CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

A Case of Multiple Intracranial Major Artery Stenoses With Coexisting *PCSK9* p.E32K and *RNF213* p.R4810K Variants

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Neurol Genet 2023;9:e200099. doi:10.1212/NXG.0000000000200099

Abstract

Objectives

Familial hypercholesterolemia (FH), caused by *PCSK9* p.E32K, is characterized by early-onset coronary artery disease. However, the relationship between *PCSK9* p.E32K and cerebrovascular disease is unclear. One of our patients with the *PCSK9* p.E32K had several intracranial artery stenoses (ICAS). The objective of this case series was to identify factors that may be associated with ICAS in the variant carriers.

Methods

A 75-year-old Japanese woman with FH carrying *PCSK9* p.E32K was found to have 5 asymptomatic ICAS when brain magnetic resonance angiography (MRA) was performed. We retrospectively investigated additional patients with FH who underwent brain MRA at our institution to explore the unknown factors accelerating ICAS.

Results

We investigated an additional 5 patients with FH who underwent brain MRA. Of them, only one had mild ICAS. The *RNF213* p.R4810K that is an established genetic risk for ICAS, particularly in East Asians, was identified only in the patient with 5 ICAS.

Discussion

PCSK9 and RNF213 play an important role in lipid metabolism and endothelial integrity. Therefore, together, these variants could be involved in the development of multiple ICAS. Our case series indicated that *PCSK9* p.E32K carriers should undergo early brain screening to obtain appropriate stroke prevention measures in the asymptomatic stage.

Introduction

Gain-of-function *PCSK9* variants induce low-density lipoprotein (LDL) receptor degradation with a subsequent increase in serum LDL cholesterol levels, potentially exacerbating clinical phenotype of familial hypercholesterolemia (FH).[1] Among Japanese patients with FH, the p.E32K variant is one of the most common pathogenic gain-of-function *PCSK9* variants, accounting for 88.5% of the cases.[2] Although FH is characterized by early-onset coronary artery disease, the relationship between cerebrovascular diseases, such as intracranial major artery stenosis (ICAS) and FH, is still controversial.[3] However, a risk for large-artery atherosclerosis stroke was reportedly higher in Han-Chinese patients with FH.[4]

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Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

In our previous study, we identified the RING finger protein 213 (*RNF213*) p.R4810K variant as an established genetic risk for ICAS in East Asians,[5] and it was observed in approximately 25% of the Japanese patients with ICAS.[6] By contrast, 0.5%–2.5% of the East Asian general population also harbor the *RNF213* p.R4810K variant.[5,7] Therefore, it implies that additional factors are required to develop ICAS associated with the *RNF213* p.R4810K variant.

In this report, we present an atypical clinical feature as a blended phenotype of FH accompanied by *PCSK9* p.E32K and *RNF213* p.R4810K variants.

Case Description

A 75-year-old Japanese woman was diagnosed with FH carrying *PCSK9* p.E32K. She has been visiting a lipid clinic at the National Cerebral and Cardiovascular Center (NCVC) in Japan from age 48 years. She had hypertension and was a smoker. She had no history of diabetes mellitus, stroke, or coronary artery disease. She had tendon xanthomas, not corneal arcus. Her LDL cholesterol level was 178 mg/dL during her initial visit, and since then, she was prescribed pitavastatin (2 mg/d). At the age 74 years, her brain magnetic resonance angiography (MRA) revealed 5 asymptomatic ICAS lesions in the right intracranial internal carotid artery, bilateral A1 segments of the anterior cerebral artery, M1 segment of the right middle cerebral artery, and M2 segment of the left middle cerebral artery (Patient 1 in Table, Figure A).

It was unclear why this patient had multiple ICAS lesions despite having started the appropriate medication since the initial visit. Patients carrying *PCSK9* p.E32K are generally not susceptible to cerebrovascular diseases. To explore the unknown factors accelerating this patient's multiple ICAS, we performed a retrospective study on other patients diagnosed with FH due to *PCSK9* p.E32K at NCVC based on the diagnostic criteria of the Japan Atherosclerosis Society between May 2005 and March 2020. This study was approved by the Research Ethics Committee of NCVC (Approval Nos. M17-056, M24-080 and M30-145).

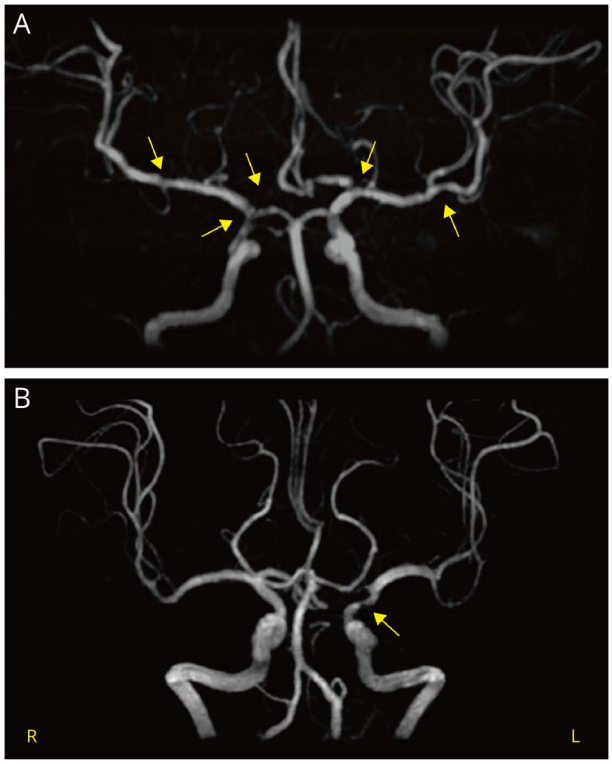
A written informed consent was obtained from all the patients. Among the 167 patients who were diagnosed with FH and underwent brain MRA, 6 patients carrying *PCSK9* p.E32K were found. However, 1 of the 6 patients harboring *PCSK9* p.E32K was eliminated. The left intracranial internal

Table Baseline and Neuroradiologic Characteristics of the Patients

Patient no.	1	2	3	4	5	6
***PCSK9* p.E32K**	+	+	+	+	+	+
***RNF213* p.R4810K**	+	–	–	–	–	–
Age	75	72	70	69	62	56
Sex	F	F	F	F	F	F
Smoking	–	–	+	–	–	–
Corneal arcus	–	–	+	–	–	–
Tendon xanthomas	+	–	+	+	–	–
LDL-C (mg/dL)	178	228	183	296	241	234
HDL-C (mg/dL)	48	90	44	64	88	65
Triglyceride (mg/dL)	154	90	225	139	89	196
Hypertension	+	–	+	+	–	–
Diabetes mellitus	–	–	–	–	–	–
PMH of stroke	–	–	–	–	–	–
PMH of coronary artery disease	–	–	+	–	–	–
Lipid-lowering drugs	+	+	+	+	+	+
Antihypertensive drugs	+	–	–	+	+	–
Antidiabetic drugs	–	–	–	–	–	–
Number of ICAS	5	0	0	1	0	0

Abbreviations: F = female; HDL-C = high-density lipoprotein cholesterol; ICAS = intracranial major artery stenosis; LDL-C = low-density lipoprotein cholesterol; PMH = past medical history.

Figure Brain Magnetic Resonance Angiography of the 2 Patients With *PCSK9* p.E32K Variant



Patient 1 with the *RNF213* p.R4810K showing five ICAS lesions (A) and Patient 4 without the *RNF213* p.R4810K showing only 1 ICAS lesion (B). Yellow arrows indicate ICAS lesions. Abbreviations: R, right; L, left.

carotid artery was not precisely assessed because the signal intensity of the artery in the brain MRA was attenuated possibly due to severe extracranial carotid artery stenosis or occlusion.

Among the 5 patients except Patient 1, only 1 patient (Patient 4) had mild ICAS (Table and Figure B). There were no baseline factors specific to Patient 1, and the environmental factors responsible for accelerating multiple ICAS remained unknown. These findings prompted us to identify *RNF213* p.R4810K. *RNF213* p.R4810K was genotyped using a fully automated gene analysis system (LightCycler 96; Roche, Basel, Switzerland). Only Patient 1 was found to carry the *RNF213* p.R4810K in addition to the shared *PCSK9* p.E32K.

Discussion

We demonstrated that the Japanese patient with both *PCSK9* p.E32K and *RNF213* p.R4810K variants had multiple asymptomatic ICAS lesions. FH is not generally associated with cerebrovascular disease, although it is reported that Asian Han-Chinese patients with FH seemed to have a higher risk for large-artery atherosclerosis stroke.[4] That could be derived from a higher frequency of *RNF213* p.R4810K variant in East Asian countries.

Notably, PCSK9 and RNF213 are both involved in lipid metabolism. RNF213, an E3 ubiquitin ligase, colocalizes with and stabilizes lipid droplets.[5,8] We have reported that statin use is associated with slower progression of ICAS in *RNF213* p.R4810K carriers.[9] Alternatively, endothelial damage could be promoted by *RNF213* p.R4810K with exacerbating inflammation[10] and by *PCSK9* p.E32K with the activation of apoptosis and reactive oxygen species.[11]

There are several limitations. This case series included only 6 patients. Multicenter study is warranted to recruit more patients. Owing to the cross-sectional nature of this care series, it focused primarily on elucidating the association between ICAS and the 2 variants.

In conclusion, our patient harboring both variants may provide new insights for future studies on the development of cerebrovascular diseases in patients with FH. We propose that a genetic interaction contributes to the development ICAS in patients with FH. Thus, *PCSK9* variant carriers should undergo early brain screening, and if an inherently rare ICAS is discovered, appropriate stroke prevention measures should be taken at the asymptomatic stage.

Study Funding

JSPS KAKENHI, 21K16961.

Disclosure

M. Ihara: Daiichi Sankyo, Eisai, Bristol-Myers Squibb, Shimadzu Corporation (outside of the submitted work). Go to Neurology.org/NG for full disclosures.

Publication History

Received by *Neurology: Genetics* June 20, 2023. Accepted in final form July 25, 2023. Submitted and externally peer reviewed. The handling editor was Associate Editor Raymond P. Roos, MD, FAAN.

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Continued

Appendix *(continued)*

Name	Location	Contribution
Mika Hori, PhD	Department of Endocrinology, Research Institute of Environmental Medicine, Nagoya University, Japan	Major role in the acquisition of data; analysis or interpretation of data
Mariko Harada-Shiba, MD, PhD	Cardiovascular Center, Osaka Medical and Pharmaceutical University Takatsuki, Japan	Analysis or interpretation of data
Masafumi Ihara, MD, PhD	Department of Neurology, National Cerebral and Cardiovascular Center, Suita, Japan	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data

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