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***RNF213* Variants in a Child with PHACE Syndrome and Moyamoya Vasculopathy**

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Abstract

Segmental infantile hemangiomas (IH) can be associated with congenital anomalies in a regional distribution. PHACE refers to large cervicofacial segmental IH in association with congenital anomalies of the aortic arch and medium-sized arteries of the head and neck, as well as structural anomalies of the posterior fossa and eye. A subset of PHACE patients have arterial anomalies that progress to moyamoya vasculopathy (MMV). MMV is defined as stenosis of the supraclinoid segment of the internal carotid arteries and/or their major branches, with subsequent development of a compensatory collateral vessel network. We describe a patient with MMV and segmental IH on the back and lower body who meets diagnostic criteria for PHACE based on a posterior segment eye anomaly and cerebral arterial anomalies. Whole exome sequencing demonstrated two inherited heterozygous variants in *RNF213*. Variants in *RNF213* are associated with increased susceptibility to MMV. Our findings suggest that *RNF213* variants may play a role in the development of MMV in patients with hemangioma syndromes associated with congenital cerebral arterial anomalies.

Keywords

PHACE; segmental hemangioma; moyamoya vasculopathy; *RNF213*

INTRODUCTION

Infantile hemangiomas (IH) are benign vascular tumors of the skin and subcutaneous tissue. Segmental IH can be associated with congenital anomalies. The acronym PHACE (OMIM # 606519), first proposed in 1996, describes the association of posterior fossa brain

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malformations, hemangiomas, arterial anomalies, cardiac defects, and eye abnormalities characteristic of the disease [Frieden et al., 1996]. Midline ventral defects such as sternal clefting or supraumbilical raphe can also be present. A subset of patients with PHACE have segmental IH on the upper body, rather than the face, in association with arch anomalies, structural brain, or cervical and cerebral arterial anomalies. This was initially described as “PHACE without face” [Nabatian et al., 2011]. More recently, the diagnostic criteria for definite or possible PHACE have been expanded to include patients with segmental IH on the trunk, or even lacking a hemangioma [Garzon et al., 2016].

Head and neck arterial anomalies are the most common extracutaneous findings in PHACE, affecting 91% of patients, and can include complete absence, dysplasia, narrowing, aberrant course or origin, and persistence of fetal-pattern vasculature [Haggstrom et al., 2010]. Rarely the cervical and cerebrovascular anomalies observed in PHACE are progressive, leading to moyamoya vasculopathy. Moyamoya vasculopathy is defined as stenosis or occlusion of the internal carotid arteries or their distal branches with subsequent development of a compensatory network of fine collateral vessels, occurring in patients with another underlying disorder [Fujimura and Tominaga, 2015]. MMV is variably referred to as “quasi-moyamoya disease,” “moyamoya syndrome,” or “secondary moyamoya disease” to distinguish this phenomenon from idiopathic moyamoya disease (MMD) [Fujimura and Tominaga, 2015]. MMD differs from MMV in that MMD arterial changes occur in the absence of underlying disease [Kamada et al., 2011].

All reported PHACE patients with MMV have associated congenital anomalies of cervical and/or cerebral arteries, but not all PHACE patients with congenital arterial anomalies will develop MMV. In fact, MMV is a relatively uncommon finding in PHACE, affecting fewer than 7% of patients [Heyer et al., 2008]. However, because the population of known PHACE patients is young, and MMV develops progressively, the incidence of MMV in PHACE may be underestimated. Importantly, severe cerebral and/or cervical arterial anomalies, especially if progressive with the development of MMV, confer an increased risk for stroke in PHACE [Siegel et al., 2012]. It is unknown why a particular subset of individuals with PHACE develops this MMV phenotype.

RNF213 has been identified as a susceptibility gene for MMD [Kamada et al., 2011]. The *RNF213* founder variant p.R4810K has been strongly associated with both MMD and MMV in patients of Asian descent [Liu et al., 2012; Morimoto et al., 2016]. However, additional rare *RNF213* variants have also been described in non-Asian MMD patients [Cecchi et al., 2014]. Of note, the vast majority of variants associated with MMD identified to date are missense variants, suggesting that more significant alteration of the gene may not be tolerated. In this report, we describe two compound heterozygous *RNF213* variants in association with MMV in a patient with PHACE. To our knowledge, this is the first report of *RNF213* variants in a hemangioma syndrome.

CLINICAL REPORT

The patient is a female of Caucasian and African-American descent who presented to dermatology clinic as a 5 year old for evaluation for PHACE. She had a history of a large

segmental infantile hemangioma located on the sacral back extending to the right posterior thigh and ankle. Magnetic resonance imaging (MRI) of the lower spine was unremarkable. Treatment of the infantile hemangioma during the first year of life included oral corticosteroids and pulsed-dye laser therapy. She had a history of decreased visual acuity in the left eye (20/800). She had normal development and no neurologic symptoms until age 4 years, when she began to have episodes of vomiting. By age 5 years, she was able to articulate that the episodes of vomiting were associated with headaches. She had no seizures and an electroencephalogram was normal. There is no family history of epilepsy or early strokes.

Physical examination was notable for a faint, telangiectatic patch on the lower back over the sacrum, extending to the buttocks and right popliteal fossa. There was no vascular stain or hemangioma on the face or scalp. There were no skeletal abnormalities, and neurologic exam was normal.

Magnetic resonance imaging/angiography (MRI/MRA) of the brain and neck revealed no brain parenchymal structural malformations or hemangiomas. The globe of the left eye was dysmorphic with a posterior staphyloma (Fig. 1a). Significant dysplasia of the cervical and cerebral arteries included tortuous course of the right internal carotid, marked tortuosity of the cervical and petrous segments of the left internal carotid artery, terminal left internal carotid artery, and left posterior communicating artery. There was focal high-grade narrowing in the distal M1 segment of the left middle cerebral artery (Fig. 1b) with numerous lenticulostriate collaterals. Left posterior cerebral artery was small in caliber. A MR perfusion study (performed with dynamic susceptibility contrast technique) showed mild compensated hypoperfusion in the left middle and posterior cerebral artery vascular territories (Fig. 1c). Due to the increased risk of stroke based on the cerebral arterial anomalies, aspirin 40 mg per day was started. She subsequently developed progression of the cerebral arteriopathy, and the patient underwent a successful pial synangiosis for treatment of her moyamoya vasculopathy.

METHODS

DNA from a skin biopsy of hemangioma tissue on the right thigh and peripheral blood from the patient as well as parent peripheral blood were extracted using standard protocols. Whole exome sequencing data were generated on the Illumina HiSeq 2000 platform (Illumina Inc; San Diego, CA). Overall mean depth of coverage was 81X in tissue and 103X in blood. The mean depth of coverage for *RNF213* (NM_001256071) was 83X in tissue and 106X in blood. Variants were called via the Genome Analysis Toolkit best practices methods (GATK, Broad Institute). Variants were analyzed using an in-house developed application called CarpeNovo [Worthey et al., 2011]. *In silico* modeling was performed using domain and protein ordered/disordered prediction tools and was modeled using the I-TASSER suite of protein modeling tools. The model was inserted into an aqueous environment with a pH of 7.4, energy minimized using YASARA and molecular dynamic simulations performed using AMBER03. Variants were confirmed with orthogonal Sanger-based clinical grade sequencing at the Children's Hospital of Wisconsin (Fig. 2).

RESULTS

Whole exome sequencing analysis of the proband samples revealed compound heterozygous variants in *RNF213* (Fig. 2). The c.14030G>T (p.W4677L) variant in *RNF213* was maternally inherited. This variant was identified in the ExAC database at 0.99% variant allele frequency in the total population, but was enriched in European versus African ethnic groups (ExAC frequency, European/African: 0.0151/0.0035). The tryptophan at this position is highly conserved through multiple species, and the p.W4677L protein change was predicted to be damaging by *in silico* modeling (Fig. 3a, 3b) and molecular dynamic simulations (Fig. 3c). The second variant c.1470G>T (p.Q469H) was inherited from the father and identified in the ExAC database at 0.22% variant allele frequency of the total population, but was seen nearly exclusively in individuals of African descent (ExAC frequency, European/African: 0/0.0240). The glutamine at this position is variable across species (Fig. 3b), however the p.Q469H protein change is predicted to be possibly damaging by changing the protein domain dynamics with *in silico* modeling (Fig. 3c). The p.W4677L variant was predicted to be “possibly damaging” by PolyPhen (PolyPhen-2 HumVar score = 0.879) and “damaging” by SIFT (SIFT score = 0.000), while the p.Q469H variant was predicted to be “possibly damaging” by Polyphen (0.533) and “tolerated” by SIFT (0.112).

Whole exome sequencing was performed on 27 additional blood samples from PHACE patients, 6 of whom had MMV. The p.W4677L variant seen in the proband was present in one other patient; the p.Q469H variant was not present in any. WES of additional samples and validation of variants is ongoing.

DISCUSSION

Large segmental IH on the head and neck are most commonly associated with cervical and cerebral arterial anomalies, structural brain anomalies, and congenital heart defects in PHACE. When located on the lower body, segmental hemangiomas can be associated with developmental anomalies described by the acronym LUMBAR (**L**ower body hemangiomas, **U**rogenital anomalies, **U**lceration, **M**yelopathy, **B**one deformities, **A**norectal/Arterial malformations, **R**enal anomalies) [Iacobas et al., 2010]. Less commonly, lower body hemangiomas co-occur with anomalies typical of PHACE. In this report we describe a patient with lower back and extremity segmental IH in association with staphyloma, an ocular structural anomaly associated with PHACE, as well as the classic arterial anomalies described in PHACE. This constellation of findings meets diagnostic criteria for definite PHACE according to recently updated diagnostic guidelines [Garzon et al., 2016].

This report highlights the association of MMV with compound heterozygous inherited rare variants within *RNF213* in a patient with PHACE syndrome and MMV. The p.W4677L variant observed in our patient was recently associated with MMD in a Caucasian patient from a cohort from the Czech Republic and Slovakia [Kobayashi et al., 2016]. This variant is located at the C-terminal end of the protein within a region of unknown function that contains the Japanese founder variant p.R4810K, as well as other variants previously associated with MMV [Kamada et al., 2011; Miyatake et al., 2012]. The second variant p.Q469H occurs closer to the N-terminus, and is predicted to fall into the potassium

dependent sodium calcium exchanger domain, potentially affecting the activity of the protein [Miyatake et al 2012].

The severity of the MMV seen in this patient may be explained by the compound heterozygous inheritance of these two potentially damaging variants. In a study of Japanese MMD patients, homozygous individuals had an earlier onset and more severe presentation [Miyatake et al., 2012]. Moyamoya disease has a reported female preponderance, an observation that has also been made in PHACE. Thus it is possible that disease severity is related to a combination of the number and type of variants present, as well as other modifiers affected by gender or genetic/environmental factors [Nanba et al., 2006; Miyatake et al., 2012].

With this report, we have identified *RNF213* as a potential modifier of phenotypic features seen in PHACE. Further study of a larger cohort of individuals with PHACE and LUMBAR, especially those with intracranial arterial anomalies and MMV, is needed to further elucidate the frequency and importance of *RNF213* in these hemangioma syndromes.

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Fig. 1.

- a) Dysmorphic appearance of the left globe consistent with posterior staphyloma (arrow) is demonstrated on T2 weighted axial image of the brain and orbits.
- b) The brain MRA demonstrated focal high-grade narrowing in the distal M1 segment of the left middle cerebral artery (arrow) and left side moyamoya collaterals.
- c) A Time to peak (TTP) map obtained from DSC MR perfusion study revealed delayed contrast peak in the left middle and posterior cerebral artery vascular territories. Mild increase in mean transit time (MTT) and relative cerebral blood volume (rCBV) was also seen in these territories (not shown) indicating mild compensated hypoperfusion as blood supply to these areas is reinforced by lenticulostriate and pial collaterals from other intracranial arteries.

Fig. 2. Visualization of exome sequencing results in the Integrative Genomics Viewer (IGV) demonstrates heterozygous mutations in the *RNF213* gene. a) *RNF213* c.1470G>T (p.Q469H) mutation in the proband and father. b) *RNF213* c.14030G>T (p.W4677L) mutation in the proband and mother. c) Sanger sequencing validation of *RNF213* c.1470G>T and c.14030G>T mutations in the proband.

Fig. 3.

- a) Molecular models of the domains for W4677 (amino acids 4535–4858) and Q469 (amino acids 343–599). Both structures have fair z-score predictions (W4677 -2.65, Q469 -3.84) relative to all structures solved in the protein data bank suggesting power to interpret the structure models. Amino acid of interest for each is colored red.
- b) Sequence alignments of 88 species for both sites of interest with variant amino acids shown in red. “*” represent amino acids conserved in all 88 species and “.” those amino acids with functional conservation.
- c) Molecular dynamic simulations were performed on either the wild type protein structures (W4677- green, Q469- blue) or structures containing the patient variants (W4677L- purple, Q469H- red). The total protein movement was tracked throughout the simulation using the carbon alpha Root Mean Square Deviation (RMSD, in angstroms). Both protein variants resulted in changes to the protein dynamics.