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ORIGINAL RESEARCH

Relationship Between *RNF213* p.R4810K and Echocardiographic Findings in Patients with Cerebrovascular Diseases: A Multicenter Prospective Cohort Study

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BACKGROUND: RING finger protein 213 (*RNF213*) p.R4810K is an established risk factor for moyamoya disease and intracranial artery stenosis in East Asian people. Recent evidence suggests its potential association with extracranial cardiovascular diseases, including pulmonary hypertension. We hypothesized that insidious abnormal cardiac functions are detected in *RNF213* p.R4810K carriers with cerebrovascular diseases.

METHODS AND RESULTS: We investigated patients registered in the National Cerebral and Cardiovascular Center Genome Registry between May 2017 and August 2021 who underwent echocardiography. All patients had cerebrovascular diseases. Patients with a medical history of chronic heart or pulmonary diseases were excluded. *RNF213* p.R4810K was genotyped in all the patients. Of 2089 patients registered in the registry, 71 carriers and 1241 noncarriers were eligible for our analyses. The carriage of *RNF213* p.R4810K emerged as a significant predictor for longer right ventricular outflow tract acceleration time in multivariable linear regression analysis (β=8.33 [95% CI, 0.92–15.74]; *P*=0.028). Additionally, the carriers showed increased odds of having right ventricular outflow tract acceleration time values ≥150 milliseconds (odds ratio, 2.22 [95% CI, 1.18–4.18]; *P*=0.014) in multivariable logistic regression analysis.

CONCLUSIONS: A longer right ventricular outflow tract acceleration time may reflect an increased pulmonary vascular bed induced by abnormal vascular collateral networks and dilation of capillary vessels in peripheral pulmonary arteries in the preclinical stage of *RNF213*-related pulmonary hypertension. Thus, the right ventricular outflow tract acceleration time marker in *RNF213* p.R4810K carriers suggests a biphasic course from the presymptomatic to symptomatic phase. Furthermore, vascular neurologists should carefully examine multiple organs because *RNF213*-related vasculopathy covers systemic cardiovascular diseases.

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Key Words: echocardiography ■ pulmonary hypertension ■ right ventricular outflow tract acceleration time ■ *RNF213* p.R4810K ■ stroke

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CLINICAL PERSPECTIVE

What Is New?

- The RING finger protein 213 (*RNF213*) p.R4810K is an established risk factor for cerebrovascular diseases, such as moyamoya disease and intracranial artery stenosis, particularly in East Asian people, and extracerebrovascular diseases, such as pulmonary hypertension.
- This was independently associated with longer right ventricular outflow tract acceleration time, a well-established marker of right ventricular function and pulmonary artery pressure, in patients with cerebrovascular diseases.
- This suggests insidious cardiovascular functional changes associated with the *RNF213* p.R4810K variant in patients without symptomatic heart failure.

What Are the Clinical Implications?

- Longer right ventricular outflow tract acceleration time observed in the *RNF213* p.R4810K carriers without symptomatic heart failure was paradoxical considering that symptomatic pulmonary hypertension typically manifests with shorter right ventricular outflow tract acceleration time. *RNF213* p.R4810K–related pulmonary hypertension is intractable to conventional treatment of pulmonary hypertension; thus, *RNF213* p.R4810K carriers would develop a distinct form of pulmonary hypertension.
- Longer right ventricular outflow tract acceleration time in patients with cerebrovascular diseases can prompt to genotype the *RNF213* p.R4810K; furthermore, vascular neurologists should carefully examine multiple organs because the brain–heart axis contributes to the development of stroke, and *RNF213*-related vasculopathy covers systemic cardiovascular diseases.

Nonstandard Abbreviations and Acronyms

ACT	acceleration time
GGOs	ground-glass opacities
NCVC	National Cerebral and Cardiovascular Center
PA/AA	pulmonary artery diameter/ascending aorta diameter ratio
PH	pulmonary hypertension
RNF213	RING finger protein 213

The RING finger protein 213 (*RNF213*) p.R4810K variant (c.14429G>A, rs112735431) is a susceptibility variant for moyamoya disease in East Asian people.[1] Approximately 80% to 90% of Japanese patients with moyamoya disease harbor this variant,[1–3] whereas its frequency in White individuals is rare, with a maximum allele frequency of 0.00064.[4,5] *RNF213* encodes adenosine ATPase associated with various cellular activities plus ATPase units and a RING finger domain that acts as an E3 ubiquitin ligase.[6] The p.R4810K variant lies at the C-terminus of *RNF213*, downstream of the RING finger domain[7] and has been reported to play a crucial role in the development of intracranial major artery stenosis/occlusion, despite not meeting the diagnostic criteria for moyamoya disease.[4,8–10] From a radiological perspective, individuals carrying the variant exhibit intracranial major artery stenosis/occlusion with a reduced outer diameter of the intracranial arteries. This is in contrast to conventional large-artery atherosclerosis, which typically demonstrates positive remodeling.[11] This observation led to the emergence of a novel disease entity: *RNF213*-related vasculopathy. Regarding promising therapies, statin use has garnered attention owing to its association with slower progression of intracranial artery stenosis.[12]

The impact of the *RNF213* p.R4810K variant extends beyond intracranial arteries, encompassing a spectrum of extracranial cardiovascular diseases, including vasospastic angina,[13] pulmonary hypertension (PH),[14] aortic dissection,[15] and abdominal and superior mesenteric artery stenosis.[16] The concept of *RNF213*-related vasculopathy appears to encompass a broader array of cardiovascular disorders than initially assumed.

Among extracranial cardiovascular diseases, PH refers to elevated pressures in the pulmonary circulation that are attributed to either pulmonary vascular remodeling and inflammation or increased downstream pressures (precapillary versus postcapillary PH),[17] ultimately resulting in right heart failure. The primary susceptibility gene associated with pulmonary artery hypertension includes transforming growth factor ß family members, such as bone morphogenic protein receptor type 2, which is found in ≈30% of patients with idiopathic or heritable pulmonary artery hypertension.[18,19] Several case series and observational studies have indicated an association between *RNF213* p.R4810K and severe PH. Intriguingly, symptomatic patients with PH harboring *RNF213* p.R4810K, *RNF213*-related PH, demonstrated resistance to targeted therapies for PH.[20] This suggests that *RNF213*-related PH should have distinct mechanisms from PH with other mutations/variants. Accordingly, it is possible that asymptomatic carriers of *RNF213* p.R4810K have distinct abnormal cardiac functions.

We aimed to investigate asymptomatic cardiac functional changes using echocardiography in our registry, which primarily included patients with cerebrovascular diseases. This study investigated whether echocardiography parameters can provide a clue to suspect the *RNF213* p.R4810K variant in patients with cerebrovascular diseases.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design

The National Cerebral and Cardiovascular Center (NCVC) Genome Registry, a multicenter prospective study, was conducted at the NCVC and 8 other comprehensive stroke institutions in Japan.[12,21–23] The NCVC Genome Registry was conducted according to the standards of Declaration of Helsinki, approved by the Research Ethical Committee of the NCVC (approval number: M29-003), and registered at the University Hospital Medical Information Network (study ID: UMIN000050750). Written informed consent was obtained from all participants between May 2017 and August 2021. This study in the NCVC Genome Registry enrolled patients who met the following criteria: (1) aged ≥20 years; (2) patients who provided written informed consent of NCVC Biobank for *RNF213* p.R4810K genotyping; and (3) those who visited the NCVC as outpatients or inpatients.

Clinical data, including age; sex; smoking and drinking habits; comorbidities, such as hypertension, dyslipidemia, and diabetes; and medical history of ischemic stroke, transient ischemic attack, intracranial hemorrhage, chronic heart disease, chronic pulmonary disease, and chronic kidney disease, were obtained from the NCVC Genome Registry. We excluded patients with chronic heart or pulmonary diseases to appropriately evaluate cardiac function associated with *RNF213* p.R4810K and minimize the influence of confounders on echocardiographic and radiological findings (Figure 1).

Genotyping of RNF213 p.R4810K

RNF213 p.R4810K was genotyped at enrollment using a fully automated gene analysis system (GTS-7000, Shimadzu Corporation, Kyoto, Japan; or LightCycler 96 system, Roche, Basel, Switzerland). These systems allow the direct detection of single-nucleotide polymorphisms from 1 μL of whole blood samples using real-time polymerase chain reaction.[24] The primer sequences used in this analysis were 5′-TTCCAGAACGTCCAGCAAGT-3′ (forward) and 5′-ACAGTCCTGGTCCTGTCAGA-3′ (reverse). The probe sets were 5′-CTCCATCAGAGGCTTCCT-3′ and 5′-CTCCATCAAAGGCTTCCT-3′. Based on the results, patients with the homozygous (AA genotype) and heterozygous (GA genotype) variants for p.R4810K were defined as variant carriers, whereas those with the wild-type homozygous (GG genotype) variant were defined as noncarriers.

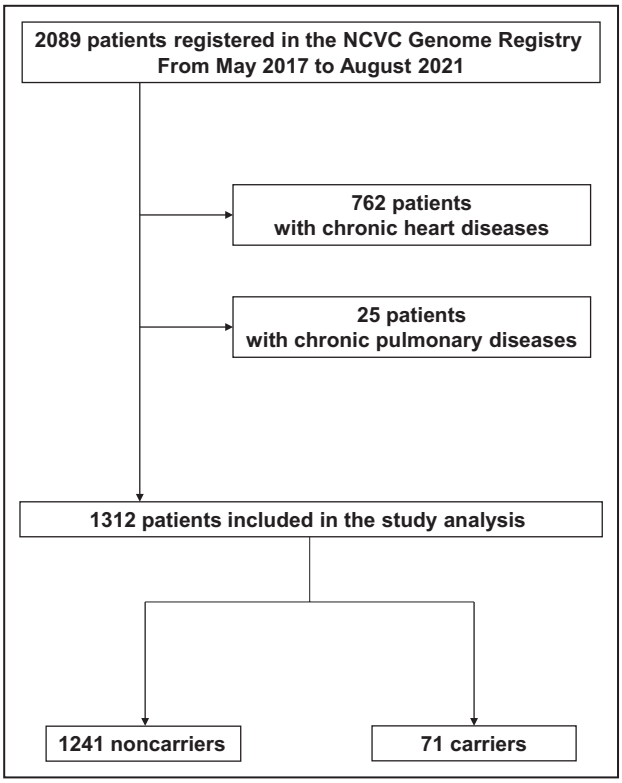

Figure 1. Study flowchart.
NCVC indicates National Cerebral and Cardiovascular Center.

Echocardiographic Evaluation

Two-dimensional echocardiography and Doppler imaging were clinically performed on all patients following the guidelines of the American Society of Echocardiography. All evaluations were performed by well-trained medical laboratory technicians at the NCVC. We retrospectively collected clinical data on ejection fraction (EF), cardiac index, cardiac output, early-to-late diastolic transmitral flow velocity, early diastolic mitral annular tissue velocity, right ventricular outflow tract acceleration time (RVOT-ACT), ejection time, tricuspid regurgitation peak gradient, right ventricular diastolic diameter, and tricuspid annular plane systolic excursion. EF was measured using Simpson's or Teichholz's method.

Chest Computed Tomography Evaluation

We evaluated chest computed tomography (CT) scans of the patients. Septal thickening, centrilobular

ground-glass opacities (GGOs), geographic GGOs, combined GGOs, and pulmonary artery diameter/ascending aorta diameter ratio (PA/AA) were individually evaluated by 2 certified radiologists (T.N. and H.H.). These findings have been defined elsewhere.[25] Patients with radiological findings indicating infectious pneumonia, interstitial pneumonia, bronchitis, and emphysema or those with poor-quality chest CT were excluded. Abnormal radiological findings, such as septal thickening was graded as mild, moderate, or severe according to previously described methods.[25] We investigated the prevalence of patients with more than mild septal thickening and those with any type and number of GGOs. The scorers were blinded to any genetic or clinical information regarding the patients during this evaluation. To assess intrarater reliability, each radiologist independently reviewed all chest CT findings twice, with an interval of >2 months.

Statistical Analysis

Data are presented as mean±SD or median (interquartile range) for continuous variables and as percentages for categorical variables. Differences in categorical or ordinal variables were evaluated using Fisher's exact test or the Mann–Whitney *U* test. The Mann–Whitney *U* test or *t* test was employed to evaluate differences in continuous variables between groups, as appropriate.

Generalized liner models with normal distribution were performed for echocardiography parameters. Model 1 was univariable, model 2 was adjusted for age and sex, and model 3 was further adjusted for hypertension, dyslipidemia, diabetes, smoking, EF <40%, and early-to-late diastolic transmitral flow velocity. Additionally, we performed a generalized linear model with normal distribution using robust estimation (Table S1). Absolute RVOT-ACT values beyond the upper 95% confidence limits defined in healthy volunteers were considered abnormal.[26–31] Therefore, RVOT-ACT values >150 milliseconds were deemed abnormal. To identify significant predictors of RVOT-ACT >150 milliseconds, univariable and multivariable logistic regression models were constructed. The multivariable model was adjusted for age, sex, diabetes, hypertension, EF <40%, and early-to-late diastolic transmitral flow velocity as potential confounders for RVOT-ACT >150 milliseconds.

We performed sensitivity analyses in 2 ways: (1) by including patients with chronic heart diseases (n=762; Figure 1) as a first sensitivity analysis and (2) by excluding patients with EF values <40% (n=21) as a second sensitivity analysis.

Cohen's κ values were calculated to gauge the consistency of interobserver scoring in CT imaging. The interpretation of the coefficient was as follows: values ≥0.81 indicated excellent agreement, 0.80 to 0.61 substantial agreement, 0.60 to 0.41 moderate agreement, 0.40 to 0.21 fair agreement, and ≤0.20 poor agreement.

Odds ratios with 95% CIs were calculated. All reported *P* values were 2-tailed, and $P<0.05$ was considered statistically significant. All analyses were performed using the SPSS version 27 (IBM, Armonk, NY) and the R statistical software package version 4.4.0 (R Development Core Team, 2010).

RESULTS

Baseline Characteristics

Of 2089 patients registered in the NCVC Genome Registry, 103 (4.9%) harbored the *RNF213* p.R4810K variant. After excluding patients with newly diagnosed chronic heart or pulmonary disease, 71 variant carriers and 1241 noncarriers were analyzed (Figure 1). The mean age was 54.4±1.8 years for carriers and 68.9±0.4 years for noncarriers ($P<0.001$; Table 1). The prevalence of patients with a medical history involving stroke, transient ischemic attack, and intracranial hemorrhage showed no significant difference between the 2 groups. The prevalence of hypertension was significantly higher in the noncarriers (carriers, 53.5%; noncarriers, 77.3%; $P<0.001$), but those of dyslipidemia (carriers, 56.3%; noncarriers, 60.9%; $P=0.43$) and

Table 1. Baseline Characteristics

	Noncarriers	Carriers	
	n=1241	n=71	*P* value
Age, y	68.9±0.4	54.4±1.8	<0.001
Male sex, n (%)	780 (62.9)	32 (45.1)	0.003
Current smoker, n (%)	268 (21.6)	11 (15.5)	0.21
Current drinker, n (%)	447 (36.0)	20 (28.2)	0.17
Cause of admission			
Ischemic stroke, n (%)	943 (76.0)	30 (42.3)	<0.001
Small-vessel occlusion	239	6	…
Large-artery atherosclerosis	236	14	…
Cardioembolic stroke	69	1	…
Others	359	9	…
Transient ischemic attack, n (%)	91 (7.3)	10 (14.1)	0.038
Intracranial hemorrhage, n (%)	77 (6.2)	1 (1.4)	-
Others	130 (10.5)	30 (42.3)	<0.001
Medical history			
Ischemic stroke, n (%)	168 (13.5)	12 (16.9)	0.42
Transient ischemic attack, n (%)	16 (1.3)	3 (4.2)	0.079
Intracranial hemorrhage, n (%)	28 (2.3)	1 (1.4)	…
Hypertension, n (%)	959 (77.3)	38 (53.5)	<0.001
Dyslipidemia, n (%)	756 (60.9)	40 (56.3)	0.43
Diabetes, n (%)	272 (21.9)	15 (21.1)	0.87
Chronic kidney disease, n (%)	22 (1.8)	0 (0)	…

Table 2. Comparison of Echocardiography Findings

	Noncarriers	Carriers	*P* value
EF<40%, n (%)	21 (2.0%)	0	…
Cardiac index (L/min per m^2)	2.8±0.0	2.5±0.1	0.13
Cardiac output (mL/min)	4.6±0.1	4.1±0.2	0.21
E/A	1.0±0.1	1.1±0.1	<0.001
E/e′	10.6±0.2	9.8±0.5	0.35
RVOT-ACT, ms	122.2±0.8	139.2±4.0	<0.001
RVOT-ACT/RVOT-ejection time ratio	0.41±0.0	0.44±0.0	0.001
TRPG, mm Hg	22.6±0.3	21.4±1.1	0.039
TAPSE, mm	20.9±0.2	22.4±0.6	0.009

ACT indicates acceleration time; E/A, early to late diastolic transmitral flow velocity; E/e′, early diastolic mitral annular tissue velocity; EF, ejection fraction; RVOT, right ventricular outflow tract; TAPSE, tricuspid annular plane systolic excursion; and TRPG, tricuspid regurgitation peak gradient.

diabetes (carriers, 21.1%; noncarriers, 21.9%; *P*=0.87) were comparable between the 2 groups.

RNF213 p.R4810K Was an Independent Predictor of Longer RVOT-ACT

Echocardiography was performed in 55 variant carriers and 1083 noncarriers. Echocardiographic assessments of right ventricular function revealed significant differences between variant carriers and noncarriers: 139.2±4.0 milliseconds versus 122.2±0.8 milliseconds for RVOT-ACT (*P*<0.001), 21.4±1.1 mm Hg versus 22.6±0.3 mm Hg for tricuspid regurgitation peak gradient (*P*=0.039), and 22.4±0.6 mm versus 20.9±0.2 mm for tricuspid annular plane systolic excursion (*P*=0.009), respectively. Furthermore, the RVOT-ACT/RVOT–ejection time ratio was significantly higher in carriers than in noncarriers (0.44±0.0 versus 0.41±0.0; *P*=0.001; Table 2). Regarding variables determining the left ventricular function, all the variant carriers had an EF of ≥40%, whereas 21 noncarriers had EF <40%. Conversely, the variant carriers and noncarriers showed comparable cardiac index (2.5±0.1 L/min per m^2 versus 2.8±0.0 L/min per m^2; *P*=0.13) and cardiac output (4.1±0.2 mL/min versus 4.6±0.1 mL per min; *P*=0.21), respectively.

Generalized linear models with normal distribution for parameters associated with pulmonary artery pressure revealed that the carriage of *RNF213* p.R4810K was an independent predictor for longer RVOT-ACT (adjusted β [adjusted mean difference in RVOT-ACT between the variant carriers and noncarriers as reference]=8.86 [95% CI, 1.57–16.15]; *P*=0.017; Table 3). In addition, robust regression estimation in generalized linear model showed similar results in RVOT-ACT (adjusted β [mean difference in RVOT-ACT between the variant carriers and noncarriers as reference]=8.86 [95% CI, 0.77–16.95]; *P*=0.032; Table S1). Multiple logistic regression analyses indicated that the presence of *RNF213* p.4810K was an independent predictor of RVOT-ACT >150 ms (odds ratio, 2.40 [95% CI, 1.29–4.47]; *P*=0.006; Table 4).

RNF213 p.R4810K Remained an Independent Predictor for Longer RVOT-ACT in the Sensitivity Analyses

In the first sensitivity analysis (Table S2), RVOT-ACT was significantly longer in *RNF213* p.4810K carriers than in noncarriers (134.6±29.4 milliseconds versus 117.8±27.6 milliseconds; *P*<0.001). Carriage of *RNF213* p.R4810K was an independent predictor of longer RVOT-ACT (adjusted β [adjusted mean difference in RVOT-ACT between the variant carriers and noncarriers as reference]=9.20 [95% CI, 2.64–15.76]; *P*=0.006) in the multivariable generalized linear model (Table S3) and of RVOT-ACT >150 ms (odds ratio, 2.27 [95% CI, 1.33–3.82]; *P*=0.002; Table S4). In the second sensitivity analysis (Table S2), RVOT-ACT remained significantly longer in *RNF213* p.4810K carriers than in noncarriers (138.8±29.6 milliseconds versus 122.1±26.4 milliseconds; *P*<0.001). Carriage of *RNF213* p.R4810K was an independent predictor of longer RVOT-ACT (adjusted β [adjusted mean difference in RVOT-ACT between the variant carriers and noncarriers as reference]=8.85 [95% CI, 0.79–16.91]; *P*=0.031) in the multivariable generalized linear model (Table S3) and of RVOT-ACT >150 milliseconds (odds ratio, 2.31 [95% CI, 1.24–4.31]; *P*=0.009; Table S4).

Table 3. Generalized Linear Model With Normal Distribution for Predicting Right Ventricular Function on Echocardiography

	RVOT-ACT			RVOT-ACT/RVOT- ejection time ratio			TRPG		
	β	95% CI	*P* value	β	95% CI	*P* value	β	95% CI	*P* value
Model 1	17.05	9.27 to 24.38	<0.001	0.03	0.01 to 0.05	0.014	−1.19	−3.48 to 1.10	0.309
Model 2	10.25	3.05 to 17.44	0.005	0.01	−0.02 to 0.03	0.511	0.44	−1.76 to 2.64	0.693
Model 3	8.86	1.57 to 16.15	0.017	0.01	−0.02 to 0.03	0.573	0.88	−1.31 to 3.07	0.432

Model 1: univariable. Model 2: adjusted for age and sex. Model 3: model 2 + hypertension, dyslipidemia, diabetes, smoking, ejection fraction <40%, and early diastolic mitral annular tissue velocity. ACT indicates acceleration time; RVOT, right ventricular outflow tract; and TRPG, tricuspid regurgitation peak gradient.

Table 4. Logistic Regression Analysis for Predicting Right Ventricular Outflow Tract Acceleration Time of >150 ms

	OR	95% CI	*P* value
Univariable	3.70	2.09–6.53	<0.001
Multivariable	2.40	1.29–4.47	0.006

Multivariable analysis adjusted for age, sex, hypertension, dyslipidemia, diabetes, smoking, ejection fraction <40%, and early diastolic mitral annular tissue velocity. OR indicates odds ratio.

PA/AA Ratio Evaluated Using Chest CT Was Larger in Variant Carriers

Five variant carriers and 112 noncarriers underwent chest CT. The variant carriers and noncarriers showed comparable prevalence of septal thickening (80.0% versus 72.3%; *P*>0.99) and GGOs (40.0% versus 58.9%; *P*=0.65), respectively. However, the *RNF213* p.R4810K variant carriers had a significantly higher prevalence of PA/AA >1 compared with the noncarriers (20.0% versus 2.7%; *P*=0.037) (Figure 2).

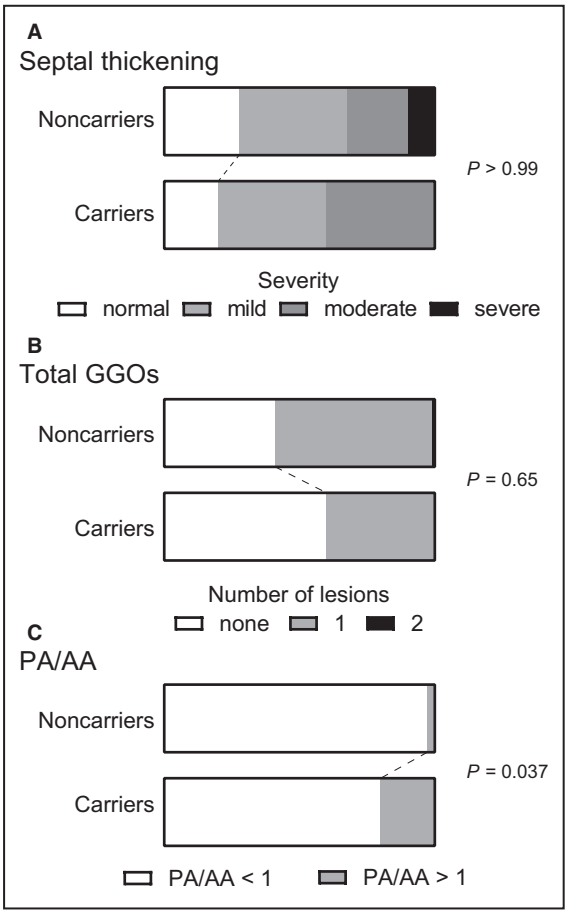

Figure 2. Comparison of chest computed tomography findings between *RNF213* p.R4810K variant carriers and noncarriers.
Frequency of radiological findings, septal thickening (**A**), GGOs (**B**), and PA/AA ratios (**C**) were compared. GGOs indicates ground-glass opacities; PA/AA, pulmonary artery diameter/ascending aorta diameter; and *RNF213*, Ring finger protein 213.

The intra- and interobserver reproducibility values of CT scores ranged from substantial to excellent (Table S5).

DISCUSSION

This study showed that the *RNF213* p.R4810K variant was independently associated with longer RVOT-ACT, a well-established marker of right ventricular function and pulmonary artery pressure. Furthermore, PA/AA ratios >1 were significantly more prevalent in variant carriers than in noncarriers. These findings suggest insidious cardiovascular functional changes associated with the *RNF213* p.R4810K variant in patients with cerebrovascular disease.

A longer RVOT-ACT may reflect low pulmonary artery pressure or an increased pulmonary vascular bed. Several reports have indicated that abnormal vascular collateral networks in peripheral pulmonary arteries on conventional angiography[32] and dilation of capillary vessels in lung histology are observed in *RNF213*-related PH,[33] suggesting that these findings are attributed to the longer RVOT-ACT, although symptomatic PH (not associated with *RNF213* variants) is characterized by shorter RVOT-ACT (<105 milliseconds). The dilation and proliferation of capillaries in the lung share similarities with the observations noted in the intracranial arteries affected by moyamoya angiopathy where these arteries are characterized by an abnormal vascular network, distinct from the patterns observed in conventional large-artery atherosclerosis. Thus, *RNF213*-related PH could form a new disease entity that is different from conventional PH. However, because the penetrance of the variant is low, all variant carriers do not always develop these phenotypes.

The contradictory finding suggests another explanation that the RVOT-ACT marker demonstrates a biphasic course from the presymptomatic to the symptomatic phase. Several other examples of disease markers have shown a biphasic course from the presymptomatic to the symptomatic phase. Regarding cerebral blood flow in *APOE4* carriers, a risk factor for Alzheimer disease, compared with noncarriers, cerebral blood flow in the resting gray matter is higher in preclinical young *APOE4* carriers and subsequently lower in older *APOE4* carriers.[34] The younger adults could display compensatory mechanisms for potential cognitive decline.[35] Amyloid-β_{1-42} levels in cerebrospinal fluid generally decrease during the symptomatic stage of Alzheimer disease. However, an increase in cerebrospinal fluid amyloid-β_{1-42} was found in the preclinical phase in mouse models of Alzheimer disease[36] and patients with early Alzheimer disease.[37] Furthermore, the estimated glomerular filtration rate

in patients with diabetic nephropathy shows hyperfiltration in the initial phase, followed by a progressive decrease in estimated glomerular filtration rate values that largely exceed what is expected for age. This initial phase of hyperfiltration may be a compensatory mechanism to counteract endothelial dysfunction, arterial stiffness, and chronic low-grade inflammation in the kidneys. Thus, the biphasic nature of disease markers may improve patient stratification for preventive treatment strategies.

Chest CT showed another notable finding: the frequency of patients with dilated main pulmonary artery was significantly higher in carriers than in noncarriers. Dilated main pulmonary artery, generally observed in patients with various diseases, such as PH, may induce high or turbulent pulmonary flow, including arteriovenous malformation, left-to-right shunting, and congenital abnormalities.[38] This potential mechanism may be explained by the dysfunction of filamin A, a substrate of the RNF213 E3 ubiquitin ligase[39] and an actin cytoskeleton crosslinking protein involved in vascular remodeling. A mutation in *FLNA* (IVS2-2A>G [c.374-2A>G in NM_001456]) encoding filamin A has been reported to be responsible for main pulmonary artery dilation.[40] Furthermore, dysfunctional filamin A in cerebrovascular smooth muscle cells was reported to increase artery diameter in a mouse model.[41] Thus, the abnormality in filamin A could contribute to the development of main pulmonary artery dilation.

This study has several limitations. First, this was a cross-sectional study; therefore, we did not longitudinally monitor cardiac function using cardiac ultrasonography. The longitudinal biphasic course of RVOT-ACT should be confirmed in future studies. The primary purpose of cardiac evaluation through echocardiography was predominantly aimed at assessing heart diseases associated with development of stroke, such as intra-atrial/ventricular thrombi, an enlarged left atrium, and wall motion, rather than to detect the existence of PH. However, right heart function, such as RVOT-ACT, RVOT-ejection time, tricuspid annular plane systolic excursion, early-to-late diastolic transmitral flow velocity, and early diastolic mitral annular tissue velocity, was routinely evaluated. Second, this study included only Japanese patients. The findings in this study would need to be confirmed in a broader sample of patients from other East Asian countries, including South Korea and China. Third, the relative difference in age, sex, and prevalence of hypertension and stroke between the carriers and noncarriers, which cannot be entirely adjusted, could have affected the echocardiographic parameters. Finally, the number of carriers who underwent chest CT was small because chest CT was not routinely performed in patients with cerebrovascular diseases.

In conclusion, this study showed that the *RNF213* p.R4810K variant was associated with longer RVOT-ACT. Further pathological examinations are required to establish clear pathomechanisms and preventive strategies for *RNF213*-related PH. Furthermore, vascular neurologists should carefully examine multiple organs because the brain–heart axis contributes to the development of stroke and *RNF213*-related vasculopathy underlying systemic cardiovascular diseases.

ARTICLE INFORMATION

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Supplemental Material

Tables S1–S5

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