |               |                       |               |               |                 |     |      |
|          | p.R4810K     | Shinya et al. [40]   | 2017 | Japan   | 104, 100   | 88.80 ± 15.8    | – – –         | 94/10/0, 98/2/0 | 9.62, 2.00 | 5.21 (1.11–24.42) | 9   | 0.92 |

**MMD** moyamoya disease, **ICASO** non-moyamoya intracranial artery stenosis/occlusion disease, – not available

<sup>a</sup>Genotype presented as wild type/heterozygous/homozygous

<sup>b</sup>OR(95% CI) was calculated in the dominant model

<sup>c</sup>We applied a half-integer continuity correction to all four cells if the event rates were zero

<sup>d</sup>Mean age of 352 intracranial stenosis patients (including MMD and ICASO) in this study.

<sup>e</sup>Median age at onset and interquartile range

<sup>f</sup>For this case-only study, we use 1516 general Korean individuals reported by Jang MA et al. as control [39]
Table 2  Main results of the pooled ORs in meta-analysis for the association between RNF213 rare variants and MMD or ICASO

| Variants | N | Sample size (case/control) | Dominant model | OR (95% CI) | p² (%) | p¹ | p² | Recessive model | OR (95% CI) | p² (%) | p¹ | p² | Allelic model | OR (95% CI) | p² (%) | p¹ | p² |
|----------|---|---------------------------|----------------|-------------|--------|----|----|----------------|-------------|--------|----|----|----------------|-------------|--------|----|----|
| **MMD**  |    |                           |                |             |        |    |    |                |             |        |    |    |                |             |        |    |    |
| (1) p.R4810K (rs112735431) |    |                           |                |             |        |    |    |                |             |        |    |    |                |             |        |    |    |
| Total    | 23 | 2311/5476                 | 85.91 (56.36–130.95) | 51.6 | 0.002 | <0.0001 | 13.19 (6.37–27.31) | 0.0 | 0.886 | <0.0001 | 46.54 (36.73–58.97) | 0.0 | 0.497 | <0.0001 |
| **Subgroup analysis** |    |                           |                |             |        |    |    |                |             |        |    |    |                |             |        |    |    |
| Country  |    |                           |                |             |        |    |    |                |             |        |    |    |                |             |        |    |    |
| Japan    | 8  | 661/1459                  | 184.04 (119.56–283.29) | 0.0 | 0.453 | <0.0001 | 19.52 (6.30–60.47) | 0.0 | 0.666 | <0.0001 | 58.64 (40.88–84.12) | 0.0 | 0.621 | <0.0001 |
| Familial | 3  | 131/1124                  | 180.44 (47.29–686.90) | 0.0 | 0.757 | <0.0001 | 51.70 (11.53–231.80) | 0.0 | 0.676 | <0.0001 | 77.40 (50.44–117.66) | 0.0 | 0.370 | <0.0001 |
| Sporadic | 8  | 530/1459                  | 134.35 (80.77–208.02) | 17.8 | 0.289 | <0.0001 | 9.93 (2.96–33.30) | 0.0 | 0.884 | <0.0001 | 52.08 (35.41–76.60) | 0.0 | 0.504 | <0.0001 |
| Korea    | 7  | 922/2278                  | 109.77 (76.30–157.93) | 0.0 | 0.889 | <0.0001 | 17.38 (308.98–07) | 386 | 0.196 | 0.001 | 42.81 (30.25–60.57) | 0.0 | 0.804 | <0.0001 |
| Familial | 1  | 46/294                    | 512.42 (130.85–2006.64) | – | – | <0.0001 | 35.09 (156–7005.8) | – | – | 0.025 | 69.41 (30.92–155.83) | – | – | <0.0001 |
| Sporadic | 7  | 876/2278                  | 99.82 (69.22–143.93) | 0.0 | 0.858 | <0.0001 | 17.62 (3.14–99.02) | 424 | 0.176 | 0.001 | 49.52 (35.11–69.85) | 0.0 | 0.933 | <0.0001 |
| China    | 8  | 748/1739                  | 31.53 (16.18–61.46) | 24.2 | 0.236 | <0.0001 | 5.48 (1.64–18.35) | 0.0 | 0.999 | 0.006 | 31.51 (16.02–62.00) | 22.0 | 0.3 | <0.0001 |
| Familial | 2  | 40/807                    | 110902 (99.39–12,375.41) | 0.0 | 0.943 | <0.0001 | 338.33 (12.09–947.51) | – | – | 0.001 | 57.05 (60.07–5505.59) | 0.0 | 0.989 | <0.0001 |
| Sporadic | 8  | 743/1739                  | 30.52 (15.63–59.59) | 21.9 | 0.256 | <0.0001 | 5.12 (1.40–18.77) | 0.0 | 0.998 | 0.014 | 29.77 (15.19–58.35) | 5.3 | 0.390 | <0.0001 |
| Total    | 6  | 217/2225                  | 111656 (462.75–2684.12) | 0.0 | 0.849 | <0.0001 | 51.86 (14.18–189.64) | 0.0 | 0.720 | <0.0001 | 85.35 (59.09–123.27) | 22.7 | 0.156 | <0.0001 |
| Sporadic | 23 | 2139/5476                 | 75.03 (50.67–1110.99) | 43.6 | 0.014 | <0.0001 | 9.41 (43.6–203.2) | 0.0 | 0.957 | <0.0001 | 46.34 (36.32–59.13) | 0.0 | 0.633 | <0.0001 |
| (2) p.R2092C (rs139265462) |    |                           |                |             |        |    |    |                |             |        |    |    |                |             |        |    |    |
| Total    | 2  | 80/80                     | 3.27 (0.32–33.80) | 0.0 | 0.949 | 0.321 | – | – | – | – | 3.13 (0.31–31.28) | 0.0 | 0.974 | 0.331 |
| (3) p.D4013N (rs397514563) |    |                           |                |             |        |    |    |                |             |        |    |    |                |             |        |    |    |
| Total    | 3  | 285/432                   | 6.47 (0.96–43.55) | 0.0 | 0.443 | 0.055 | – | – | – | – | 6.18 (0.92–41.33) | 0.0 | 0.433 | 0.06 |
| (4) p.R4062Q |    |                           |                |             |        |    |    |                |             |        |    |    |                |             |        |    |    |
| Total    | 3  | 400/559                   | 4.64 (0.72–29.96) | 0.0 | 0.798 | 0.107 | – | – | – | – | 4.62 (0.72–29.76) | 0.0 | 0.798 | 0.107 |
### Table 2: Main results of the pooled ORs in meta-analysis for the association between RNF213 rare variants and MMD or ICASO (Continued)

| Variants | N   | Sample size (case/control) | Dominant model | Recessive model | Allelic model |
|----------|-----|-----------------------------|----------------|-----------------|---------------|
|          |     |                             | OR (95% CI)    | p^2 (%)         | p^2 (%)       |
|          |     |                             |                |                 |               |
|          |     |                             | p^2 (%)        |                 |               |
|          |     |                             |                |                 |               |
| (5) p.A4399T (rs148731719) |     |                             |                |                 |               |
| Total    | 5   | 585/924                    | 1.15 (0.41–3.25)^a | 70.8            | 0.008         | 0.79          |
|          |     |                             | 4.80 (0.49–47.02) | 0.0             | 0.611         | 0.178         |
|          |     |                             | 1.19 (0.45–3.19)^a | 69.2            | 0.011         | 0.727         |
| Subgroup analysis |     |                             |                |                 |               |
| Japan    | 2   | 267/241                    | 0.85 (0.43–1.68) | 0.0             | 0.374         | 0.636         |
|          |     |                             | 2.78 (0.11–68.63) | –               | –             | 0.532         |
|          |     |                             | 0.90 (0.46–1.76) | 0.0             | 0.426         | 0.767         |
| China    | 3   | 318/683                    | 1.13 (0.06–21.30)^a | 80.1            | 0.007         | 0.933         |
|          |     |                             | 8.98 (0.36–221.54) | –               | –             | 0.179         |
|          |     |                             | 1.04 (0.07–15.72)^a | 79.6            | 0.007         | 0.975         |
| (6) p.E4950D (rs371441113) |     |                             |                |                 |               |
| Total    | 3   | 477/957                    | 9.06 (1.49–55.27) | 0.0             | 0.915         | 0.017         |
|          |     |                             | –              | –               | –             | –             |
|          |     |                             | 9.00 (1.48–54.78) | 0.0             | 0.917         | 0.017         |
| (7) p.A5021V (rs138130613) |     |                             |                |                 |               |
| Total    | 4   | 324/827                    | 5.01 (1.57–15.98) | 0.0             | 0.738         | 0.006         |
|          |     |                             | –              | –               | –             | –             |
|          |     |                             | 4.91 (1.55–15.53) | 0.0             | 0.733         | 0.007         |
| ICASO    |     |                             |                |                 |               |
| p.R4810K (rs112735431) |     |                             |                |                 |               |
| Total    | 11  | 1778/3140                  | 13.89 (8.01–2409) | 37.0            | 0.140         | <0.0001       |
|          |     |                             | 2.70 (0.28–26.38) | 0.0             | 0.764         | 0.394         |
|          |     |                             | 13.01 (7.55–22.42) | 18.3            | 0.269       | <0.0001       |
| Subgroup analysis |     |                             |                |                 |               |
| Japan    | 3   | 229/235                    | 10.71 (3.97–28.91) | 0.0             | 0.537         | <0.0001       |
|          |     |                             | 1.89 (0.07–48.17) | –               | –             | 0.7           |
|          |     |                             | 10.00 (3.74–26.77) | 0.0             | 0.570         | <0.0001       |
| Korea    | 4   | 517/1750                   | 28.52 (11.04–73.67) | 0.0             | 0.779         | <0.0001       |
|          |     |                             | –              | –               | –             | –             |
|          |     |                             | 24.16 (9.71–60.13) | 0.0             | 0.845         | <0.0001       |
| China    | 4   | 1022/1155                  | 5.59 (2.12–14.75) | 11.6            | 0.335         | 0.001         |
|          |     |                             | 3.80 (0.15–94.95) | –               | –             | 0.416         |
|          |     |                             | 5.76 (7.55–22.42) | 13.0            | 0.328         | <0.0001       |

N number of studies, – not available
^aORs were calculated under random-effects model
^b p value for Q test
^c p value for Z test, I^2, Higgins I^2 statistic
significant associations were observed between the other four variants and the susceptibility of MMD in this meta-analysis (Table 2).

**Association between RNF213 p.R4810K and ICASO**

The association between p.R4810K and ICASO was investigated by 11 studies, including 1778 ICASO patients and 3140 controls. Result showed that p.R4810K was significantly associated with the risk of ICASO (dominant model: OR 13.89, 95% CI 8.01–24.09, \( p < 0.0001 \) (Table 2 and Fig. 4a).

Subgroup analysis showed that the strongest association was observed in Korea (dominant model: OR 28.52; 95% CI 11.04–73.67, \( p < 0.0001 \)), followed by that in Japan (dominant model: OR 10.71, 95% CI 3.97–28.91, \( p < 0.0001 \)) and China (dominant model: OR 5.59, 95% CI 2.12–14.75, \( p = 0.001 \) (Table 2, Fig. 4b).

**Fig. 2** Forest plots for the association of **RNF213** p.R4810K with MMD under the dominant model. a Forest plots of **RNF213** p.R4810K and sporadic MMD in different subgroup populations under the dominant model. b Forest plots of **RNF213** p.R4810K and familial MMD in different subgroup populations under the dominant model.

**Fig. 3** Forest plots for the association of **RNF213** p.E4950D and p.A5021V with MMD under the dominant model. a Forest plots of **RNF213** p.E4950D and MMD in Chinese population under the dominant model. b Forest plots of **RNF213** p.A5021V and MMD in Chinese population under the dominant model.
Publication bias
Owning to the association between RNF213 p.R4810K and MMD that was investigated by 23 studies, we used Begg’s funnel plot and the Egger regression asymmetry test to assess the publication bias of these studies. In the dominant model, the results of Begg’s funnel plot (continuity corrected p value, 0.561) and the Egger regression asymmetry test (t = −1.27, p = 0.218) did not find significant asymmetry (Fig. 5a). For the association between RNF213 p.R4810K and ICASO, no significant publication bias was observed (Fig. 5b).

Sensitivity analysis
Sensitivity analysis was performed by sequentially excluding individual study for each meta-analysis to assess the stability of the results. For the association between RNF213 p.R4810K and MMD or ICASO, corresponding pooled ORs showed no significant change when sequentially excluded one study from each meta-analysis, which indicated that these results are stable and reliable (Additional file 1: Figure S1).

Systematic review of other RNF213 rare variants and MMD
Except variants mentioned above, various other rare variants of RNF213 were identified in Japanese, Chinese, European, and Hispanic American populations (Fig. 6 and Additional file 2: Table S1) [11–14, 16, 17, 28]. These variants were not found in control subjects and were
detected in only one patient, suggesting that they had potential causative effects in MMD development.

Discussion
MMD is a rare idiopathic intracranial vascular disorder with strong genetic components. Genetic study of familial MMD clearly indicated autosomal dominant inheritance pattern [44]. RNF213 was the first identified susceptibility gene for MMD recently. We performed this study to quantitatively evaluate whether or to what extent the rare variants of RNF213 contribute to MMD and ICASO in different populations. The main results showed that RNF213 p.R4810K significantly increased familial MMD risk in Japanese, Korean, and Chinese population (dominant model ORs 1802.44, 512.42, and 1109.02), with 5 ~ 36 times larger effect sizes than that for sporadic cases (dominant model ORs 134.35, 99.82, and 30.52) (Table 2). The pooled results were similar to the original report by Liu et al. [12] and illustrated that genetic screening of RNF213 p.R4810K in Japanese, Korean, and Chinese population especially in the people with familial history of MMD would be an effective approach to identify asymptomatic patients [44]. For the sporadic cases, significant effect sizes difference was observed in different countries. The effect sizes of RNF213 p.R4810K were 3 ~ 4 times greater in Japanese and Korean population than that in Chinese. This illustrated that distinct genetic background may exist and other environmental or genetic factor(s) may contribute to sporadic MMD. In this study, we found another two rare variants—p.E4950D and p.A5021V—in RNF213 significantly increased MMD risk in Chinese population in the pooled analysis (pooled ORs 9.06 and 5.01, 95% CIs 1.49 – 55.27 and 1.57 – 15.98, respectively, in the dominant model). In addition, more than 40 other rare missense variants of RNF213 were identified in Chinese MMD cases but absent in controls (such as p.D4013N, p.R4062Q, p.D4863N, p.D5160E, and p.E5176G) [12, 13, 19]. Of them, p.D4013N and p.R4062Q have been independently reported by different studies, highly indicating the causative effects [12–14, 19]. Recently, Kobayashi et al. found that RNF213 p.D4013N-transfected human umbilical vein endothelial cells displayed significant lowered migration activity which was similar with the experiment result of p.R4810K transfection and strongly indicated the disease-causing effect [28]. However, due to the low allele
frequency and the limited sample size, it was difficult to get association evidences for them. Furthermore, except for the rare variants mentioned above, more than half of Chinese MMD has not been identified the possible disease-causing variants of RNF213 [19]. MMD appears to have more complex determiners in China. In addition, even in Japan and Korea, the majority of carriers with RNF213 p.R4810K remain unaffected with MMD [26]. Unknown factors are considered to overlay the genetic predisposition to develop MMD [45]. Both genetic and environmental triggers should be explored in the future studies.

Except for the variants mentioned above in the Asian population, various RNF213 rare variants were identified in MMD cases worldwide [12, 14]. Even RNF213 p.R4810K was not identified in European, Hispanic, or Black descent MMD patients, other rare variants in RNF213 were identified in these populations, such as p.A529del, p.R3922Q, p.N3962Q, p.C3997Y, p.D4013N, and p.R4019C (Fig. 6) [12, 14, 20, 28]. Due to the low allele frequency and the limited sample size, no associations were observed between these variants and MMD. However, there is evidence suggesting that many of these variants are disease causing. First, the variants are either not present or present at extremely low frequencies (MAF < 0.001) in the Exome Variant Server database. Second, most of these variants located in the C terminus of RNF213 protein, which is where the RNF213 p.R4810K founder variant located [29]. Even with limited information about these variants, causative effect was highly suspected. The genetic heterogeneity may partly explain why manifestations of MMD vary by geographic regions and ethnic groups.

In this study, we also found that RNF213 p.R4810K was significantly associated with ICASO in Japan and Korea (pooled OR 10.71 and 28.52, 95% CI 5.59–28.91 and 11.04–73.67, respectively) and to a less degree in Chinese population (pooled OR 5.59; 95% CI 2.12–14.75). About the association results, there are two possible explanations: (1) MMD has been misclassified as ICASO due to the atypical manifestation with the absence of one or two diagnostic criteria and lead to the spurious association between RNF213 p.R4810K and ICASO or (2) RNF213 p.R4810K is indeed associated with ICASO. Currently, MMD was diagnosis based on the findings of magnetic resonance angiography or digital subtraction angiography: (1) steno-occlusive lesions around the terminal portions of the internal carotid arteries (including proximal portions of the anterior and middle cerebral arteries), (2) moyamoya vessels at the base of the brain appearing as abnormal vascular networks, (3) findings 1 and 2 are present bilaterally [1]. Bang et al. analyzed 352 consecutive ischemic patients within the middle cerebral artery distribution and found that the occurrence of RNF213 p.R4810K increased with the number of observed angiographic criteria. They demonstrated that the current criteria is limited in distinguishing MMD and ICASO, and a substantial proportion of patients with adult-onset MMD may be misclassified as having ICASO [23]. However, they found that more than one fifth of ICASO patients confirmed by high-resolution magnetic resonance imaging and conventional angiography had RNF213 p.R4810K variant in a subsequent research, which demonstrated that the RNF213 p.R4810K is also a high-risk variant for ICASO [24]. We prefer to agree that there exist a new entity of ICASO caused by the RNF213 p.R4810K variant, which can be differentiated from ICASO caused by atherosclerosis by using genetic analysis [21, 22]. However, similar with MMD, geographic and ethnic discrepancies are also highly indicated for ICASO. In China, RNF213 p.R4810K variant contributed less extent of ICASO risk compared to that in Korea and Japan (pooled ORs were 5.59, 28.52, and 10.71 in China, Korea, and Japan, respectively). Similar with MMD, ethnicity-specific genetic and environmental factors may contribute to this discrepancy. Further well-designed genetic epidemiology studies focusing on ethnicity-specific risk factors such as choosing the relative genetically homogenous population and comprehensively collecting the detailed environmental factors of ICASO are needed.

To date, the mechanisms of how RNF213 p.R4810K and other rare variants lead to intracranial vascular lesions are still unknown [29]. An in vitro functional study revealed that RNF213 p.R4810K affected neither the transcription level nor the ubiquitin ligase activity of the protein [12]. Knockdown of RNF213 in zebrafish leads to abnormal sprouting and irregular diameter of intracranial vessels, suggesting some role of RNF213 in the vascular formation [12]. Hitomi et al. observed reduced angiogenic activity and genomic instability in endothelial cells derived from induced pluripotent stem cells of p.R4810K-mutated patients [46, 47]. However, ablation of Rnf213 in mice did not induce any apparent abnormality of the vascular system [45, 48]. Unknown factors are considered to overlay the genetic predisposition in the RNF213 p.R4810K carrier to develop vascular lesions [49]. Recently, Kobayashi et al. found that RNF213 p.R4810K showed a reduced angiogenesis of transgenic mouse response to hypoxia in vivo [49]. Scholz et al. found that Rnf213 was a co-regulated gene for the WNT signaling enhancer R-spondin3 (RSPO3) and identified that endothelial RSPO3-driven non-canonical WNT/Ca(2+)/NFAT signaling as a critical maintenance pathway of the remodeling vasculature [50]. Banh et al. found that protein-tyrosine phosphatase-1B (PTP1B) controlled non-mitochondrial oxygen consumption by regulating RNF213 to promote tumor survival during hypoxia and concluded that PTP1B/RNF213/α-KGDD pathway was
critical for survival of tumors in the hypoxic microenvironment [51]. The investigation of WNT signaling and PTP1B/RNF213/α-KGDD pathway in cells expressing RNF213 R4810K and other rare variants under different environmental condition such as hypoxia and chronic inflammation is expected to provide answers to the pending questions.

The limitations of this study should be considered. First, due to the fact that we analyzed the association between rare variants and diseases, the number of cases and controls involved in the meta-analysis for moderate effect rare variants may be less powered, studies with larger sample size and high quality are needed to explore the associations in the future; second, MMD and ICASO appears to have complex determiners, with both genetic predisposition and environmental triggers. Unknown modifier factor(s) may also be contributory to MMD and ICASO. Multivariate analysis to adjust for the confounding factors such as behavior or clinical or biochemical factors in our meta-analysis was not available. Further comprehensive studies focusing on multiple ethnicity-specific factors are needed; third, ICASO may represent a broad spectrum of diseases and there are various phenotypes (i.e., bilateral M1 occlusion or unilateral M2 stenosis and so on), which may belong to different clinical entities. Due to no more clinical information available in the original papers, subgroup analysis could not be performed according to these factors, which may lead to bias. Further studies with detailed clinical features are needed; fourth, this analysis was constrained to studies which were published and deposited in English and Chinese databases, the other databases were not available, and selection bias could not be excluded (Additional file 3).

Conclusions
This comprehensive systematic review and meta-analysis reveals that the critical roles of RNF213 p.R4810K in MMD especially familial MMD and ICASO in Japan, Korea, and China. It significantly increases MMD and ICASO risk in Japanese and Korean population and to a less degree in Chinese population. Except for RNF213 p.R4810K, another two rare variants—p.E4950D and p.A5021V—increased MMD risk in Chinese population. MMD seems to have more complex determiners in China. Distinct genetic background exists, and other environmental or genetic factor(s) may contribute to MMD. Studies focused on delineating the ethnicity-specific factors and pathological role of RNF213 variants in MMD and ICASO are needed.

Additional files

Additional file 1: Figure S1. Sensitivity analysis of the association of RNF213 p.R4810K with MMD and ICASO under a dominant model (TIFF 1024 kb)

Additional file 2: Table S1. Other rare variants of RNF213 identified in different populations (XLSX 14 kb)

Additional file 3: Literature list (XLSX 67 kb)
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