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Scientific Letter

Identification of Alpha Thalassemia, *RNF213* p.R4810K and *PROC* p.R189W among Children with Moyamoya Disease/Syndrome

Keywords: Alpha thalassemia, *RNF213* p.R4810K, *PROC* p.R189W, Moyamoya.

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To the editor.

Moyamoya is an occlusive vasculopathy characterized by progressive bilateral or unilateral stenosis/occlusion of the distal internal carotid artery and the abnormal development of a hazy network of basal collateral vessels.[1] Moyamoya, classified into moyamoya syndrome and moyamoya disease, is more common in Asian than in Western countries. Moyamoya syndrome is associated with underlying conditions, such as hemoglobinopathy and post-radiation, while moyamoya disease is not.[1] The etiology of moyamoya remains unknown; however, genetic analyses identified the variable incidence rates of *RNF213* p.R4810K of 23.1-90.1% in most East Asian populations.[2] The genetic variants of moyamoya reported from Southeast Asian countries are limited. Therefore, we reported the underlying disease of non-deletional α-thalassemia, antiphospholipid antibodies (APAs), and genetic variants of *PROC* p.R189W, and less frequent *RNF213* p.R4810K in moyamoya disease/syndrome, using whole-exome sequencing (WES).

We describe a cross-sectional study involving 14 patients (7 with moyamoya syndrome and 7 with moyamoya disease), female: male 1.33:1, with a median age of 9.3 years (1.7-12.6) at diagnosis. Their median follow-up time was 6.3 years. The diagnosis was confirmed using magnetic resonance imaging and angiographic study. Recurrent hemiparesis and seizure were the most common presentations. All patients had no family history of moyamoya. Genomic DNA was extracted from peripheral blood samples and sent for WES, performed on a Novaseq 6000 System (Illumina, San Diego, CA, USA). WES data were analyzed using the commercial software, Sophia DDM, V4.4 (Sophia Genetics). This study was approved by the Ethics Committee of the Faculty of Medicine Ramathibodi Hospital (ID: COA. MURA2020/1788).

Among the seven patients with moyamoya syndrome, three were diagnosed with non-deletional α-thalassemia disease [hemoglobin H/Constant Spring (--SEA/CS)], one with Williams syndrome, and one post cranial radiation. Two of the three α-thalassemia patients also had APAs. The WES study demonstrated a heterozygous variant of a prothrombotic gene of heterozygous *PROC* gene mutation (c.565C > T, p.R189W) in three patients. Two patients had low protein (PC) activities (42% and 61%). WES study also confirmed the genetic information of thalassemia [α^{CS} (HBA2: c.427T > C)/ $--^{SEA}$ (NG_000006.1: g.26264_45564del19301)] among three patients. One of the three heterozygous p.R189W mutation patients also had homozygous HBB c.79G>A, p.E27K, causing mild anemia (**Table 1** and **Supplement Figure 1**). For the seven patients with moyamoya disease, the WES study identified genetic variants of heterozygous *RNF213* mutation (c.14429G>A, p.R4810K) in two patients (**Table 1** and **Supplement Figure 1**).

Neurological outcomes revealed neurologic deficits among seven patients. The modified Rankin scale (mRS) was higher in moyamoya syndrome (score 3 in craniopharyngioma post cranial radiation, score 2 in alpha thalassemia disease, and score 1 in Williams syndrome), indicating higher disability when compared with moyamoya disease (score 1 in two of seven patients).

Sickle cell anemia and β thalassemia had been reported in moyamoya syndrome.[1] The present study reported non-deletional α-thalassemia in moyamoya. The α-thalassemia disease usually involves moderate anemia and requires occasional red blood cell (RBC) transfusion. However, non-deletional α-thalassemia may exhibit more severe phenotypes, similar to patients with β-thalassemia.[3] The patients in this report, before the diagnosis of moyamoya, received occasional RBC transfusions. As a result, the high proportion of phosphatidylserine exposing RBC increased the hypercoagulable state[4] and may contribute to the

Table 1. Characteristics of the 14 patients with moyamoya. Patients 1-7 were classified as moyamoya syndrome, and Patients 8-14 as moyamoya disease.

No.	Sex	Age diagnosis)year(	FU time)year(	Underlying disease	Presentation		Current treatment			MRS##	Outcome		WES result#	PC activity)%(**
					Motor	Seizure	Medication	Revascularization	Other		Motor	Other		
1	F	12.0	7.7	$--^{SEA}/\alpha^{CS}\alpha$, APS***	recurrent right hemiparesis	✓	rivaroxaban	not done	regular RBC Tx	0	no weakness	epilepsy	no pathogenic	85
2	M	8.2	15.7	craniopharyngioma postradiation	recurrent left hemiparesis	✓	aspirin	not done	none	3	mild hemiparesis left*	epilepsy,	no pathogenic	89
3	F	9.9	11.1	$--^{SEA}/\alpha^{CS}\alpha$, APS***	right hemiparesis	✓	aspirin	left EC-IC bypass, Right EDAMS	regular RBC Tx, risperidol, VPA	2	increased muscle tone	epilepsy cognitive impairment	no pathogenic	88
4	M	1.7	6.1	Williams syndrome	alternating hemiparesis		aspirin	bilateral EDAS		1	mild weakness	delayed development	no pathogenic	63
5	F	9.6	0.8	$--^{SEA}/\alpha^{CS}\alpha$	right hemiparesis		aspirin	not done	regular RBC Tx	2	mild hemiparesis left	motor aphasia	heterozygous *PROC* p.189W	42
6	F	12.6	1.8	none	right hemiparesis	✓	aspirin	not done	none	0	normal	none	heterozygous *PROC* p.R189W	92
7	F	11.9	2.4	β^E/β^E	alternating hemiparesis		clopidogrel	not done	none	0	normal	none	heterozygous *PROC* p.R189W	61
8	F	7.3	2.5	none	right hemiparesis	✓	aspirin	not done	TPM	1	right arm weakness	epilepsy	no pathogenic	ND
9	F	7.2	12.2	none	hemiparesis	✓	aspirin	not done	CBZ	0	normal	epilepsy	no pathogenic	ND
10	M	3.0	3.4	none	hemiparesis	✓	aspirin	not done	none	1	mild weakness	epilepsy	no pathogenic	ND
11	M	9.1	0.5	none	recurrent alternating hemiparesis	✓	aspirin	not done	phenytoin	0	normal	epilepsy	no pathogenic	ND
12	F	6.5	11.9	none	none	✓	aspirin	not done	none	0	normal	none	heterozygous *RNF213* p.R4810K	100
13	M	11.0	5.7	none	none	✓	aspirin	bilateral STA-MCA bypass	none	0	normal	none	no pathogenic	150

14	M	11.9	6.3	none	recurrent right hemiparesis	aspirin	left STA-MCA bypass	none	1	mild hemiparesis	none	heterozygous *RNF213* p.R4810K	90

Abbreviation: APS, antiphospholipid syndrome; CBZ, Carbamazepine; CS, Constant Spring; EC, external carotid; EDAMS, encephalo-duro-arterio-myo-synangiosis; EDAS, Encephaloduroarteriosynangiosis; F, Female; FU, Follow-up; IC, Internal carotid; M, Male; MCA, Middle cerebral artery; ND, No data; No, Number; PC, Protein C; RBC, Red blood cell; MRS, Modified Rankin Scale; STA, Superficial temporal artery; TPM, Topiramate; Tx, Transfusion; VPA, Sodium valproate.

*Chronic bilateral optic neuropathy with blindness right eye, panhypopituitarism and hydrocephalus s/p VP shunt)secondary to craniopharyngioma(, ** normal protein C activity (laboratory reference rang; 64.0-141.0%, *** positive lupus anticoagulant, anticardiolipin antibody (IgG > 120 GPI), and anti β2 glycoprotein I (IgG > 200 U/mL) in Patient 1, and positive lupus anticoagulant and anticardiolipin antibody (IgG 24 GPI) in Patient 3.# The candidate variants were classified based on ACHMG 2015 and confirmed by Sanger sequencing. Common single-nucleotide polymorphisms with allele frequency > 5% was removed, .## 0; No symptoms at all, 1; No significant disability despite symptoms; able to carry out all usual duties and activities, 2; Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance, 3; Moderate disability; requiring some help, but able to walk without assistance, 4; Severe disability; bedridden, incontinent and requiring constant nursing care and attention, 5; Dead

Note: $-\alpha^{3.7}$; g.34164_37967 del 3804, α^{CS} ; HBA_2 c.427 T > C, $--^{SEA}$; g.26264_45564 del 19,301, $-\beta^{E}$; HBB:c.79G > C

developing moyamoya. The additional prothrombotic risk factors were APAs in two α-thalassemia patients and *PROC* p.R189W in one patient. The p.R189W mutation, resulted in low or slightly low levels of PC activity. The related report suggested that p.R189W had a low binding affinity to endothelial PC receptors; however, some patients with p.R189W had normal PC activity.[5,6] WES demonstrated the prothrombotic genetic variants of the *PROC* p.R189W mutation in three patients. These findings indicated the overall prothrombotic risk factors in 35.7% of patients and 71.4% of moyamoya syndrome, including two patients with APS and three with *PROC* p.R189W mutation. A related study reported prothrombotic risk factors consisting of APAs and PS deficiencies in 40% of the investigated ten patients.[7]

In addition to prothrombotic risk factors, one patient in this study presented Williams syndrome associated with vascular abnormality, including peripheral pulmonary stenosis and moyamoya syndrome.[1] One patient developed moyamoya syndrome after 18 months of 54 Gy cranial radiation. Altogether, 14.2% of our reported patients and 28.6% of moyamoya disease demonstrated *RNF213* p.R4810K, which was lower than that reported among Japanese (90.1%) and Korean (78.9%). Still, the same as in Chinese (23.2%) patients.[2] The incidence was higher than the prevalence in the general population (0-11.4%).[2] The *RNF213* gene, located on chromosome 17q25.3, is related to angiogenesis and vascular inflammation with an unknown physiologic function.

Although the number of enrolled patients was small due to the rarity of the disease, our report demonstrated a non-deletional type α-thalassemia disease (--SEA/α^csα), and APAs in moyamoya syndrome. In addition to the prothrombotic risk factor of genetic variants of *PROC* p.R189W. Moreover, the (*RNF213* p.R4810K was identified in 28.6% of moyamoya disease. In addition, the *RNF213* p.R4810K was identified in 28.6% of moyamoya disease.

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Competing interests: The authors declare no conflict of Interest.

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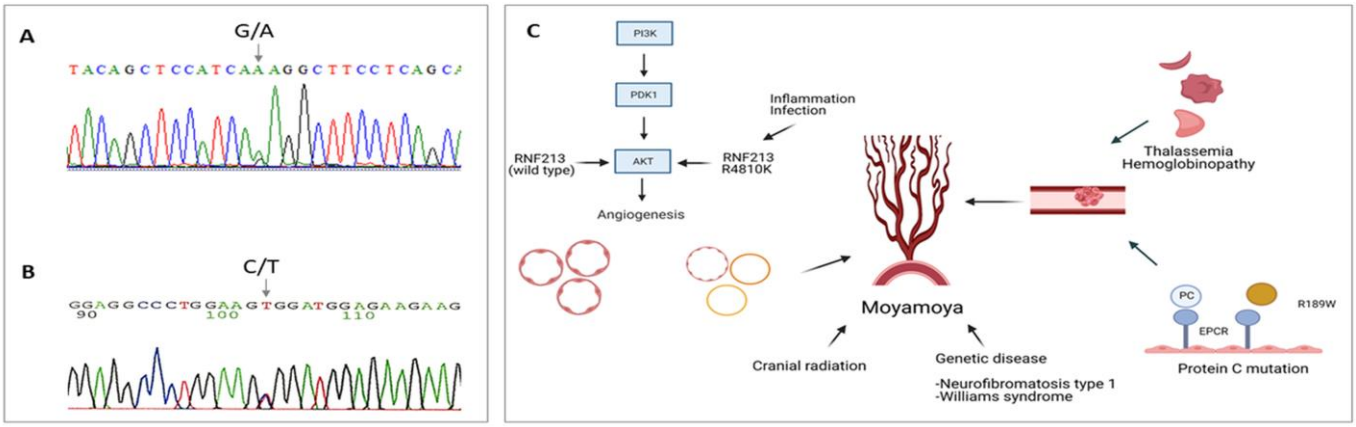
Supplement Figure:



Supplement Figure 1. Sequencing results of: (A), *RNF213* c.14429G>A, p.R4810K in patients 11 and 13; and (B), *PROC* c.565C>T, p.R189W in patients 5, 7, and 9. Diagram (C) demonstrates the possible pathogeneses of moyamoya from this report, including genetic variants of *RNF213* p.R4810K resulting in abnormal angiogenesis; *PROC* p.R189W resulting in increased thrombus formation, and contributing factors such as infection, inflammation, thalassemia disease, neurofibromatosis type I, cranial radiation and Williams syndrome.