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Detailed phenotype of *RNF213* p.R4810K variant identified by the Chinese patients with acute ischaemic stroke or transient ischaemic attack

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ABSTRACT

Background and purpose The ring finger protein 213 gene (*RNF213*) p.R4810K variant increased the risk of acute ischaemic stroke (AIS) attributable to intracranial arterial stenosis (ICAS) in the Japanese and Korean populations. In this study, we aimed to examine the prevalence of the *RNF213* p.R4810K variant in Chinese patients with AIS or transient ischaemic attack and identify the phenotype of the carriers.

Methods We analysed data from the Third China National Stroke Registry. All included participants were divided into two groups by carrier status of the p.R4810K variant. The aetiological classification was conducted according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria. The presence of ICAS and extracranial arterial stenosis (ECAS) was defined as 50%–99% stenosis or occlusion of any intracranial and extracranial artery. Logistic regression models and Cox regression models were used to evaluate the association of the p.R4810K variant with TOAST classification, stenosis phenotypes and clinical outcomes.

Results A total of 10 381 patients were enrolled, among which 56 (0.5%) had the heterozygote GA genotype for p.R4810K. The variant carriers were younger (p=0.01), and more likely to suffer from peripheral vascular disease (p=0.04). The p.R4810K variant was associated with large-artery atherosclerosis (LAA) (adjusted OR=1.94, 95% CI 1.13 to 3.33), anterior circulation stenosis (adjusted OR=2.12, 95% CI 1.23 to 3.65) and ECAS (adjusted OR=2.29, 95% CI 1.16 to 4.51). Nevertheless, the p.R4810K variant was not associated with recurrence, poor functional outcome and mortality at 3 months and 1 year.

Conclusions The *RNF213* p.R4810K variant was associated with LAA, anterior circulation stenosis and ECAS in Chinese patients. Given the low carrying rate and only 1-year follow-up information, caution should be taken to interpret our findings in no statistically significant association between the p.R4810K variant and stroke prognosis in Chinese patients.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The ring finger protein 213 gene (*RNF213*) p.R4810K variant has been found to increase the susceptibility to acute ischaemic stroke (AIS) attributable to intracranial arterial stenosis in the Japanese and Korean populations.

WHAT THIS STUDY ADDS

⇒ This study first identified the *RNF213* p.R4810K variant was associated with large-artery atherosclerosis, anterior circulation stenosis and extracranial arterial stenosis in Chinese patients with AIS or transient ischaemic attack.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The *RNF213* p.R4810K variant carriers are recommended to undergo high-resolution MRI for aetiological diagnosis and complete systemic vascular screening.

INTRODUCTION

The ring finger protein 213 gene (*RNF213*) is situated on chromosome 17q25.3 and encodes a sizeable 591 kDa protein named the mysterin, which comprises a pair of AAA (+) ATPases that are associated with cellular activities, as well as a RING (really interesting new gene) finger domain that is involved in mediating ubiquitination.[1 2] The previous clinical investigations have demonstrated a strong association between the founder variant *RNF213* p.R4810K (rs112735431, c.14429G>A) and moyamoya disease (MMD) in East Asian populations, especially Japanese, Korean and Chinese.[3 4]

Intracranial arterial stenosis (ICAS) stands out as a prominent aetiology for acute ischaemic stroke (AIS) and transient ischaemic attack (TIA) worldwide, and it assumes a significant role in stroke recurrence.[5] Given the comparable susceptible population and involved arteries between ICAS and MMD, it has been hypothesised that a specific subset of ICAS may share a similar genetic background and pathological mechanism with MMD.[6 7] Several recent studies have suggested that the *RNF213* p.R4810K variant is associated with a

heightened risk of AIS attributable to large-artery atherosclerosis (LAA) in the Japanese and Korean populations, and exhibits a strong correlation with ICAS.[1 8 9] However, insufficient large-scale studies exist pertaining to the association between the *RNF213* p.R4810K variant and ischaemic cerebrovascular disease in Chinese patients.

Thus, we aimed to investigate the prevalence of the *RNF213* p.R4810K variant in Chinese patients with AIS or TIA, and identify the phenotype exhibited by the carriers.

METHODS

Design and study population

The Third China National Stroke Registry (CNSR-III) was a nationwide prospective cohort study that recruited patients with AIS or TIA (≥18 years old; within 7 days from the onset). The study was conducted between 3 August 2015 and 5 March 2018 and involved 201 hospitals spanning 22 provinces and four municipalities in China. The detailed design of the CNSR-III has been published previously.[10] All participants underwent a standardised aetiological evaluation that consisted of physical examination, imaging assessment and laboratory examination. Imaging data and biological specimens were procured to delve deeper into prospective prognostic indicators for ischaemic cerebrovascular disease. Prior to participating in the study, each participant or a legally authorised representative provided signed informed consent. The enrolled patients were followed up at 3 months and 1 year.

Baseline characteristics

Trained research coordinators at each site procured baseline information from the medical records, encompassing demographics, risk factors, medical history, family history and the primary diagnosis. Furthermore, they performed face-to-face interviews with patients on admission to collect the pre-stroke modified Rankin Scale (mRS) and evaluate the National Institutes of Health Stroke Scale scores. The information was uploaded to the online platform through an electronic data capture system.

The Tiantan Neuroimaging Centre of Excellence at Beijing Tiantan Hospital performed central aetiological classification utilising the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria, which distinguished five subtypes of stroke: LAA, cardioembolism, small-vessel occlusion, other determined aetiology and undetermined aetiology.[11] The diagnosis of hypertension was determined by one of the following criteria: blood pressure ≥140/90 mm Hg, the use of antihypertensive medications, or self-reported hypertension.[12] The diabetes mellitus (DM) diagnosis was defined as fasting plasma glucose level ≥126 mg/dL (7.0 mmol/L), haemoglobin A1c ≥6.5% (48 mmol/mol), self-reported history of physician-diagnosed diabetes or the use of antidiabetic agents.[13] The diagnosis of dyslipidaemia was based on self-reported history of physician-diagnosed dyslipidaemia or the use of lipid-lowing agents.

Imaging data collection and analysis

All eligible patients were required to undergo imaging procedures as per the study protocol, including baseline MRI or CT (if contraindicated to MRI), and at least one vascular assessment for intracranial arteries (magnetic resonance angiography, CT angiography (CTA) or digital subtraction angiography (DSA)) and extracranial arteries (carotid doppler, CTA, contrast-enhanced magnetic resonance angiography or DSA).[10] The images were stored in the Digital Imaging and Communications in Medicine format on discs and centrally interpreted by two neurologists. Differences of opinion were resolved via discussion with a third reviewer.

Definite MMD diagnosis was based on the guidelines established by the Research Committee on the Pathology and Treatment of Spontaneous Occlusion of the Circle of Willis and Health Labour Sciences Research Grant for Research on Measures for Intractable Diseases, Japan.[14]

The presence of ICAS and extracranial arterial stenosis (ECAS) was defined as 50% to 99% stenosis or occlusion of any intracranial and extracranial arteries, according to the Warfarin–Aspirin Symptomatic Intracranial Disease Trial and the North American Symptomatic Carotid Endarterectomy Trial, respectively.[15 16] In detail, intracranial arteries assessed included distal internal carotid arteries, middle cerebral arteries (M1 and M2), anterior cerebral arteries (A1 and A2), posterior cerebral arteries (P1 and P2), basilar artery and vertebral arteries (V4). Specifically, the anterior circulation was evaluated via distal internal carotid arteries, middle cerebral arteries (M1 and M2) and anterior cerebral arteries (A1 and A2), while the posterior circulation was evaluated via posterior cerebral arteries (P1 and P2), basilar artery and vertebral arteries (V4). Moreover, extracranial arteries assessed included common carotid arteries, proximal internal carotid arteries and vertebral arteries (V1, V2, V3).

Clinical outcome

The clinical outcomes included recurrence, functional outcome and mortality, which were obtained by face-to-face interviews at 3 months or telephone interviews at 1 year. We evaluated a new stroke, a new ischaemic stroke and new composite vascular events (ischaemic stroke, haemorrhagic stroke, myocardial infarction or vascular death), respectively. The poor functional outcome was defined as an mRS score ranging from 3 to 6, indicating disability or death.[17] Mortality included death from any cause.

Genotyping

Peripheral blood samples were collected from the 171 study sites involved in the genetic substudy. The genomic DNA was extracted from peripheral leukocytes using the DNA Isolation Kit (Bioteke, AU1802, Beijing, CHN). The genotype for p.R4810K was determined using a customised panel that employs high-throughput sequencing technology to detect genetic variants associated with cerebrovascular disease, as described previously.[18]

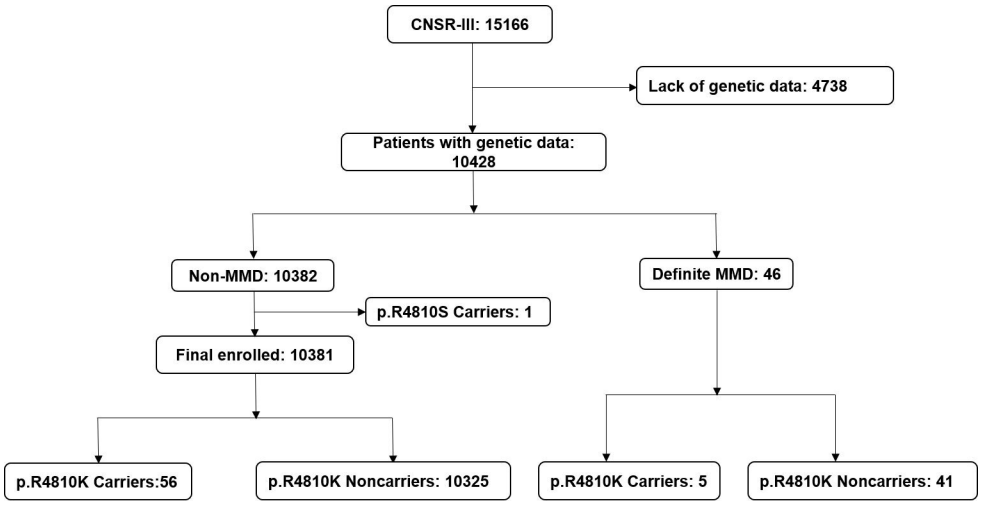

Figure 1 Flowchart of the study participants. CNSR-III, Third China National Stroke Registry; MMD, moyamoya disease.

Statistical analysis

Continuous variables were demonstrated using mean±SD or median with IQRs and categorical variables using percentages. The baseline characteristics of different groups were assessed by utilising Wilcoxon tests for continuous variables and $\chi 2$ test for categorical variables. Multiple logistic regression models for ORs and Cox regression models for HRs were used to assess the correlation between the *RNF213* p.R4810K variant and TOAST classification, large artery stenosis phenotypes and clinical outcomes. Adjusted variables included age, sex, BMI, smoking status and medical history (hypertension, DM and dyslipidaemia). All tests were conducted as two-sided tests, and statistical significance was determined using a threshold of $p<0.05$. All statistical analyses were conducted with SAS statistical software, V.9.4, developed by SAS Institute, Cary, North Carolina.

RESULTS

A total of 10 428 participants had complete genetic data. After excluding 46 definite MMD patients and 1 patient with the *RNF213* p.R4810S variant (c.14430A>C), 10 381 patients were included. Among these participants, 56 (0.5%) had the heterozygote GA genotype for p.R4810K locus on the *RNF213* gene, and no one had the homozygote AA genotype (figure 1). The included participants were classified into two groups, the *RNF213* p.R4810K variant carriers and non-carriers. Table 1 shows the demographics and clinical characteristics of the study population. Compared with non-carriers, the variant carriers were inclined to be younger (58.3±10.1 vs 62.3±11.3, p=0.01), more likely to suffer from peripheral vascular disease (3.6% vs 0.9%, p=0.04). Other baseline characteristics, including medical history, family history and risk factors, were not statistically different between the two groups.

The proportion of LAA in carriers was higher than that in non-carriers (39.3% vs 25.5%). The crude OR for LAA was 1.90 (95% CI 1.11 to 3.25). After adjustment for age, sex, BMI, smoking status and medical history (hypertension, DM and dyslipidaemia), the strong association still persisted (adjusted OR=1.94, 95% CI 1.13 to 3.33). Meanwhile, there was no association of the p.R4810K variant with cardioembolism, small-vessel occlusion, other determined aetiology and undetermined aetiology (table 2).

87.9% (9126/10381) of patients have imaging data. Both ICAS and ECAS were more frequent in carriers than non-carriers (ICAS 58.5% vs 46.8%; ECAS 20.8% vs 11.4%). The p.R4810K variant has a significant association with anterior circulation stenosis (adjusted OR=2.12, 95% CI 1.23 to 3.65) and ECAS (adjusted OR=2.29, 95% CI 1.16 to 4.51). The correlation between the p.R4810K variant and ICAS did not reach statistical significance (adjusted OR=1.73, 95% CI 0.99 to 3.01). Besides, the p.R4810K variant was not associated with the prevalence of both ICAS and ECAS in patients with ischaemic cerebrovascular disease (adjusted OR=1.43, 95% CI 0.56 to 3.66) (table 3).

All patients completed 3-month and 1-year follow-ups for recurrent events and mortality. 10 262 (98.9%) patients were evaluated with mRS Score at 3 months and 10 130 (97.6%) patients at 1 year. During the 3-month follow-up period, 6.3% (653/10381) patients had a recurrent stroke, 1.5% (157/10381) were dead and 13.6% (1392/10262) patients had the poor functional outcome. Compared with the non-carriers group, the p.R4810K variant carriers tended to increase the risk of a recurrent stroke but did not reach statistical significance (adjusted HR=1.19, 95% CI 0.44 to 3.17). None of the patients with the p.R4810K variant died within 3 months. Meanwhile, the p.R4810K variant did not correlate with the poor functional outcome (adjusted OR=0.91, 95% CI 0.39 to 2.14). In addition, the p.R4810K variant had no statistical association with recurrence, mortality and poor functional outcome within 1 year (table 4).

Table 1 Baseline characteristics between the *RNF213* p.R4810K variant carriers and non-carriers

Characteristics	All patients N=10381	Carriers n=56	Non-carriers n=10325	P value
Demographic				
Age, years, mean (SD)	62.3±11.3	58.3±10.1	62.3±11.3	0.01
Women, n (%)	3263 (31.4)	18 (32.1)	3245 (31.4)	0.91
BMI, kg/m^2, mean (SD)	24.7±3.3	24.9±3.0	24.7±3.3	0.65
Systolic pressure, mm Hg	150.3±22.1	152.8±27.6	150.2±22.1	0.97
Diastolic pressure, mm Hg	87.5±13.2	86.0±13.2	87.5±13.2	0.32
Medical history, n (%)				
Hypertension	6509 (62.7)	35 (62.5)	6474 (62.7)	0.98
Diabetes mellitus	2480 (23.9)	16 (28.6)	2464 (23.9)	0.41
Dyslipidaemia	878 (8.5)	4 (7.1)	874 (8.5)	0.72
Stroke or TIA	2334 (22.5)	15 (26.8)	2319 (22.5)	0.44
Coronary heart disease	1145 (11.0)	4 (7.1)	1141 (11.1)	0.35
Atrial fibrillation/flustter	738 (7.1)	4 (7.1)	734 (7.1)	0.99
Peripheral vascular disease	98 (0.9)	2 (3.6)	96 (0.9)	0.04
Smoking status, n (%)				
Never	5734 (55.2)	33 (58.9)	5701 (55.2)	0.82
Previous	1338 (12.9)	6 (10.7)	1332 (12.9)	
Current	3309 (31.9)	17 (30.4)	3292 (31.9)	
Index event, n (%)				
Ischaemic stroke	9688 (93.3)	50 (89.3)	9638 (93.4)	0.22
TIA	693 (6.7)	6 (10.7)	687 (6.7)	
Family history of stroke, n (%)	1392 (13.4)	6 (10.7)	1386 (13.4)	0.55
Pre-stroke mRS 0~2, n (%)	9934 (95.7)	54 (96.4)	9880 (95.7)	0.79
NIHSS score on admission, median (IQR)	3 (1–6)	3 (1–5)	3 (1–6)	0.30

BMI, body mass index; mRS, modified Rankin Scale; NIHSS, National Institute of Health Stroke Scale; TIA, transient ischaemic attack.

DISCUSSION

In the present study, a few (0.5%) Chinese patients with AIS or TIA carried the *RNF213* p.R4810K variant, rarer than in Japanese and Korean populations.[1 8 19] The p.R4810K variant was related to LAA, anterior circulation stenosis and ECAS in Chinese patients. Given the low-carrying rate and only 1-year follow-up information, caution should be taken to interpret our findings of no statistically significant association between the p.R4810K variant and stroke prognosis in Chinese patients.

A previous study reported that the prevalence of the *RNF213* p.R4810K variant in the normal Chinese population is 0.5%, which is similar to the prevalence of the p.R4810K variant in Chinese stroke patients (0.5%), but

Table 2 Association of the *RNF213* p.R4810K variant with TOAST classification

TOAST classification	Number (%) All patients (N=10381)	Carriers (n=56)	Non-carriers (n=10325)	OR (95% CI)	P value	Adjusted OR (95% CI)*	Adjusted P value*
LAA	2652 (25.6)	22 (39.3)	2630 (25.5)	1.90 (1.11 to 3.25)	0.02	1.94 (1.13 to 3.33)	0.02
CE	2183 (21.0)	9 (16.1)	2174 (21.1)	0.72 (0.35 to 1.47)	0.36	0.69 (0.34 to 1.41)	0.31
SVO	662 (6.4)	4 (7.1)	658 (6.4)	1.13 (0.41 to 3.13)	0.81	1.55 (0.55 to 4.35)	0.41
Others†	4884 (47.1)	21 (37.5)	4863 (47.1)	0.67 (0.39 to 1.16)	0.15	0.64 (0.37 to 1.11)	0.11

*Adjusted model: adjusted by age, sex, BMI, smoking status and medical history (hypertension, diabetes mellitus and dyslipidaemia).
†Others were combined by other determined cause and undetermined cause of TOAST classification.
BMI, body mass index; CE, cardioembolism; LAA, large-artery atherosclerosis; SVO, small-vessel occlusion; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

Table 3 Association of the *RNF213* p.R4810K variant with large artery stenosis phenotypes

Large artery stenosis phenotypes	Number (%)			OR (95% CI)	P value	Adjusted OR (95% CI)*	Adjusted P value*
	All patients (N=9126)	Carriers (n=53)	Non-carriers (n=9073)				
ICAS	4275 (46.8)	31 (58.5)	4244 (46.8)	1.60 (0.93 to 2.77)	0.09	1.73 (0.99 to 3.01)	0.05
Anterior	2953 (32.4)	26 (49.1)	2927 (32.3)	2.02 (1.18 to 3.47)	0.01	2.12 (1.23 to 3.65)	0.007
Posterior	2674 (29.3)	13 (24.5)	2661 (29.3)	0.78 (0.42 to 1.47)	0.45	0.85 (0.45 to 1.61)	0.62
Anterior without posterior	1601 (17.5)	18 (34.0)	1583 (17.5)	2.43 (1.38 to 4.01)	0.002	2.39 (1.35 to 4.24)	0.003
Posterior without anterior	1322 (14.5)	5 (9.4)	1317 (14.5)	0.62 (0.25 to 1.55)	0.30	0.65 (0.26 to 1.63)	0.36
Anterior and posterior	1352 (14.8)	8 (15.1)	1344 (14.8)	1.02 (0.48 to 2.17)	0.95	1.13 (0.53 to 2.43)	0.75
ICAS without ECAS	3595 (39.4)	26 (49.1)	3569 (39.3)	1.49 (0.87 to 2.55)	0.15	1.54 (0.90 to 2.66)	0.12
Anterior	2508 (27.5)	22 (41.5)	2486 (27.4)	1.88 (1.09 to 3.26)	0.02	1.92 (1.11 to 3.32)	0.02
Posterior	2173 (23.8)	11 (20.8)	2162 (23.8)	0.84 (0.43 to 1.63)	0.60	0.89 (0.46 to 1.75)	0.74
Anterior without posterior	1422 (15.6)	15 (28.3)	1407 (15.5)	2.15 (1.18 to 3.92)	0.01	2.10 (1.15 to 3.83)	0.02
Posterior without anterior	1087 (11.9)	4 (7.6)	1083 (11.9)	0.60 (0.22 to 1.67)	0.33	0.63 (0.23 to 1.76)	0.38
Anterior and posterior	1086 (11.9)	7 (13.2)	1079 (11.9)	1.13 (0.51 to 2.50)	0.77	1.22 (0.54 to 2.72)	0.64
ECAS	1047 (11.5)	11 (20.8)	1036 (11.4)	2.03 (1.04 to 3.96)	0.04	2.29 (1.16 to 4.51)	0.02
ECAS without ICAS	367 (4.0)	6 (11.3)	361 (4.0)	3.08 (1.31 to 7.25)	0.01	3.39 (1.43 to 8.06)	0.006
ICAS and ECAS	680 (7.5)	5 (9.4)	675 (7.4)	1.30 (0.51 to 3.27)	0.58	1.43 (0.56 to 3.66)	0.45

*Adjusted model: adjusted by age, sex, BMI, smoking status and medical history (hypertension, diabetes mellitus and dyslipidaemia).
BMI, body mass index; ECAS, extracranial arterial stenosis; ICAS, intracranial arterial stenosis.

slightly lower than the prevalence in LAA (0.8%) and in ICAS (0.7%).[4] The wide discrepancy in the prevalence between our study and prior investigations may be attributed to genetic heterogeneity.[8 9] Numerous epidemiological studies have demonstrated notable ethnic differences in the prevalence of the p.R4810K variant among Japanese, Korean and Chinese patients with MMD.[20] Although sizeable clinical evidence confirmed a strong association of the *RNF213* p.R4810K variant with ischaemic stroke, especially with LAA, in the Japanese and Korean populations, recent literature has also found other *RNF213* variants closely related to the high prevalence of ICAS in Chinese patients, such as p.A5021V (rs138130613, c.15062C>T).[8 19 21] It suggests that ICAS and MMD may have a similar frequency spectrum of the *RNF213* variants in different ethnicities, that is, the variant sites in the *RNF213* gene among the Japanese and Korean populations are concentrated in the p.R4810K, but among the Chinese population are scattered, and among other populations are few.[22 23]

Table 4 Association of the *RNF213* p.R4810K variant with clinical outcomes

Outcomes	Events (number of events/total patients, %)			OR/HR (95% CI)	P value	Adjusted OR/HR (95% CI)*	Adjusted P value*
	All patients	Carriers	Non-carriers				
3 months							
Stroke	653/10381 (6.3)	4/56 (7.1)	649/10325 (6.3)	1.13 (0.42 to 3.01)	0.81	1.19 (0.44 to 3.17)	0.74
Ischaemic stroke	609/10381 (5.9)	4/56 (7.1)	605/10325 (5.9)	1.21 (0.45 to 3.24)	0.70	1.27 (0.48 to 3.41)	0.63
CVD†	677/10381 (6.5)	4/56 (7.1)	673/10325 (6.5)	1.09 (0.41 to 2.91)	0.86	1.15 (0.43 to 3.08)	0.77
Mortality	157/10381 (1.5)	0/56 (0)	157/10325 (1.5)	–	0.97	–	0.97
mRS 3~6	1392/10262 (13.6)	6/55 (10.9)	1386/10207 (13.6)	0.78 (0.33 to 1.82)	0.57	0.91 (0.39 to 2.14)	0.83
1 year							
Stroke	1029/10381 (9.9)	7/56 (12.5)	1022/10325 (9.9)	1.27 (0.60 to 2.66)	0.53	1.34 (0.64 to 2.81)	0.45
Ischaemic stroke	940/10381 (9.1)	7/56 (12.5)	933/10325 (9.0)	1.39 (0.66 to 2.93)	0.38	1.47 (0.70 to 3.10)	0.31
CVD†	1087/10381 (10.5)	7/56 (12.5)	1080/10325 (10.5)	1.20 (0.57 to 2.52)	0.63	1.27 (0.60 to 2.67)	0.53
Mortality	349/10381 (3.4)	0/56 (0)	349/10325 (3.4)	–	0.95	–	0.95
mRS 3~6	1346/10130 (13.3)	2/55 (3.6)	1346/10075 (13.3)	0.25 (0.06 to 1.01)	0.05	0.30 (0.07 to 1.25)	0.10

*Adjusted model: adjusted by age, sex, BMI, smoking status and medical history (hypertension, diabetes mellitus and dyslipidaemia).
†CVD included ischaemic stroke, haemorrhagic stroke, myocardial infarction and vascular death.
BMI, body mass index; CVD, composite vascular events; mRS, modified Rankin Scale.

Previous studies have demonstrated that patients with the *RNF213* p.R4810K variant exhibit earlier onset age and had a higher rate of women.[24] The variant carriers were younger than non-carriers in our participants. Nevertheless, there was no sex difference between the two groups. Recent findings from high-resolution MRI (HR-MRI) have provided evidence that the pathological mechanism underlying ICAS in patients with the *RNF213* p.R4810K variant was not only non-atherosclerotic but also atherosclerotic.[25] Given no difference in atherosclerotic risk factors between patients with and without variants in our study, the contradiction may be potentially elucidated by a higher prevalence of atherosclerosis in Chinese patients with the *RNF213* p.R4810K variant, which may be linked to a greater proportion of male patients.[26]

Our study first demonstrated that the *RNF213* p.R4810K variant was associated with an elevated risk of LAA among Chinese patients with ischaemic cerebrovascular disease. Besides, the p.R4810K variant was related to anterior circulation stenosis in Chinese patients, which bears similarity to the characteristics of vascular stenosis in Japanese patients.[1] However, the trend towards a higher proportion of ICAS in Chinese patients with the p.R4810K variant did not reach statistical significance. Previous meta-analysis and systematic review explored the critical roles of the *RNF213* p.R4810K in Chinese patients with ICAS.[27] An early study demonstrated that the carriers in Chinese patients with ICAS were comparatively less symptomatic and more likely to possess collateral vessels.[28] Considering our study mainly included hospitalised patients, there exists a possibility that individuals with mild symptoms carrying the p.R4810K variant related to ICAS may have been overlooked. It is necessary to verify the correlation between p.R4810K polymorphism and ICAS in the asymptomatic and symptomatic Chinese ICAS population.

The novel findings in the present study were the high rate of peripheral vascular disease history in carriers and a strong association of the p.R4810K variant with ECAS. A recent study verified that the p.R4810K variant carriers exhibit smaller outer diameters of the cervical arteries compared with non-carriers with ischaemic stroke.[29] In addition, some previous cases have demonstrated that the *RNF213* p.R4810K variant impacts arteries throughout the body, such as the abdominal aorta, renal artery and pulmonary artery.[30–32] The evidence suggests that the variant of *RNF213* might play an essential role in the multiple circulatory systems. However, the clinical phenotypes displayed may vary owing to interindividual variations. In-depth and methodical research on the spectrum of *RNF213*-related vasculopathy holds the potential to shed novel insights into the aetiology and progression of vascular disease.[2 8 30]

The *RNF213* p.R4810K variant showed a tendency to increase the risk of stroke recurrence in Chinese patients while reducing the risk of poor functional outcome after 1 year. However, these observations did not attain statistical significance. Although we did not distinguish between atherosclerosis and non-atherosclerosis using HR-MRI or pathological examination, given the disparity in sample size between the two groups, we could not directly conclude that conventional secondary prevention is helpful for all variant carriers. A recent case report discovered that patients with atherosclerotic moyamoya syndrome exhibit the development of new moyamoya vessels despite receiving antiplatelet and statin therapy.[33] Besides, many studies have reported that the *RNF213* p.R4810K variant carriers with ICAS or intracranial artery dissection gradually developed into MMD.[34 35] Screening for the *RNF213* polymorphism, completing HR-MRI to evaluate stenosis and receiving long-term imaging follow-up is of great significance for enhancing individualised treatment and improving stroke prognosis.

The pathological mechanism underlying the effect of the *RNF213* variant on abnormal angiogenesis remains unknown. Preclinical research has found that the loss of *RNF213* function did not trigger the spontaneous development of smog-like vessels but caused irregular vascular wall formation as a critical regulator of cerebral endothelium integrity.[22 36] According to research conducted on mice deficient in *RNF213*, several metabolites have been found to be associated with the occurrence of abnormal angiogenesis, such as matrix metalloproteinase—9 and caveolin-1.[37 38] Besides, some basic studies have demonstrated that biological agents, such as microRNAs and interferon—β, are capable of regulating the expression of *RNF213*.[39 40] The present evidence suggests that *RNF213* is implicated in the multifaceted pathogenesis of anomalous vascular structures. Further investigation into the upstream and downstream pathways of *RNF213* is warranted to enhance comprehension of the underlying mechanism and identify new targets for the prevention and treatment of *RNF213*-related vasculopathy.

This study has several limitations. First, due to the low prevalence of the *RNF213* p.R4810K variant, there may be a deviation in the statistical analysis between the two groups. However, the differences in sample size between the two subgroups may be closer to the real-world's situation in China and provide more contributing information for clinical applications. Second, because some patients have contraindications to MRA or CTA, the imaging methods for evaluating arterial conditions are not uniform, which may affect classification accuracy. Third, a lack of HR-MRI or pathological analysis might dilute the effect of the variant on non-atherosclerotic blood vessels. Fourth, the present study is limited by its enrolment of only Chinese patients, which raises concerns regarding the generalisability of the findings to other populations. Fifth, this study solely discussed the relationship between the *RNF213* p.R4810K variant and ischaemic cerebrovascular disease. More comprehensive genetic analysis, such as the whole genome sequencing analysis, is essential to discuss whether patients with large artery stenosis have an association with other variants of the *RNF213* or other genes. Sixth, the assessment of the involved vessels remains confined to a cross-sectional result. Further longitudinal studies are necessary to

confirm whether the ICAS with variant has progressed to MMD.

CONCLUSIONS

In summary, this study first confirmed that the *RNF213* p.R4810K variant is associated with LAA, anterior circulation stenosis and ECAS in Chinese patients. Given the low carrying rate and only 1-year follow-up information, caution should be taken to interpret our findings in no statistically significant association between the p.R4810K variant and stroke prognosis in Chinese patients. The *RNF213* p.R4810K variant carriers are recommended to undergo HR-MRI for aetiological diagnosis and complete systemic vascular screening.

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