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A novel pathogenic *RHOA* variant in a patient with patterned cutaneous hypopigmentation associated with extracutaneous findings

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Abstract

RHOA-related neuroectodermal syndrome is characterised by linear skin hypopigmentation along Blaschko's lines associated with alopecia, leukoencephalopathy, facial and limb hypoplasia, and ocular, dental, and acral anomalies. Herein, we report a patient with patterned cutaneous hypopigmentation with a similar phenotype due to a novel postzygotic *RHOA* variant (c.210G>T; p.Arg70Ser). This illustrates that the complexity of the orchestration of morphogenesis and organogenesis can be affected by different variants in the same gene.

KEYWORDS
acral, brain anomalies, hypopigmentation; genetics; mosaicism; hypomelanosis of Ito, ocular, *RHOA* variants

1 | BACKGROUND

Hypomelanosis of Ito was first described by Minor Ito in 1952 in a 22-year-old woman with linear symmetric hypopigmentation of her trunk and arms.[1] Over the years, hypomelanosis of Ito became an umbrella term referring to cutaneous hypopigmentation along Blaschko's lines, presenting with or without associated extracutaneous findings in various systems, particularly neurological, skeletal, and ophthalmological.[2] Diagnostic criteria have been proposed by Ruiz-Maldonado and al. in 1992,[3] but no consensus or formal definition has been adopted to this day. Unjustified investigations for the patient and unnecessary stress for the parents could be avoided with a better delineation of syndromic patterned hypopigmentation.

Identification of postzygotic variants in vascular anomalies, such as port-wine stains and Sturge–Weber syndrome (*GNAQ*), and hamartomatous skin disorders, such as sebaceous nevi and Schimmelpenning syndrome (*KRAS* and *HRAS*),[4,5] stimulated the search for the genetic basis of pigmentary mosaicism. To this day, three distinct mosaic hypopigmentation syndromes[6–8] have been identified with postzygotic variants in *MTOR*, *TFE3*, and *RHOA* genes.

2 | CASE REPORT

A girl born at term by spontaneous delivery to non-consanguineous French–Canadian (Caucasian) parents after an uneventful pregnancy

presented at birth with right-sided hypoplasia of the face. Leg hypoplasia became apparent in infancy and led over time to leg-length discrepancy requiring three embolisations of the left distal femoral growth plate and a left leg epiphysiodesis at age 7 years. She also presented with congenital colorectal atresia surgically corrected by a colectomy and terminal–terminal anastomosis at day 1 of life. The surgery was complicated by perforation peritonitis which required intravenous antibiotics and four more surgeries. An anterior anus and mild left pyelocalyceal ectasia were noted at birth. She had multiple right eye anomalies: persistent hyperplastic primary vitreous (PHPV) associated with microphthalmia, and cataract and optic nerve hypoplasia. Right microdontia, retention of left deciduous teeth,and class 3 malocclusion were observed during her dental follow-ups. Brain magnetic resonance imaging (MRI) at day 12 of life showed mild dilation of lateral ventricles with prominence of interventricular foramina, mild asymmetry of extra-axial spaces, and mild left cerebral hypoplasia. Leukoencephalopathy and cystic structures were not reported. Hearing, cognitive, and motor development were normal. The patient was first evaluated in pediatric dermatology during the neonatal period because of the congenital right facial hypoplasia and a diagnosis of Parry Romberg was entertained. She was seen again at 18 months of age for linear cutaneous hypopigmentation on her right arm, leg, cheek, nose, chest, axilla, and lateral thigh that became apparent towards 10 months of age. There were no hair, nail, mucosal, or acral anomalies. She was then diagnosed with hypomelanosis of Ito. Figures 1 and 2 illustrate these clinical findings.

Our patient had a normal female karyotype (46, XX). Ultra-deep sequencing of *RHOA* (NM_001664.4) was performed, as previously described, [8] on the patient's blood sample and affected skin as well as on her parental blood samples. A postzygotic *RHOA* variant (c.210G>T; p. Arg70Ser) was found at an allele fraction of 34% on the DNA derived from her skin biopsy but not from the patient's blood lymphocytes nor from her parents. Figure 3 shows the variant and sequencing data.

3 | DISCUSSION

Mosaicism refers to cells with different genotypes derived from otherwise a genetically homogeneous zygote. Cutaneous pigmentary mosaicism represents different patterns of hyper or hypopigmentation due to the genetic heterogeneity of cells and their ability to produce melanin. Six archetypal patterns of cutaneous mosaicism have been classified by Happle: narrow and broad bands along Blaschko lines (type 1a and type 1b respectively), blocklike (type 2), phylloid (type 3), patchy without midline demarcation (type 4), lateralised with a clear midline separation (type 5), and sash-like (type 6).[9]

In our retrospective study of 106 children with patterned cutaneous hypopigmentation (PCH), the predominant types of patterns were along Blaschko lines type 1a and type 1b, whether or not extracutaneous anomalies were present.[10] Although not statistically significant, type 1a pattern of hypopigmentation was more frequently associated with extracutaneous findings than type 1b, 34.1% vs. 21%, respectively. The anterior and posterior trunk were most frequently affected, 69% and 56%, respectively. The reported frequency of extracutaneous involvement in patients with PCH is highly variable. In our series, extracutaneous involvement, especially neurological and developmental, was present in 28.3% of patients and was significantly associated with ≥4 involved body sites. Half of the patients (4/8) with a block-like pattern had extracutaneous manifestations.

Since 2016, various mosaic variants have been described in patients with PCH and extracutaneous anomalies, often referred to as hypomelanosis of Ito. *RHOA* encodes a Ras-related Rho GTPase, also known as the transforming protein RhoA, that controls important biological functions, such as morphogenesis, axonal guidance, cell adhesion, cell migration, and cell proliferation.[11] Pathogenic germline variants in *RHOA* are yet to be described in humans. However, recurrent somatic variants have been described in numerous malignancies, such as angioimmunoblastic T-cell lymphoma, adult T-cell

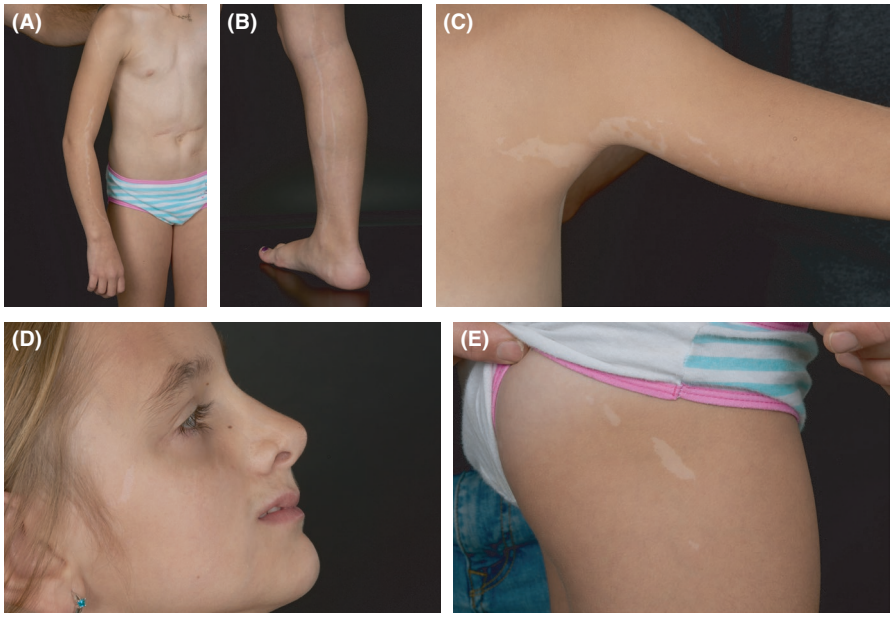

FIGURE 1 (A–E) Our patient presented different types of patterned hypopigmentation: type 1a (narrow bands along the lines of Blaschko) and type 1b (broad bands along the lines of Blaschko)

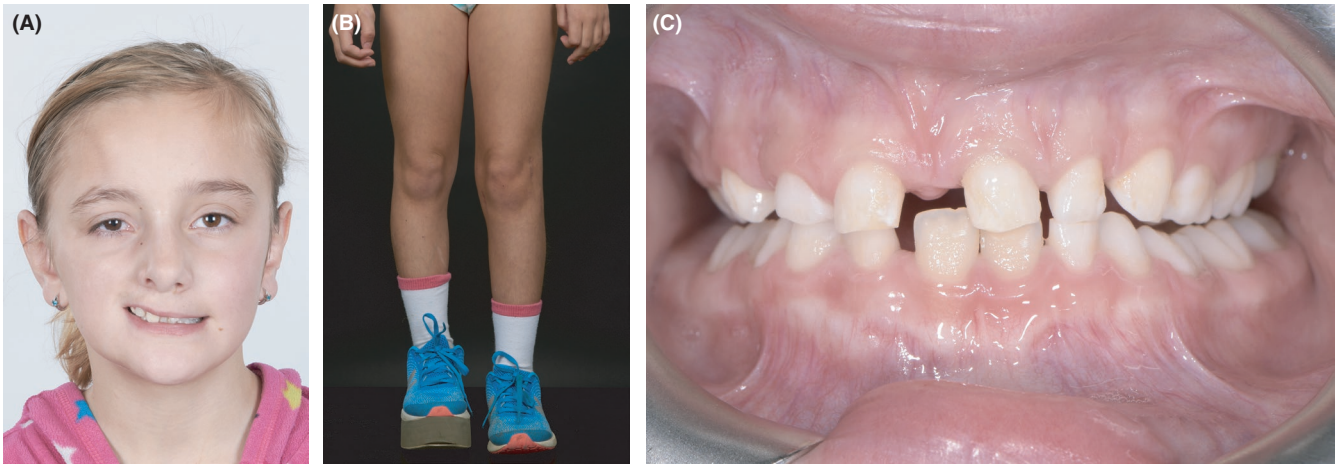

FIGURE 2 Musculoskeletal and dental anomalies in our patient: (A) Right facial hemihypotrophy (B) Lower limb hemihypotrophy (C) Dental anomalies

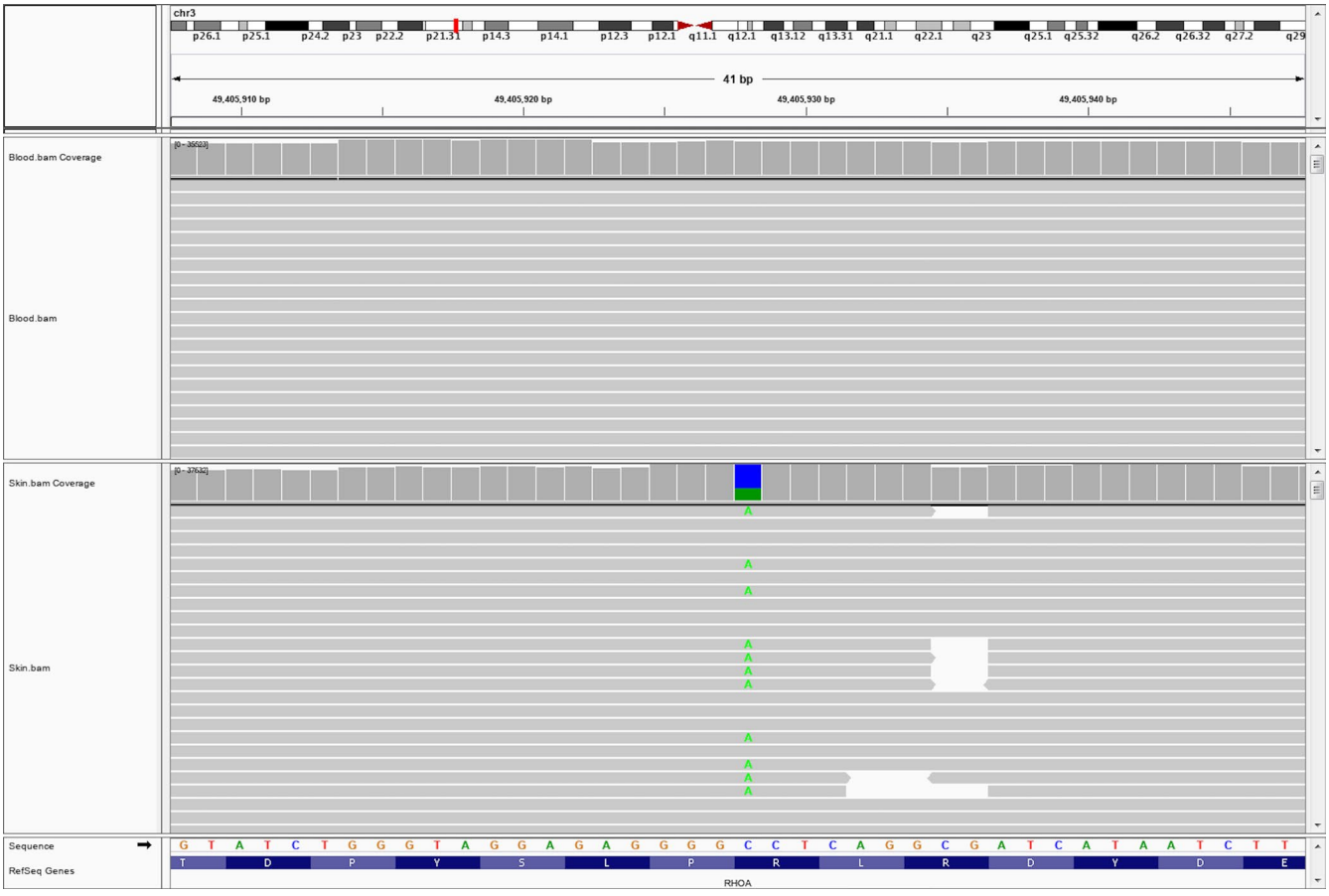

FIGURE 3 Integrative Genomics Viewer (IGV) screenshots of the RHOA c.210G>T substitution (encoding p. Arg70Ser) in blood (top) and skin (bottom) of the patient. The variant site was covered by 31,557 and 37,119 sequencing reads in the blood and skin samples, respectively

leukemia/lymphoma, and diffuse gastric cancer.[12-14] *RHOA* is considered essential for cell viability due to the low tolerance to amino acid substitution of the transforming protein RhoA. Therefore, *RHOA*-related neuroectodermal syndrome is considered a disorder caused by lethal DNA variants surviving only by mosaicism. [15]

As of now, 11 patients, 10 of whom were female, have been described with neuroectodermal syndrome and a mosaic *RHOA* variant. Vabres et al.[8] and Yigit et al. [16] reported seven and four patients, respectively, with syndromic PCH associated with multiple extracutaneous anomalies. We include our patient (case 12) in Table 1. *RHOA* missense variant c.139G>A (p. Gu47Lys) was identified from skin biopsies of the affected skin in 9/12 patients. Case 3 showed a novel *RHOA* variant c.211C>T (p. Pro71Ser); the biological material of case 6 was inadequate for genetic testing; and case 12 (our patient)

TABLE 1 Anomalies associated with syndromic patterned cutaneous hypopigmentation

Case[ref]	RHOA variant c.139G>A p.(Glu47Lys)	Sex	Skin patterned hypopigmentation				Body sites	Face		Scarring alopecia	Eyes
			1a	1b	2	3		Hypoplasia	Other features		
1[8]	+	F	+	+	–	–	Neck Lower limbs	R	Broad nasal bridge Thick alae nasi	+	Peripapillary chorioretinal atrophy (R)
2[8]	+	M	+	+	–	–	Neck Upper limbs Lower limbs	R	Broad nasal bridge Thick alae nasi Short philtrum	+	Cataracts Corectopia Retinal atrophy Papillary dysversion Congenital nystagmus
3[8]	c.211C>T p.(Pro71Ser)	F	–	+	–	–	Neck Upper limbs Lower limbs	L	Thick alae nasi Narrow palpebral fissure (L) Cleft lip	+	Strabismus
4[8]	+	F	+	+	–	–	Upper limbs Lower limbs	L	–	NA	Microphthalmia (L) Optical nerve atrophy (L) Oculo–motor dyssynergia
5[8]	+	F	+	–	–	–	Upper limbs Lower limbs	R	–	+	–
6[8]	NA	F	NA				Limbs	L	–	NA	–
7[8]	+	F	+	–	–	–	Upper limbs Lower limbs	R	Broad nasal bridge	–	Congenital cataracts (R) Congenital glaucoma (R) Retinal dystrophy
8[16]	+	F	+	–	–	–	Scalp Lower limb (R)	R	–	–	–
9[16]	+	F	+	+	+	–	Upper limbs Lower limbs	–	Prominent forehead Narrow palpebral fissures Short philtrum Micrognathia Microstomia	+	Cataracts Anterior segment dysgenesis Glaucoma Optic nerve hypoplasia (L) Ocular prosthesis (R)
10[16]	+	F	+	+	–	–	Upper limb (L) Lower limb (L)	–	–	NA	–
11[16]	+	F	+	–	–	–	Lower limb (L)	–	Prominent forehead Relative macrocephaly Micrognathia Microstomia	–	Vision loss Asymmetric eye shape
12 (Our case)	c.220G>T p.(Arg70Ser)	F	+	+	–	+	Face (R) Chest (R) Upper limb (R) Lower limb (R)	R	–	–	Cataracts (R) Optic nerve hypoplasia (R) Persistent hyperplastic primary vitreous (R) Microphthalmia (R)

Abbreviations: –, negative; +, positive; C, CT-scan; F, Female; L, Left; M, Male; M, MRI; NA, Not Available; R, Right; ref, reference.

Limbs hypotrophy	Digits	Teeth	Hearing loss	Brain Imaging MRI[M]/ CTScan[C]	Normal development	GI	GU
NA	Toe asymmetry	Conical teeth Oligodontia Microdontia Persistent decidual dentition	NA	Leukoencephalopathy[M]	+	+	+
NA	Toe brachydactyly	Conical teeth Oligodontia Microdontia	+	Leukoencephalopathy[M] Posterior fossa arachnoid cyst[M]	+	NA	NA
Lower limbs (L)	Toe polydactyly Toe syndactyly	Dental agenesis	NA	Ventriculomegaly[C]	+	NA	+
NA	Toe brachydactyly	Conical teeth	NA	Leukoencephalopathy[C] Posterior fossa arachnoid cyst[C] Basal ganglia calcifications[C]	+	NA	NA
NA	Toe clinodactyly	Dental agenesis (R)	NA	Leukoencephalopathy[M]	+	+	NA
NA	Toe brachydactyly	Dental agenesis Microdontia	NA	NA	+	NA	NA
Lower limbs (L)	Toe brachydactyly Toe clinodactyly Sandal gap	Premature eruption of teeth (R)	+	–	+	NA	NA
R	Toe brachydactyly	Microdontia (R)	NA	Leukoencephalopathy[M]	+	NA	+
Lower limbs (L)	Fingers syndactyly Long great toes Overlapping toes	Oligodontia Microdontia	+	Leukoencephalopathy[M] Basal ganglial and thalamic cysts[M]	Focal clonic seizures Difficulties in reading and writing	NA	+
L	Toes syndactyly	Anomalies of the incisor (R)	NA	Leukoencephalopathy[M]	+	NA	NA
L	–	–	+	Leukoencephalopathy[M] Ventriculomegaly[M] Small pituitary[M]	Global developmental delay	NA	+
Lower limbs (L)	Toes brachydactyly	Microdontia (R) Persistent deciduous teeth (L)	–	Ventriculomegaly[M] Brain hemisphere asymmetries[M] (L >R)	+	+	-

presented with a novel *RHOA* variant (c.210G>T; p. Arg70Ser). All patients presented PCH, type 1a and 1b. Case 12 mainly presented with PCH of type 1a. Limbs and trunk were involved in in 100% and 8%, respectively. Associated findings included the following: prominent facial dysmorphism (11/12) with the most frequent finding being hemifacial hypotrophy (9/12), followed by broad nasal bridge (3/12), and thick alae nasi (3/12). Other frequent anomalies were as follows: dental (11/12), acral (11/12), ocular (8 vs 10/12), scarring alopecia (5/12), and hearing loss (4/12). Although Vabres et al. reported body asymmetry in only 2/7 patients, Yigit et al. report ipsilateral hemihypotrophy in all four patients presented. Case 12 also presented ipsilateral facial and lower limb hemihypotrophies. Although leukoencephalopathy with ventriculomegaly and cystic structures on brain MRI was a recurrent finding in these patients, none presented major intellectual deficiency nor neurological impairment. (Table 1).

Beside *RHOA*, two other genes associated with PCH have been described. Postzygotic mammalian target of rapamycin (*MTOR*) variants are associated with PCH, severe epilepsy, focal cortical dysplasia, and megalencephaly or hemimegalencephaly.[6] Transcription factor E3 (*TFE3*) variants have been described in numerous patients with PCH as well as coarse facial features, severe intellectual disability, epilepsy, skeletal anomalies, obesity, and growth retardation.[7] Cutaneous biopsies and genetic testing should be considered in patients with patterned cutaneous hypopigmentation with systemic involvement reminiscent of *RHOA*, *MTOR*, or *TFE3* mutations.

In conclusion, postzygotic variants in *RHOA* are responsible for *RHOA*-related neuroectodermal syndrome. The importance of the Eph/Ephrin receptor–ligand system sheds more light on this syndromic dysplasia.[17] This system plays an important role in embryogenesis, principally by influencing cell behavior through signaling pathways, resulting in modification of the cell cytoskeleton and cell adhesion. Eph/Ephrin protein signaling results in the activation of several cytoplasmic downstream signaling pathways. Several of these pathways are dependent on the activity of the Rho family GTPases, including RhoA. Furthermore, Ephrin-B ligand proteins play a dual role in the guidance of neural crest cell migration of the melanoblasts along the dorsolateral pathway and of the neurons and glial cells migrating ventrally.[18] In addition, Ephrin-A5 has been shown to be a critical regulator for primary vitreous regression in the eyes of mice.[19] Therefore, Eph/Ephrin pathway dysfunction may partially explain our patient's patterned cutaneous hypopigmentation, brain MRI anomalies, and PHPV.

Unraveling the genetic basis of syndromic PCH still is a challenge due to the complexity of embryogenesis and morphogenesis, the phenotype and genetic heterogeneity, the technical difficulties in detecting mosaic variants by standard sequencing approaches, and obtaining affected skin tissues. With the identification of responsible postzygotic variants, such as in *MTOR*, *TFE3*, and *RHOA*, for PCH associated with extracutaneous anomalies, physicians will be able to better counsel patients or parents. One interesting query would be to evaluate if the previously identified variants causing syndromic pigmentary mosaicism are also capable of generating isolated cutaneous findings, similar to *GNAQ* variants causing either Sturge–Weber syndrome or isolated port-wine stains.[4]

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

INSTITUTIONAL BOARD APPROVAL

The study was approved by our Institutional Review Board (# MP-21–2018–1591).

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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