**ORIGINAL INVESTIGATION**

# Monogenic causes of pigmentary mosaicism

Ken Saida[1] · Pin Fee Chong[2] · Asuka Yamaguchi[3] · Naka Saito[4] · Hajime Ikehara[5] · Eriko Koshimizu[1] ·
Rie Miyata[3] · Akira Ishiko[6] · Kazuyuki Nakamura[7] · Hidenori Ohnishi[8] · Kei Fujioka[9] · Takafumi Sakakibara[10] ·
Hideo Asada[11] · Kohei Ogawa[11] · Kyoko Kudo[12] · Eri Ohashi[13] · Michiko Kawai[13] · Yuichi Abe[13] · Naomi Tsuchida[1,14] ·
Yuri Uchiyama[1,14] · Kohei Hamanaka[1] · Atsushi Fujita[1] · Takeshi Mizuguchi[1] · Satoko Miyatake[1,15] · Noriko Miyake[1,16] ·
Mitsuhiro Kato[17] · Ryutaro Kira[2] · Naomichi Matsumoto[1]

## Abstract

Pigmentary mosaicism of the Ito type, also known as hypomelanosis of Ito, is a neurocutaneous syndrome considered to be predominantly caused by somatic chromosomal mosaicism. However, a few monogenic causes of pigmentary mosaicism have been recently reported. Eleven unrelated individuals with pigmentary mosaicism (mostly hypopigmented skin) were recruited for this study. Skin punch biopsies of the probands and trio-based blood samples (from probands and both biological parents) were collected, and genomic DNA was extracted and analyzed by exome sequencing. In all patients, plausible monogenic causes were detected with somatic and germline variants identified in five and six patients, respectively. Among the somatic variants, four patients had *MTOR* variant (36%) and another had an *RHOA* variant. De novo germline variants in *USP9X*, *TFE3*, and *KCNQ5* were detected in two, one, and one patients, respectively. A maternally inherited *PHF6* variant was detected in one patient with hyperpigmented skin. Compound heterozygous *GTF3C5* variants were highlighted as strong candidates in the remaining patient. Exome sequencing, using patients' blood and skin samples is highly recommended as the first choice for detecting causative genetic variants of pigmentary mosaicism.

✉ Naomichi Matsumoto
naomat@yokohama-cu.ac.jp

1 Department of Human Genetics, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

2 Department of Pediatric Neurology, Fukuoka Children's Hospital, Fukuoka, Japan

3 Department of Pediatrics, Tokyo-Kita Medical Center, Tokyo, Japan

4 Department of Pediatrics, Tsuruoka Municipal Shonai Hospital, Yamagata, Japan

5 Department of Pediatrics, Chiba University Graduate School of Medicine, Chiba, Japan

6 Department of Dermatology, Toho University School of Medicine, Tokyo, Japan

7 Department of Pediatrics, Faculty of Medicine, Yamagata University, Yamagata, Japan

8 Department of Pediatrics, Gifu University Graduate School of Medicine, Gifu, Japan

9 Center of General Internal Medicine and Rheumatology, Gifu Municipal Hospital, Gifu, Japan

10 Department of Pediatrics, Nara Medical University School of Medicine, Nara, Japan

11 Department of Dermatology, Nara Medical University School of Medicine, Nara, Japan

12 Department of Dermatology, Fukuoka Children's Hospital, Fukuoka, Japan

13 Division of Neurology, National Center for Child Health and Development, Tokyo, Japan

14 Department of Rare Disease Genomics, Yokohama City University Hospital, Yokohama, Japan

15 Clinical Genetics Department, Yokohama City University Hospital, Yokohama, Japan

16 Department of Human Genetics, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan

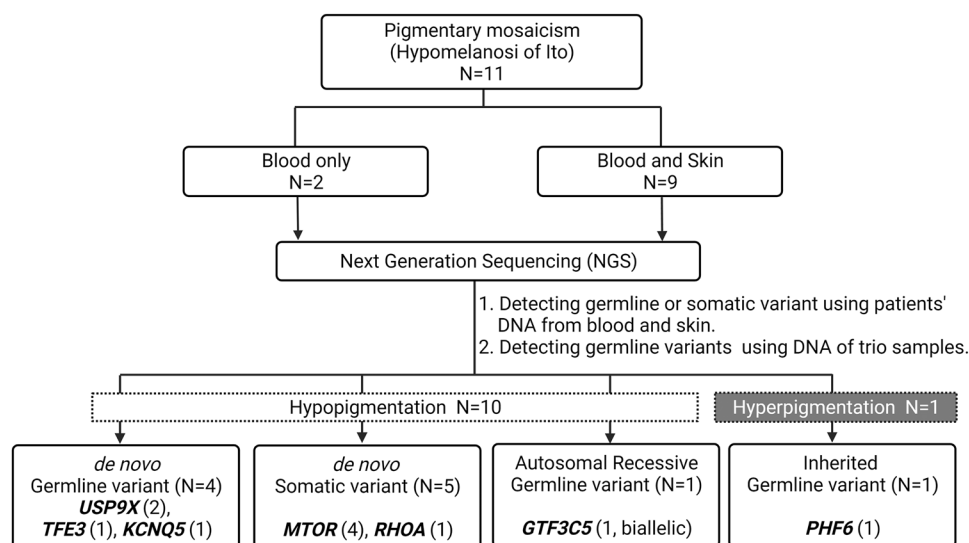17 Department of Pediatrics, Showa University School of Medicine, Shinagawa-ku, Tokyo, Japan

**Fig. 1** Flowchart of the study. DNA was extracted from blood leukocytes and skin lesion biopsies of patients with pigmentary mosaicism and analyzed by next-generation sequencing. Rare variants only in the skin (somatic variants) or in both the skin and blood (germline or somatic variants) were investigated. Candidate germline variants were confirmed by Sanger sequencing of trio-samples (from patients and their parents). Exome sequencing of 11 individuals with pigmentary mosaicism revealed four somatic *MTOR* variants, a somatic *RHOA* variant; de novo variants in *USP9X* ($n=2$), *TFE3* ($n=1$), and *KCNQ5* ($n=1$); biallelic germline variants in *GTF3C5*; and one inherited germline variant in *PHF6*

## Introduction

Pigmentary mosaicism, or hypomelanosis of Ito (HI, MIM# 300337), is a rare neurocutaneous syndrome characterized by hypopigmented skin whorls along Blaschko's lines. It is associated with nervous system abnormality and other ectodermal defects involving fingers, toes, and teeth (Kouzak et al. 2013; Sybert 1994). Hypopigmented lesions have a reduced number of melanocytes, which originate from neural crest cells (Carmignac et al. 2021; Kuster and Konig 1999; Lee et al. 1999). In some cases, it is difficult to determine whether the affected skin is hypopigmented or hyperpigmented (Nehal et al. 1996), and the term "pigmentary mosaicism" refers to patterned hypopigmentation or hyperpigmentation resulting from a clone of skin cells with altered melanin-producing ability (Kromann et al. 2018; Shaffer and Chernoff 2020). The prevalence of HI is unknown, but it is estimated to be between 1/10,000 and 1/8,500, making it one of the most predominant rare neurocutaneous syndromes (Barbel et al. 2015). It affects both sexes, with a female-to-male prevalence ratio of 2:1. It has long been considered that somatic mosaicism is involved in HI, because the differently pigmented skin areas correspond to the varying distribution of two mosaic cell lines in each individual. Up to 60% of individuals with HI present with multiple types of mosaic cytogenetic abnormalities, including numerical (e.g., trisomy 18 or 20, triploidy) and structural chromosomal aberrations (e.g.,

deletions, duplications, or translocations) (Kromann et al. 2018; Salas-Labadia et al. 2019; Shaffer and Chernoff 2020; Thomas et al. 1989). However, no medical treatments are currently available for such chromosomal abnormalities, even if they were detected. Thus, skin biopsies, only to confirm chromosomal abnormalities, have been less motivated.

Recently, a few monogenic causes of HI have been reported. Germline *TFE3* (MIM* 314310) and somatic *RHOA* (MIM* 165390) variants were found in pigmentary mosaicism (Lehalle et al. 2020; Vabres et al. 2019; Yigit et al. 2020). Somatic or germline *MTOR* (MIM* 601231) variants have also been occasionally associated with pigmentary mosaicism (Gordo et al. 2018; Hadouiri et al. 2020; Handoko et al. 2019; Mirzaa et al. 2016) and a large cohort of *MTOR*-related HI has recently been reported (Carmignac et al. 2021). This study aims to further explore the monogenic causes of pigmentary mosaicism.

## Materials and methods

### Patients

Individuals who were clinically diagnosed with pigmentary mosaicism were recruited through the collaborative activity of the Japanese Society of Child Neurology between April 2018 and March 2021. A total of 11 patients with pigmentary mosaicism (clinically diagnosed as HI and predominant

skin hypopigmentation) were enrolled (Fig. 1). Skin samples were obtained from the patients, whereas blood samples were collected from the patients and their parents. Fresh skin tissues were obtained by punch biopsies of hypopigmented skin. Genomic DNA was extracted from the skin and peripheral blood leukocytes. Informed consent was obtained from the patients' parents. This study protocol was approved by the Institutional Review Board of the Yokohama City University Faculty of Medicine.

## Exome sequencing (ES)

The flowchart of the study design is shown in Fig. 1. DNA was extracted from skin biopsies using proteinase K and sodium dodecyl sulfate with RNase and phenol/chloroform. DNA extraction from peripheral blood leukocytes was performed using the QuickGene DNA Extraction Whole Blood Kit L (Kurabo Industries Ltd., Osaka, Japan). ES was performed at a standard depth (approximately, 40–70×) as previously described (Sakamoto et al. 2021). In brief, genomic DNA was captured with a SureSelect Human All Exon V6 Kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced on a Illumina Novaseq 6000 Sequencing System. Reads were aligned to the human reference genome (hg19) using Novoalign v3.02.13 (http://www.novocraft.com/).

## Single nucleotide variant (SNV) and copy number variation (CNV) analysis

Germline variants in exons and at canonical splice sites (± 2 bp) were selected based on autosomal dominant and X-linked models considering de novo occurrence. Simultaneously, rare variants, assuming autosomal recessive inheritance were also considered. These variants were absent from our in-house exome database ($n = 575$) and the genome aggregation database (https://gnomad.broadinstitute.org/) (or at extremely low frequency if recessive). Somatic variants were extracted using MuTect2 (Cibulskis et al. 2013) and Varscan2 (Koboldt et al. 2012). We focused on variants detected by both programs. Candidate somatic variants were selected if they were covered by at least three or more reads. Known gene variants which have been previously reported in neurocutaneous syndrome-related phenotype on the Human Gene Mutation Database (HGMD) or the Catalogue of Somatic Mutations in Cancer (COSMIC) databases were prioritized among candidates with > 4% of variant allele frequencies (VAF). Candidate variants were validated by Sanger sequencing. Primers were designed using Primer3Plus, a web tool for designing primers (primer information is available on request) and PCR was performed using TaKaRa Ex Taq DNA Polymerase with 30 cycles of PCR. CNV was detected using eXome Hidden Markov Model (XHMM) and Nord's program as described previously (Miyatake et al. 2015; Tsuchida et al. 2018; Uchiyama et al. 2021).

# Results

## ES analysis

ES identified plausible monogenic variants in all patients (11/11) (Fig. 1). The candidate variants information is summarized in Table 1 and Supplementary Table 1. The mean read depths of the protein coding sequences were covered with 40 reads or more. De novo somatic *MTOR* variants were identified in four patients [Patient 1, NM_004958.4: c.4448G>A, p.(Cys1483Tyr); Patient 2, c.5930C>T, p.(Thr1977Ile); Patient 3, c.6644C>T, p.(Ser2215Phe), Patient 4, c.7292T>C, p.(Leu2431Pro)]. Of these, three were exclusively detected in skin lesions, and one was identified in both skin and blood samples (Table 2). The somatic VAF of the *MTOR* mutant allele detected in skin in Patients 1, 2, 3, and 4 were 26.6% (20/70 reads), 21.3% (10/43 reads), 11.4% (6/62 reads), and 22.8% (8/35 reads), respectively. In Patient 4, the *MTOR* mutant read was also detected in the blood sample (5/28, 17.8%). All mosaic variants were confirmed to have signal peaks compared with wild-type variants in Sanger sequencing electropherograms (Supplementary Fig. 1). A somatic *RHOA* variant [NM_001664.4:c.139G>A, p.(Glu47Lys)] was found in the skin of Patient 5, with a VAF of 25.6%; this has been previously reported (Yigit et al. 2020). As for germline variants, de novo null variants in *USP9X* were found in two patients [Patient 7, NM_001039590.3: c.355dupT, p.(Arg121Profs*2); Patient 8, c.5834G>A, p.(Trp1945*)]. De novo *TFE3* and *KCNQ5* variants were identified in Patient 6 [NM_006521.6:c.560C>T, p.(Thr187Met)] and Patient 10 [NM_001160133.2:c.1477C>T, p.(Arg439Trp)], respectively. Patient 6 has been previously reported (Lehalle et al. 2020). A pathogenic *PHF6* variant [NM_032458.3: c.821G>A, p.(Arg274Gln)] was maternally inherited in Patient 9, who presented hyperpigmented skin, rather than the usual hypopigmentation pattern. In Patient 11, we could neither identify de novo mosaic variants in the skin, nor any de novo germline variants. However, trio-based ES of blood DNA revealed biallelic *GTF3C5* variants [NM_001286709.2: c.1160A>G; 1517G>T, p.(Lys387Arg); p.(Gly513Val)] as the candidates for this neurocutaneous phenotype.

## Clinical data

Clinical photographs and images from brain imaging studies of patients are shown in Figs. 2 and 3, and clinical

**Table 1** Genetic and clinical information of patients with hypomelanosis of Ito

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 | Patient 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gender | F | M | F | F | F | F | F | F | F | F | M |
| Age (years) | 7 | 7 | 2 | 37 | 12 | 11 | 4 | 18 | 5 | 4 | 12 |
| Gene | MTOR | MTOR | MTOR | MTOR | RHOA | TFE3 | USP9X | USP9X | PHF6 | KCNQ5 | GTF3C5 |
| Reference cDNA | NM_004958.4 | NM_004958.4 | NM_004958.4 | NM_004958.4 | NM_001664.4 | NM_006521.6 | NM_001039590.3 | NM_001039590.3 | NM_032458.3 | NM_001160133.2 | NM_001286709.2 |
| Variant | c.4448G>A, p.(Cys1483Tyr) | c.5930C>T, p.(Thr1977Ile) | c.6644C>T, p.(Ser2215Phe) | c.7292 T>C, p.(Leu2431Pro) | c.139G>A, p.(Glu47Lys) | c.560C>T, p.(Thr187Met) | c.355dupT, p.(Arg121Profs*2) | c.5834G>A, p.(Trp1945*) | c.821G>A, p.(Arg274Gln) | c.1477C>T, p.(Arg439Trp) | c.1160A>G, p.(Lys387Arg)/ c.1517G>T, p.(Gly513Val) |
| Inheritance | De novo | De novo | De novo | De novo | De novo | De novo | De novo | De novo | Maternal | De novo | Parental |
| Germline/somatic | Somatic mosaicism (only in skin) | Somatic mosaicism (only in skin) | Somatic mosaicism (only in skin) | Somatic mosaicism (blood and skin) | Somatic mosaicism (only in skin) | Germline | Germline | Germline | Germline | Germline | Germline |
| *Birth information* | | | | | | | | | | | |
| Gestation | 40 weeks | 38 weeks | 40 weeks | 37 weeks | 38 weeks | 36 weeks | 39 weeks | 38 weeks | 38 weeks | 36 weeks | 35 weeks |
| Birth weight | 2.73 kg (−1.17 SD) | 2.996 kg (+0.22 SD) | 3.552 kg (+0.98 SD) | 3.710 kg (+3.53 SD) | 2.562 kg (−0.45 SD) | 2.674 kg (+1.0 SD) | 2.188 kg (−2.15 SD) | 3.108 kg (+0.6 SD) | 2.258 kg (−1.67 SD) | 3.01 kg (+1.68 SD) | 2.114 kg (−0.69 SD) |
| Birth length | 48.5 cm (−0.78 SD) | 50.5 cm (+1.28 SD) | 51.6 cm (+1.02 SD) | 52.0 cm (+2.58 SD) | 49.0 cm (+0.46 SD) | 46.0 cm (−0.1 SD) | 45.5 cm (−1.69 SD) | 50 cm (+0.8 SD) | 47 cm (−0.55 SD) | 47.0 cm (+0.36 SD) | 48.0 cm (+1.36) |
| OFC | 33.5 cm (−0.10 SD) | 32.5 cm (−0.38 SD) | 35.0 cm (+1.12 SD) | 33.5 cm (+0.63 SD) | 32.0 cm (−0.74 SD) | 31.0 cm (−0.72 SD) | 33.7 cm (+0.37 SD) | 33 cm (−0.1 SD) | – | 32.5 cm (+0.28 SD) | 33.5 cm (+1.34) |
| *Data of the last measurement* | | | | | | | | | | | |
| Height | 97.5 cm (−2.05 SD) | 117.7 cm (−0.4 SD) | 84.7 cm (+1.6 SD) | 152.1 cm (−1.3 SD) | 147.0 cm (−1.1 SD) | 125 cm (−2.7 SD) | 91.9 cm (−1.11 SD) | 143.8 cm (−2.7 SD) | 100 cm (−1.59 SD) | 100.6 cm (−0.3 SD) | 123.3 cm (−3.4 SD) |
| Weight | 17.4 kg (0 SD) | 29.3 kg (+1.5 SD) | 8.725 kg (−1.4 SD) | 68.8 kg (+3.2 SD) | – | 33 kg (−0.5 SD) | 12.9 kg (−0.74 SD) | 39.8 kg (−1.7 SD) | 9.23 kg (−3.4 SD) | 19.8 kg (+1.7 SD) | 15.4 kg (−2.8 SD) |
| OFC | – | 54 cm | 43.7 cm (−2.0 SD) | 58.8 cm (+2.9 SD) | – | 55 cm | 48.0 cm (−0.47 SD) | 51.3 cm | 44.0 cm (−3.76 SD) | 48.0 cm (−1.1 SD) | 51.0 cm |
| Body/limb asymmetry | – | +(right > left) | – | – | + | + (Larger right foot than left) | – | – | – | – | – |
| Developmental delay | + DQ38 | + KABC-II 68 (62–76) | + | + Severe | – | + | + | − IQ 96 (8 yr 5 mo) | +DQ19 | +Mild | + Profound |
| Delay in walking | + | – | + | +4.5 yr | – | +No walk (kneeling walk) | + | – | + | +Instability | +No walk |
| Delay in speech | + | – | + | +No speech achieved | – | + | + | + | + | + | +No speech achieved |
| Epilepsy/seizure | + | + | + | + | – | + | – | – | – | – | + |
| First episode of seizure | Afebrile seizure at 4 mo | Febrile seizure at 10 mo Afebrile seizure at 4 yr | 10 days | 6 yr | – | 5 yr (only once) | – | – | – | – | Afebrile seizure at 1 yr 6 mo |

**Table 1** (continued)

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 | Patient 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Antiepileptic drug | PB, LEV, PER | CBZ, LEV, LCM | ZNS, PB, KBr | CBZ | – | – | – | – | – | – | TPM, KBr, CBZ, LEV, CLB |
| Brain imaging study | Left hemisphere slightly smaller than right, mild focal cortical dysplasia (suspected) | Unclear cortex at bilateral occipital lobes, focal cortical dysplasia (suspected) | Right hemisphere slightly larger than left, mild focal cortical dysplasia suspected | Pituitary adenoma | Multicystic periventricular white matter, mild ventriculomegaly | W.N.L. | W.N.L. | W.N.L. | Atrophy of the bridge capsules and superior cerebellar peduncles | W.N.L. | Polymicrogyria, mildly enlarged ventricules, left hippocampal sclerosis, loss of white matter volume |
| Vision anomalies | – | Different colors of the iris | Strabismus | Strabismus | – | Bilateral mild ptosis | Hyperopia, astigmatism | Mild hypopigmentation of the iris | Bilateral corneal clouding, left eye moving impairment | Bilateral ptosis, strabismus, retinal degeneration | Strabismus |
| Hearing loss | – | – | – | – | – | – | – | Conductive hearing loss | – | – | – |
| Scoliosis | – | + | – | + | – | + | + | – | – | – | + |
| Distinctive craniofacial features | Thick lips, depressed nasal ridge | – | – | Thick eyebrow, thick nasal alae, square face, micrognathia | – | Thick eyebrow, depressed nose, anteverted nares, hypertrichosis, thick lips, epicanthus, thick earlobes | + | Thick nasal alae, square face | Left facial palsy, micrognathism, auricular hypoplasia, corneal flab | Ptosis, broad forehead | Hypertelorism, broad forehead, ptosis |
| Skin pigmentation anomalies | + | + | + | + | + | + | + | + | + Hyperpigmentation | + | + |
| -localization | Back from lumbar to thighs | Right half of the body | Left neck to upper left arm and forearm | Body trunk | Upper left shoulder and arm and lower left buttock to knee | Back and posterior thighs | Whole body (face, trunk, and extremities) | Bilateral forearms (ulnar side), bilateral popliteal fossa | Trunk and extremities | Bilateral lower legs | Whole body |
| Dental abnormalities | – | + | – | – | + | – | + Anodontia | + Malalignment of teeth | + | – | – |
| Deformity of fingers or toes | – | – | – | – | + | – | – | – | + | – | – |
| Hirsutism | – | – | – | – | – | + | – | – | + | + | – |

**Table 1** (continued)

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 | Patient 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Respiratory | – | – | – | – | – | – | Laryngomalacia, nasal stenosis, neonatal respiratory distress | Occlusion of posterior nares, Exudative otitis media | – | Tracheomalacia | Neonatal respiratory distress, pneumothorax, mediastinal emphysema |
| Cardiac anomalies | – | – | – | – | – | – | + VSD (spontaneous closure) | – | – | + ASD, Ebstein's anomaly (operated) | – |
| Genital anomalies | – | – | – | Amenorrhea due to hyperprolactinemia | – | – | – | Precocious puberty | – | – | Right inguinal hernia (operated), left migratory testis (operated) |
| Genetic testing prior to ES | 46,XX | – | – | 46,XX | 46,XX | 46,XX | 46,XX | 46,XX | 46,XX, no variants in NEMO | 46,XX | – |
| Others | – | ADHD | – | Compatible with Smith–Kingsmore syndrome, autism, psychotropic drugs | – | – | Umbilical hernia, transient hypothyroidism, torticollis, hospitalization in NICU until day 111 due to neonatal asphyxia and respiratory distress | Spike and slow wave complex on electroencephalogram | – | – | Delayed bone age, distal femur fracture at 9 yr |

The transcript (RefSeq mRNA number): *MTOR* [NM_004958.3], RHOA [NM_001664.4], *TFE3* [NM_006521.5], *USP9X* [NM_001039590.2], *PFH6* [NM_032458.2], *KCNQ5* [NM_001160133.1], *GTF3C5* [NM_012087.4]

*OFC* occipitofrontal circumference, *ES* exome sequencing, *SD* standard deviation, *DQ* development quotient, *KABC-II* Kaufman Assessment Battery for Children, *IQ* intelligence quotient, *VSD* ventricular septal defect, *ASD* arterial septal defect, *ADHD* attention-deficit/hyperactivity disorder, *NICU* neonatal intensive care unit, *PB* phenobarbital, *LEV* levetiracetam, *PER* perampanel, *CBZ* carbamazepine, *LCM* lacosamide, *ZNS* zonisamide, *KBr* potassium bromide, *TPM* topiramate, *CLB* clobazam, *W.N.L.* within normal limits

**Table 2** Characterization of pathogenic variants

| Patient | Gene | Variant | Amino acid change | Chr | Position | Mutation type | VAF in blood | VAF in skin | SIFT | Polyphen2 | CADD | gnomAD | COSMIC | ACMG guideline | Known/Novel |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | MTOR | c.4448G>A | p.(Cys-1483Tyr) | 1 | 11217230 | Missense | 0% (0/70 reads) | 26.6% (20/75 reads) | 0 | 0.995 | 32 | None | Yes | Pathogenic | Known |
| 2 | MTOR | c.5930C>T | p.(Thr1977Ile) | 1 | 11188164 | Missense | 0% (0/52 reads) | 21.3% (10/47 reads) | 0.01 | 0.999 | 32 | None | Yes | Pathogenic | Known |
| 3 | MTOR | c.6644C>T | p.(Ser2215Phe) | 1 | 11184573 | Missense | 0% (0/60 reads) | 11.4% (6/62 reads) | 0 | 0.984 | 30 | None | Yes | Pathogenic | Known |
| 4 | MTOR | c.7292T>C | p.(Leu-2431Pro) | 1 | 11174383 | Missense | 17.8% (5/28 reads) | 22.8% (8/35 reads) | 0 | 1 | 33 | None | Yes | Pathogenic | Known |
| 5 | RHOA | c.139G>A | p.(Glu47Lys) | 3 | 49412884 | Missense | 0% (0/37 reads) | 29.6% (8/27 reads) | 0.01 | 0.996 | 31 | None | Yes | Pathogenic | Known |
| 6 | TFE3 | c.560C>T | p.(Thr187Met) | X | 48895942 | Missense | 47.6% (39/82 reads) | – | 0 | 0.997 | 26 | None | None | Pathogenic | Known |
| 7 | USP9X | c.355dupT | p.(Arg-121Profs*2) | X | 40994009 | Frameshift | 62.5% (25/40 reads) | 53.5% (15/28 reads) | – | – | – | None | None | Pathogenic | Novel |
| 8 | USP9X | c.5834G>A | p.(Trp1945*) | X | 41075654 | Nonsense | 48.0% (50/104 reads) | – | – | – | 45 | None | None | Pathogenic | Novel |
| 9 | PHF6 | c.821G>A | p.(Arg274Gln) | X | 133549137 | Missense | 50.0% (30/60 reads) | – | 0 | 1 | 35 | None | Yes | Pathogenic | Novel |
| 10 | KNCQ5 | c.1477C>T | p.(Arg439Trp) | 6 | 73843316 | Missense | 48% (25/52 reads) | 55% (33/60 reads) | 0 | 1 | 34 | None | None | Likely Pathogenic | Novel |
| 11 | GTF3C5 | c.1160A>G | p.(Lys387Arg) | 9 | 135930189 | Missense | 41.6% (15/36 reads) | 44.4% (20/45 reads) | 0.03 | 0.991 | 25 | None | None | VUS | Novel |
| | | c.1517G>T | p.(Gly513Val) | 9 | 135933324 | Missense | 40.0% (10/25 reads) | 45.0% (14/31 reads) | 0 | 1 | 27.6 | 0.00034 | None | VUS | Novel |

The transcript (RefSeq mRNA number): *MTOR* [NM_004958.3], *RHOA* [NM_001664.4], *TFE3* [NM_006521.5], *USP9X* [NM_001039590.2], *PFH6* [NM_032458.2], *KCNQ5* [NM_001160133.1], *GTF3C5* [NM_012087.4]

*VAF* variant allele frequency, *SIFT* sorting intolerant from tolerant, *CADD* combined annotation dependent depletion, *gnomAD* genome aggregation database, *COSMIC* catalogue of somatic mutations in cancer, *VUS* variant of uncertain significance
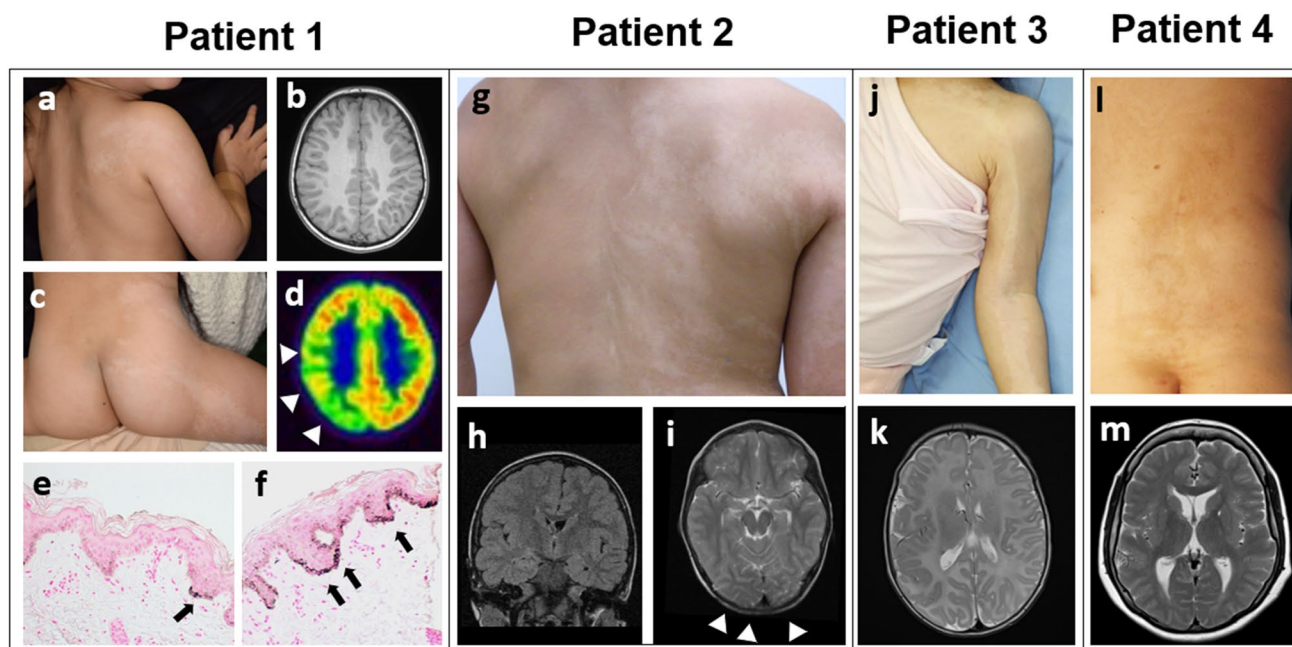
**Fig. 2** Skin lesion photographs and brain magnetic resonance imaging (MRI) studies of patients 1–4. **a–f** Patient 1 with a somatic *MTOR* somatic variant (c.4448G>A, p.Cys1483Tyr). **a, c** Hypopigmentation of the back and buttocks. **b** Axial view of T1-weighted MRI images of the brain. The brain is almost symmetrical, but the right hemisphere is slightly larger than the left. **d** Axial image of brain positron emission tomography (PET). The signals are significantly decreased in the right hemisphere (arrowhead). **e, f** Fontana–Masson stained skin biopsy of a depigmented lesion. Arrow indicates clusters of melanin-laden keratinocytes (brown areas), which are sparse in the hypopigmented skin compared to a normally pigmented skin lesion, although the number of melanocytes is not reduced (**f**). **g** Skin lesion of Patient 2. Hypopigmentation of the skin is seen only in the right side of the body. **h, i** T2-weighted coronal and axial images of brain MRI of Patient 2 with a somatic *MTOR* variant (c.5930C>T, p. Thr1977Ile). The right hemisphere is slightly larger than the left. The arrowheads show focal cortical dysplasia in bilateral occipital lobes, but predominantly in the right hemisphere. **j** Skin lesion of Patient 3 with a somatic *MTOR* variant (c.6644C>T, p.Ser2215Phe). **k** T2-weighted MRI brain finding of Patient 3 at day 21 after birth prior to surgery. Right hemimegalencephaly is observed in many slices. **l, m** Skin lesions and T2-weighted brain MRI of Patient 4 with a somatic *MTOR* variant (c.7292 T>C, p.Leu2431Pro) found in both skin lesion and blood leukocytes, respectively. Brain MRI of Patient 4 is normal and almost symmetrical

information is described in detail in Table 1 and in the Supplementary Information. Of the 11 patients, 9 (81.8%) exhibited intellectual disability. Patient 5 with a mosaic *RHOA* variant developed normally, whereas Patient 8 who had a germline *USP9X* variant had delayed developmental milestones (such as head control at 6 months, sitting at 11 months, walking at 17 months, and speaking a word at 2 years), but reached to the normal level of development by 6 years of age. Of the 11 patients, 7 (63.6%) had epilepsy, 6 (54.5%) exhibited brain abnormalities, 5 (45.5%) had dental anomalies, 3 (27.2%) experienced body/limb asymmetry, and another 3 (27.2%) demonstrated distinctive facial features. Macrocephaly was observed in only one case (Patient 4 with a somatic *MTOR* variant in blood and skin samples).

All of the four patients with somatic *MTOR* variants were diagnosed with epilepsy and developmental delay of varied severity after birth in addtion to skin hypopigmentation (Fig. 2 a, c, g, j, l). Patient 1 had a slightly enlarged right hemisphere on brain magnetic resonance imaging (MRI) (Fig. 2b). In a brain positron emission tomography scan, signals were significantly decreased in the right hemisphere (Fig. 2d). Patient 2 presented with unclear bilateral occipital cortexes (especially in the right) (Fig. 2i), but was able to walk and speak; therefore, his phenotype was considered relatively mild. Afebrile seizures occurred at 4 years, which were not improved with levetiracetam and lacosamide but were eventually well controlled with carbamazepine. Patient 3 had seizures 10 days after birth and exhibited severe developmental delay; thus, brain surgery was required to control refractory seizures. Brain MRI indicated that Patient 3 had apparent right hemimegalencephaly and focal cortical dysplasia (FCD) in the right occipital lobe, which was surgically corrected (Fig. 2k). Patient 4 showed macrocephaly, severe developmental delay, and epilepsy but without FCD or hemimegalencephaly on brain MRI (Fig. 2m), which is indicative of Smith–Kingsmore syndrome.

Patient 5 with a mosaic *RHOA* variant showed skin hypopigmentation (Fig. 3a) along with abnormally high MRI signals and cysts near the right ventricle (Fig. 3b and c). Almost all patients demonstrated skin hypopigmentation (Fig. 3a,
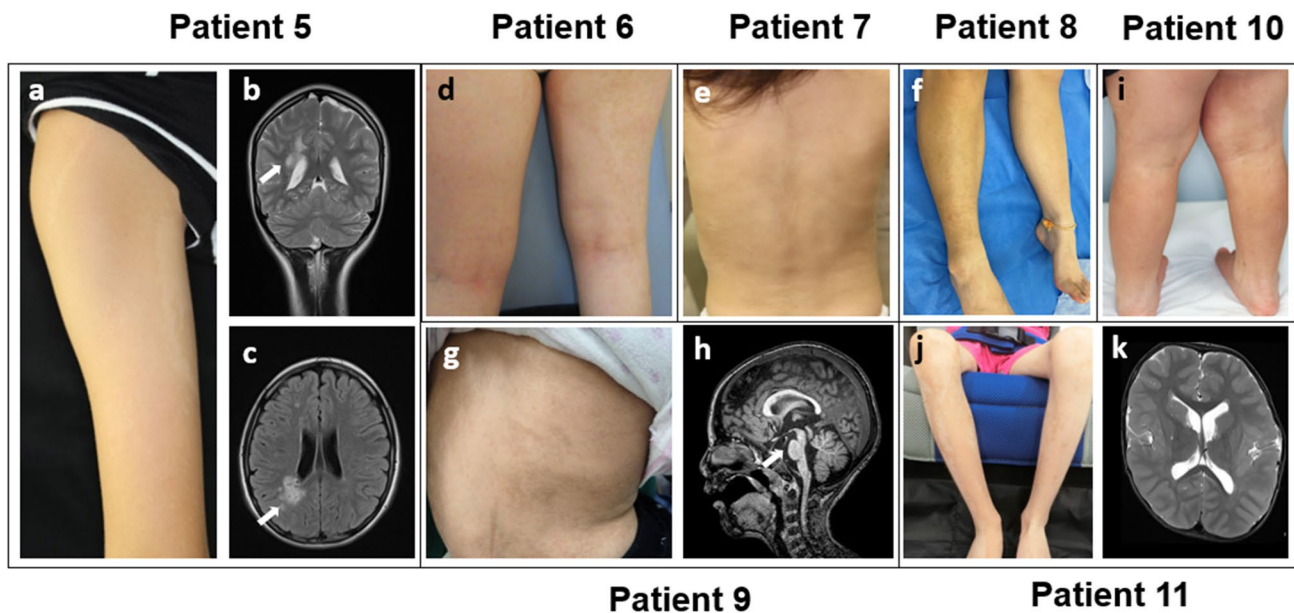
**Fig. 3** Skin lesion photographs and brain magnetic resonance imaging (MRI) studies of patients 5–11. **a** Hypopigmentation in the left leg of Patient 5 with a somatic *RHOA* variant. **b, c** Saggital and axial views of T2-weighted brain MRI, respectively. No laterality is observed. Hyperintensity with mild dilatation of ventricles and small cysts are observed. **d** Skin lesion of Patient 6 with a germline *TFE3* variant. **e, f** Skin lesions of Patients 7 and 8, respectively, both with a germline *USP9X* variant. **g, h** Skin lesion and sagittal view of T2-weighted brain MRI of Patient 9 with a germline *PHF*6 variant. The patient's skin showed hyperpigmentation rather than hypopigmentation. Atrophy of the bridge capsules and superior cerebellar peduncles is observed. **i** Skin lesion of Patient 10 with a germline *KCNQ5* variant. **j, k** Skin lesions and sagittal view of T2-weighted brain MRI of Patient 11. Brain MRI showed polymicrogyria, loss of white matter volume, and mildly enlarged lateral ventricles

d–f, i, j) except Patient 9, who had a *PHF6* variant and demonstrated skin hyperpigmentation (Fig. 3g). The *PHF6* variant was inherited from the patient's mother who also had hyperpigmented skin. The familial pedigree of Patient 9 with genetic data is shown in Supplementary Fig. 2. Regarding a *PHF6* variant, there was a report demonstrating grandparental genotyping was useful for interpreting ES results (Daum et al. 2020). We confirmed that the maternal grandmother did not possess a *PHF6* variant. Unfortunately, a sample derived from the maternal grandfather was unavailable for confirmation. Neither grandparent exhibited hyperpigmentation or any other symptoms; therefore, the mother's *PHF6* variant may have occurred de novo. Patient 11 was born at 35 weeks of gestation by cesarean section because of oligohydramnios. He was noted to have HI after birth (Fig. 3j), refractory epilepsy at 1 year, and severe growth and developmental delay. The family pedigree of Patient 11 with genetic data is shown in Supplementary Fig. 2.

## Discussion

We identified plausible monogenic causes of pigmentary mosaicism (mostly HI) in all 11 patients examined (Fig. 1). All causal genes identified in this study are highly intolerant to variation (high pLI, LOEUF, and z scores) except for the autosomal recessive *GTF3C5* variants (Table 3). The high rate of monogenic cause of pigmentary mosaicism in this study was unexpected, but may be reasonable, considering that previous genetic investigations of pigmentary mosaicism were performed using only chromosomal analysis and/or array-based comparative genomic hybridization (without ES) (Kromann et al. 2018; Salas-Labadia et al. 2019). We propose that the exome-first strategy, preferably using blood- and skin-derived DNA, should be adopted for HI because it is a neuroectodermal disorder.

De novo somatic variants were identified in almost half cases (45%, five of 11 patients). Epidermal tissue contains both keratinocytes and melanocytes. It is well known that melanocytes are neuroectoderm derived. The neural tube (differentiating to neuronal cells in the central nervous system) and neuronal crest cells (to melanocytes) are derived from the neuroectoderm (Li et al. 2020). Thus, skin hypopigmentation could accompany the brain phenotype, depending on when somatic variants arise.

Postzygotic pathogenic variants in *MTOR* cause FCD (MIM# 607341), hemimegalencephaly, and Smith–Kingsmore syndrome (MIM# 616638). Among the 11 patients in this study, somatic *MTOR* variants were the most prevalent ($n = 4$, 36.3%), and among the four variants, three (Patients

**Table 3** Genes which can cause pigmentary mosaicism

|  | RHOA | MTOR | TFE3 | USP9X | PHF6 | KCNQ5 | GTF3C5 |
|---|---|---|---|---|---|---|---|
| Mis-Z score | 3.36 | 7.02 | 2.15 | 6.41 | 2.28 | 3.32 | 0.77 |
| pLI score | 1 | 1 | 0.98 | 1 | 1 | 0.98 | 0 |
| LOEUF | 0.66 | 0.18 | 0.29 | 0.05 | 0.17 | 0.31 | 0.89 |
| ExAC_pRec | 0.1901 | 0.0000000000003 | 0.0262 | 0.0000000000236 | 0.0276 | 0.000464913 | 0.994 |
| Inheritance | De novo | De novo | XLD | XLD | XLD/XLR | De novo | AR |
| Somatic/germline | Somatic | Somatic/Germline | Somatic/Germline | Germline | Germline | Germline | Germline |
| Type of skin pigmentation | Hypopigmentation | Hypopigmentation | Hypopigmentation | Hypopigmentation | Hyperpigmentation | Hypopigmentation | Hypopigmentation |

*pLI* tolerance of a given gene to the loss of function, *LOEUF* loss-of-function observed/expected upper bound fraction, *ExAC* Exome Aggregation Consortium, *XLD* X-linked dominant, *XLR* X-linked recessive, *AR* autosomal recessive

1–3) were exclusively observed only in skin DNA, indicating that the variants may have occurred later in the postzygotic period (after epidermal differentiation), thus, only affecting the skin cells, while Patient 4 with Smith–Kingsmore syndrome had a variant in both skin and blood, indicating earlier occurrence in the postzygotic period (before epidermal differentiation). According to a review of Gordo et al. (2018), HI was observed in 14.8% (4/27) of patients with Smith–Kingsmore syndrome. Recently, *MTOR*-related HI in 14 (19.7%) of 71 patients with pigmentary mosaicism was reported (Carmignac et al. 2021), indicating that *MTOR* is the most common cause of pigmentary mosaicism. *MTOR* variants are often mosaic; therefore, skin biopsy is highly recommended for a conclusive diagnosis. All *MTOR* variants detected in this study have been previously known, and are all registered in COSMIC database as pathogenic somatic variants in cancers. The c.4448G>A, p.(Cys1483Tyr) variant in Patient 1 has been recurrently reported and is implicated as a cause of hemimegalencephaly and FCD or Smith–Kingsmore syndrome (D'Gama et al. 2015; Gordo et al. 2018). The c.5930C>T, p. (Thr1977Ile) variant found in Patient 2 has also been frequently reported as a cause of FCD and megalencephaly with pigmentary mosaicism (Carmignac et al. 2021; Handoko et al. 2019; Mirzaa et al. 2016). Interestingly, Handoko et al. (2019) confirmed that all four patients with c.5930C>T, p.(Thr1977Ile) showed cutaneous hypopigmentation along Blaschko's lines. These two variants [c.4448G>A, p.(Cys1483Tyr) and c.5930C>T, p.(Thr1977Ile)] have been reported as either somatic or germline variant. The c.6644C>T, p.(Ser2215Phe) variant in Patient 3 has also been indicated as a cause of FCD or hemimegalencephaly (D'Gama et al. 2017; Moller et al. 2016), while the c.7292 T>C, p.(Leu2431Pro) variant in Patient 4 has never been reported as the cause of such condition (previously reported only in cancer). Somatic *MTOR* variants were first described as the cause of FCD requiring brain surgery to control epilepsy (Lim et al. 2015; Nakashima et al. 2015). Three patients in our cohort with *MTOR* somatic

variants (except patient 3) did not require brain surgery, indicating that such variants can cause a relatively mild epilepsy phenotype. The VAF of *MTOR* variants in skin lesions was approximately 10–30%, which is consistent with that of a previous report regardless of the disease severity (Carmignac et al. 2021). The VAF of *MTOR* variants in skin lesions was considerably higher than that in FCD and hemimegalencephaly lesions, and it is usually less than 10% (sometimes 1–3% or less). This is advantageous for performing ES analysis of skin-derived DNA because deep sequencing may not be essential. As for patients with *MTOR* variants, mTOR inhibitors such as sirolimus and everolimus are potential treatment options.

Pigmentary mosaicism caused by somatic *RHOA* variants is usually associated with normal neurodevelopment, although apparent brain lesions can be detected on MRI, as in Patient 5 in this cohort (Vabres et al. 2019; Yigit et al. 2020). Previous reports have included patients with clinical manifestations compatible with this unique HI, but their genetic status was not confirmed (Inoue et al. 2015; Mateus et al. 2014). In two previous studies, the genetic cause of this type of HI in 9 of 11 patients (9/11) was a recurrent somatic *RHOA* variant, c.139G>A, p.(Glu47Lys), and is therefore called "*RHOA*-related mosaic ectodermal dysplasia" (Vabres et al. 2019; Yigit et al. 2020). *RHOA* variants are also frequently registered in the COSMIC database.

Pathogenic germline variants were found in three X-linked genes, *TFE3* (transcription factor E3), *USP9X* (ubiquitin-specific protease 9, MIM*300072), and *PHF6* (PHD finger protein 6, MIM*300414), which are consistent with the association of HI with structural abnormalities of the X chromosome. Germline or somatic *TFE3* variants cause intellectual disability with pigmentary mosaicism and storage disorder-like features in both males and females (Lehalle et al. 2020). TFE3 functions in the mechanistic target of rapamycin (mTOR) complex 1 signaling in the PIK3–AKT–mTOR pathway, which can explain the association of these variants with pigmentary mosaicism similar

to *MTOR* variants. Lehalle et al. (2020) found pigmentary mosaicism to be present in the majority of patients with *TFE3* mutations (71%, 12/17), including 40% of the males and 83% of the females. *USP9X* and *PHF6* variants cause distinct neurodevelopmental disorders with congenital anomalies in both sexes. X-linked recessive (MIM# 300919) and dominant (MIM# 300968) intellectual developmental disorders are caused by *USP9X* variants, whereas Borjeson–Forssman–Lehmann syndrome (BFLS, MIM# 301900) is caused by *PHF6* variants. However, pigmentary mosaicism was only seen in female patients with abnormalities in these two genes (Jolly et al. 2020; Reijnders et al. 2016; Zhang et al. 2019). The rates of skin pigment complication in females related to *USP9X* and *PHF6* variants were 65% (11/17) (Reijnders et al. 2016) and 77% (10/13) (Zhang et al. 2019), respectively. Skin lesions in females with aberrant *USP9X* and *PHF6* manifest as hypopigmentation and hyperpigmentation along Blaschko's lines, respectively. USP9X plays an important role associated with mTOR in neural development both in humans and mice (Agrawal et al. 2012; Murtaza et al. 2015; Reijnders et al. 2016), which may explain how this variant causes HI. PHF6 is implicated in chromatin remodeling by interacting with the SWI/SNF complex (Zhang et al. 2019; Zweier et al. 2013), the variants of which are occasionally found in patients with Coffin–Siris syndrome (MIM# 135900) (Zweier et al. 2013). In addition, pathogenic variants in *USP9X* and *PHF6* are also found in cancers such as pediatric leukemia (Ma et al. 2018), which is similar to the reports of *MTOR* and *RHOA* variants being frequently detected in various cancers (Kilian et al. 2021; Mirzaa and Poduri 2014; Zou et al. 2020).

Germline *KCNQ5* variants cause intellectual disability or epilepsy (Lehman et al. 2017), and only a few individuals with de novo *KCNQ5* variants have been reported. Skin hypopigmentation in Patient 10 with a de novo *KCNQ5* variant is indeed a novel phenotypic feature, which has not previously been reported in association with *KCNQ5* abnormality. Therefore, it remains uncertain whether the *KCNQ5* variant is related to the skin phenotype. *GTF3C5 (MIM*604890)* which encodes general transcription factor IIIC subunit 5 (GTF3C5), plays a crucial role in general transcription (Crepaldi et al. 2013); however, *GTF3C5* abnormalities have never been reported in human diseases. Homozygous mutant *Gtf3c5*$^{tm2a(KOMP)Wtsi}$ mice exhibited complete preweaning lethality, while heterozygous mutant mice survived (Ayadi et al. 2012). The biallelic *GTF3C5* variants may potentially cause skin hypopigmentation based on the high probability score (0.99) of gene intolerance to the biallelic loss of function (Lek et al. 2016). Further studies involving patients with *GTF3C5* variants are required to confirm the association of *GTF3C5* with this human ectodermal disorder. It is important to study and analyze more patients harboring *KCNQ5*

and *GTF3C5* variants and collect further evidence before establishing any conclusion.

HI is thought to be caused by somatic chromosomal structural abnormalities, but these structural abnormalities have been inconsistent, and genetic testing for diagnosis has not been actively performed until recently. Moreover, HI has sometimes been considered as a benign phenotype rather than a disease (Ruggieri and Pavone 2000; Sybert 1994). However, the high discovery rate of monogenic causes of HI prompts us to perform ES for patients with pigmentary mosaicism, preferably by collecting skin samples, as this may lead to the optimal therapeutic approaches in the future. Although using skin tissue instead of brain tissue may allow regular ES with 40–70× read coverage for reliably detecting somatic mosaic variants with > 4% of VAF in pigmentary mosaicism, higher read coverage in the NGS analysis is better to avoid missing low-prevalent (≦ 4%) variants.

## Declarations

# References

Agrawal P, Chen YT, Schilling B, Gibson BW, Hughes RE (2012) Ubiquitin-specific peptidase 9, X-linked (USP9X) modulates activity of mammalian target of rapamycin (mTOR). J Biol Chem 287:21164–21175. https://doi.org/10.1074/jbc.M111.328021

Ayadi A, Birling MC, Bottomley J, Bussell J, Fuchs H, Fray M, Gailus-Durner V, Greenaway S, Houghton R, Karp N, Leblanc S, Lengger C, Maier H, Mallon AM, Marschall S, Melvin D, Morgan H, Pavlovic G, Ryder E, Skarnes WC, Selloum M, Ramirez-Solis R, Sorg T, Teboul L, Vasseur L, Walling A, Weaver T, Wells S, White JK, Bradley A, Adams DJ, Steel KP, Hrabe de Angelis M, Brown SD, Herault Y (2012) Mouse large-scale phenotyping initiatives: overview of the European Mouse Disease Clinic (EUMODIC) and of the Wellcome Trust Sanger Institute Mouse Genetics Project. Mamm Genome 23:600–610. https://doi.org/10.1007/s00335-012-9418-y

Barbel P, Brown S, Peterson K (2015) Identification of hypomelanosis of Ito in pediatric primary care. J Pediatr Health Care 29:551–554. https://doi.org/10.1016/j.pedhc.2015.01.002

Carmignac V, Mignot C, Blanchard E, Kuentz P, Aubriot-Lorton MH, Parker VER, Sorlin A, Fraitag S, Courcet JB, Duffourd Y, Rodriguez D, Knox RG, Polubothu S, Boland A, Olaso R, Delepine M, Darmency V, Riachi M, Quelin C, Rollier P, Goujon L, Grotto S, Capri Y, Jacquemont ML, Odent S, Amram D, Chevarin M, Vincent-Delorme C, Catteau B, Guibaud L, Arzimanoglou A, Keddar M, Sarret C, Callier P, Bessis D, Genevieve D, Deleuze JF, Thauvin C, Semple RK, Philippe C, Riviere JB, Kinsler VA, Faivre L, Vabres P, Mosaique PN (2021) Clinical spectrum of MTOR-related hypomelanosis of Ito with neurodevelopmental abnormalities. Genet Med. https://doi.org/10.1038/s41436-021-01161-6

Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, Gabriel S, Meyerson M, Lander ES, Getz G (2013) Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol 31:213–219. https://doi.org/10.1038/nbt.2514

Crepaldi L, Policarpi C, Coatti A, Sherlock WT, Jongbloets BC, Down TA, Riccio A (2013) Binding of TFIIIC to sine elements controls the relocation of activity-dependent neuronal genes to transcription factories. PLoS Genet 9:e1003699. https://doi.org/10.1371/journal.pgen.1003699

Daum H, Mor-Shaked H, Ta-Shma A, Shaag A, Silverstein S, Shohat M, Elpeleg O, Meiner V, Harel T (2020) Grandparental genotyping enhances exome variant interpretation. Am J Med Genet A 182:689–696. https://doi.org/10.1002/ajmg.a.61511

D'Gama AM, Geng Y, Couto JA, Martin B, Boyle EA, LaCoursiere CM, Hossain A, Hatem NE, Barry BJ, Kwiatkowski DJ, Vinters HV, Barkovich AJ, Shendure J, Mathern GW, Walsh CA, Poduri A (2015) Mammalian target of rapamycin pathway mutations cause hemimegalencephaly and focal cortical dysplasia. Ann Neurol 77:720–725. https://doi.org/10.1002/ana.24357

D'Gama AM, Woodworth MB, Hossain AA, Bizzotto S, Hatem NE, LaCoursiere CM, Najm I, Ying Z, Yang E, Barkovich AJ, Kwiatkowski DJ, Vinters HV, Madsen JR, Mathern GW, Blumcke I, Poduri A, Walsh CA (2017) Somatic mutations activating the mTOR pathway in dorsal telencephalic progenitors cause a continuum of cortical dysplasias. Cell Rep 21:3754–3766. https://doi.org/10.1016/j.celrep.2017.11.106

Gordo G, Tenorio J, Arias P, Santos-Simarro F, Garcia-Minaur S, Moreno JC, Nevado J, Vallespin E, Rodriguez-Laguna L, de Mena R, Dapia I, Palomares-Bralo M, Del Pozo A, Ibanez K, Silla JC, Barroso E, Ruiz-Perez VL, Martinez-Glez V, Lapunzina P (2018) mTOR mutations in Smith-Kingsmore syndrome: four additional patients and a review. Clin Genet 93:762–775. https://doi.org/10.1111/cge.13135

Hadouiri N, Darmency V, Guibaud L, Arzimanoglou A, Sorlin A, Carmignac V, Riviere JB, Huet F, Luu M, Bardou M, Thauvin-Robinet C, Vabres P, Faivre L (2020) Compassionate use of everolimus for refractory epilepsy in a patient with MTOR mosaic mutation. Eur J Med Genet 63:104036. https://doi.org/10.1016/j.ejmg.2020.104036

Handoko M, Emrick LT, Rosenfeld JA, Wang X, Tran AA, Turner A, Belmont JW, Undiagnosed Diseases N, Lee BH, Bacino CA, Chao HT (2019) Recurrent mosaic MTOR c.5930C > T (p.Thr1977Ile) variant causing megalencephaly, asymmetric polymicrogyria, and cutaneous pigmentary mosaicism: case report and review of the literature. Am J Med Genet A 179:475–479. https://doi.org/10.1002/ajmg.a.61007

Inoue M, Fukuda M, Ishii E, Sayama K (2015) Linear leukoplakia and central nervous system lesions: a clinical clue to the diagnosis of hypomelanosis of Ito. J Pediatr 167(771):e1. https://doi.org/10.1016/j.jpeds.2015.06.024

Jolly LA, Parnell E, Gardner AE, Corbett MA, Perez-Jurado LA, Shaw M, Lesca G, Keegan C, Schneider MC, Griffin E, Maier F, Kiss C, Guerin A, Crosby K, Rosenbaum K, Tanpaiboon P, Whalen S, Keren B, McCarrier J, Basel D, Sadedin S, White SM, Delatycki MB, Kleefstra T, Kury S, Brusco A, Sukarova-Angelovska E, Trajkova S, Yoon S, Wood SA, Piper M, Penzes P, Gecz J (2020) Missense variant contribution to USP9X-female syndrome. NPJ Genom Med 5:53. https://doi.org/10.1038/s41525-020-00162-9

Kilian LS, Voran J, Frank D, Rangrez AY (2021) RhoA: a dubious molecule in cardiac pathophysiology. J Biomed Sci 28:33. https://doi.org/10.1186/s12929-021-00730-w

Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, Miller CA, Mardis ER, Ding L, Wilson RK (2012) VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res 22:568–576. https://doi.org/10.1101/gr.129684.111

Kouzak SS, Mendes MS, Costa IM (2013) Cutaneous mosaicisms: concepts, patterns and classifications. An Bras Dermatol 88:507–517. https://doi.org/10.1590/abd1806-4841.20132015

Kromann AB, Ousager LB, Ali IKM, Aydemir N, Bygum A (2018) Pigmentary mosaicism: a review of original literature and recommendations for future handling. Orphanet J Rare Dis 13:39. https://doi.org/10.1186/s13023-018-0778-6

Kuster W, Konig A (1999) Hypomelanosis of Ito: no entity, but a cutaneous sign of mosaicism. Am J Med Genet 85:346–350. https://doi.org/10.1002/(sici)1096-8628(19990806)85:4%3c346::aid-ajmg7%3e3.0.co;2-1

Lee HS, Chun YS, Hann SK (1999) Nevus depigmentosus: clinical features and histopathologic characteristics in 67 patients. J Am Acad Dermatol 40:21–26. https://doi.org/10.1016/s0190-9622(99)70524-4

Lehalle D, Vabres P, Sorlin A, Bierhals T, Avila M, Carmignac V, Chevarin M, Torti E, Abe Y, Bartolomaeus T, Clayton-Smith J, Cogne B, Cusco I, Duplomb L, De Bont E, Duffourd Y, Duijkers F, Elpeleg O, Fattal A, Genevieve D, Guillen Sacoto MJ, Guimier

A, Harris DJ, Hempel M, Isidor B, Jouan T, Kuentz P, Koshimizu E, Lichtenbelt K, Loik Ramey V, Maik M, Miyakate S, Murakami Y, Pasquier L, Pedro H, Simone L, Sondergaard-Schatz K, St-Onge J, Thevenon J, Valenzuela I, Abou Jamra R, van Gassen K, van Haelst MM, van Koningsbruggen S, Verdura E, Whelan Habela C, Zacher P, Riviere JB, Thauvin-Robinet C, Betschinger J, Faivre L (2020) De novo mutations in the X-linked TFE3 gene cause intellectual disability with pigmentary mosaicism and storage disorder-like features. J Med Genet 57:808–819. https://doi.org/10.1136/jmedgenet-2019-106508

Lehman A, Thouta S, Mancini GMS, Naidu S, van Slegtenhorst M, McWalter K, Person R, Mwenifumbo J, Salvarinova R, Study C, Study E, Guella I, McKenzie MB, Datta A, Connolly MB, Kalkhoran SM, Poburko D, Friedman JM, Farrer MJ, Demos M, Desai S, Claydon T (2017) Loss-of-function and gain-of-function mutations in KCNQ5 cause intellectual disability or epileptic encephalopathy. Am J Hum Genet 101:65–74. https://doi.org/10.1016/j.ajhg.2017.05.016

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Exome Aggregation C (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature 536:285–291. https://doi.org/10.1038/nature19057

Li M, Knapp SK, Iden S (2020) Mechanisms of melanocyte polarity and differentiation: What can we learn from other neuroectoderm-derived lineages? Curr Opin Cell Biol 67:99–108. https://doi.org/10.1016/j.ceb.2020.09.001

Lim JS, Kim WI, Kang HC, Kim SH, Park AH, Park EK, Cho YW, Kim S, Kim HM, Kim JA, Kim J, Rhee H, Kang SG, Kim HD, Kim D, Kim DS, Lee JH (2015) Brain somatic mutations in MTOR cause focal cortical dysplasia type II leading to intractable epilepsy. Nat Med 21:395–400. https://doi.org/10.1038/nm.3824

Ma X, Liu Y, Liu Y, Alexandrov LB, Edmonson MN, Gawad C, Zhou X, Li Y, Rusch MC, Easton J, Huether R, Gonzalez-Pena V, Wilkinson MR, Hermida LC, Davis S, Sioson E, Pounds S, Cao X, Ries RE, Wang Z, Chen X, Dong L, Diskin SJ, Smith MA, Guidry Auvil JM, Meltzer PS, Lau CC, Perlman EJ, Maris JM, Meshinchi S, Hunger SP, Gerhard DS, Zhang J (2018) Pan-cancer genome and transcriptome analyses of 1699 paediatric leukaemias and solid tumours. Nature 555:371–376. https://doi.org/10.1038/nature25795

Mateus AM, Silva RL, Conceicao C, Vieira JP (2014) Importance of cutaneous changes in the diagnosis of neurological diseases. Einstein (sao Paulo) 12:529–530. https://doi.org/10.1590/S1679-45082014AI2931

Mirzaa GM, Poduri A (2014) Megalencephaly and hemimegalencephaly: breakthroughs in molecular etiology. Am J Med Genet C Semin Med Genet 166C:156–172. https://doi.org/10.1002/ajmg.c.31401

Mirzaa GM, Campbell CD, Solovieff N, Goold C, Jansen LA, Menon S, Timms AE, Conti V, Biag JD, Adams C, Boyle EA, Collins S, Ishak G, Poliachik S, Girisha KM, Yeung KS, Chung BHY, Rahikkala E, Gunter SA, McDaniel SS, Macmurdo CF,

Bernstein JA, Martin B, Leary R, Mahan S, Liu S, Weaver M, Doerschner M, Jhangiani S, Muzny DM, Boerwinkle E, Gibbs RA, Lupski JR, Shendure J, Saneto RP, Novotny EJ, Wilson CJ, Sellers WR, Morrissey M, Hevner RF, Ojemann JG, Guerrini R, Murphy LO, Winckler W, Dobyns WB (2016) Association of MTOR mutations with developmental brain disorders, including megalencephaly, focal cortical dysplasia, and pigmentary mosaicism. JAMA Neurol 73:836–845. https://doi.org/10.1001/jamaneurol.2016.0363

Miyatake S, Koshimizu E, Fujita A, Fukai R, Imagawa E, Ohba C, Kuki I, Nukui M, Araki A, Makita Y, Ogata T, Nakashima M, Tsurusaki Y, Miyake N, Saitsu H, Matsumoto N (2015) Detecting copy-number variations in whole-exome sequencing data using the eXome Hidden Markov Model: an "exome-first" approach. J Hum Genet 60:175–182. https://doi.org/10.1038/jhg.2014.124

Moller RS, Weckhuysen S, Chipaux M, Marsan E, Taly V, Bebin EM, Hiatt SM, Prokop JW, Bowling KM, Mei D, Conti V, de la Grange P, Ferrand-Sorbets S, Dorfmuller G, Lambrecq V, Larsen LH, Leguern E, Guerrini R, Rubboli G, Cooper GM, Baulac S (2016) Germline and somatic mutations in the MTOR gene in focal cortical dysplasia and epilepsy. Neurol Genet 2:e118. https://doi.org/10.1212/NXG.0000000000000118

Murtaza M, Jolly LA, Gecz J, Wood SA (2015) La FAM fatale: USP9X in development and disease. Cell Mol Life Sci 72:2075–2089. https://doi.org/10.1007/s00018-015-1851-0

Nakashima M, Saitsu H, Takei N, Tohyama J, Kato M, Kitaura H, Shiina M, Shirozu H, Masuda H, Watanabe K, Ohba C, Tsurusaki Y, Miyake N, Zheng Y, Sato T, Takebayashi H, Ogata K, Kameyama S, Kakita A, Matsumoto N (2015) Somatic mutations in the MTOR gene cause focal cortical dysplasia type IIb. Ann Neurol 78:375–386. https://doi.org/10.1002/ana.24444

Nehal KS, PeBenito R, Orlow SJ (1996) Analysis of 54 cases of hypopigmentation and hyperpigmentation along the lines of Blaschko. Arch Dermatol 132:1167–1170

Reijnders MR, Zachariadis V, Latour B, Jolly L, Mancini GM, Pfundt R, Wu KM, van Ravenswaaij-Arts CM, Veenstra-Knol HE, Anderlid BM, Wood SA, Cheung SW, Barnicoat A, Probst F, Magoulas P, Brooks AS, Malmgren H, Harila-Saari A, Marcelis CM, Vreeburg M, Hobson E, Sutton VR, Stark Z, Vogt J, Cooper N, Lim JY, Price S, Lai AH, Domingo D, Reversade B, Study DDD, Gecz J, Gilissen C, Brunner HG, Kini U, Roepman R, Nordgren A, Kleefstra T (2016) De novo loss-of-function mutations in USP9X cause a female-specific recognizable syndrome with developmental delay and congenital malformations. Am J Hum Genet 98:373–381. https://doi.org/10.1016/j.ajhg.2015.12.015

Ruggieri M, Pavone L (2000) Hypomelanosis of Ito: clinical syndrome or just phenotype? J Child Neurol 15:635–644. https://doi.org/10.1177/088307380001501001

Sakamoto M, Iwama K, Sekiguchi F, Mashimo H, Kumada S, Ishigaki K, Okamoto N, Behnam M, Ghadami M, Koshimizu E, Miyatake S, Mitsuhashi S, Mizuguchi T, Takata A, Saitsu H, Miyake N, Matsumoto N (2021) Novel EXOSC9 variants cause pontocerebellar hypoplasia type 1D with spinal motor neuronopathy and cerebellar atrophy. J Hum Genet 66:401–407. https://doi.org/10.1038/s10038-020-00853-2

Salas-Labadia C, Gomez-Carmona S, Cruz-Alcivar R, Martinez-Anaya D, Del Castillo-Ruiz V, Duran-McKinster C, Ulloa-Aviles V, Yokoyama-Rebollar E, Ruiz-Herrera A, Navarrete-Meneses P, Lieberman-Hernandez E, Gonzalez-Del Angel A, Cervantes-Barragan D, Villarroel-Cortes C, Reyes-Leon A, Suarez-Perez D, Pedraza-Melendez A, Gonzalez-Orsuna A, Perez-Vera P (2019) Genetic and clinical characterization of 73 Pigmentary Mosaicism patients: revealing the genetic basis of clinical manifestations. Orphanet J Rare Dis 14:259. https://doi.org/10.1186/s13023-019-1208-0

Shaffer JV, Chernoff KA (2020) Pigmentary mosaicism (hypomela-nosis of Ito). In: Levy ML, Hand JL (eds) UpToDate, Waltham

Sybert VP (1994) Hypomelanosis of Ito: a description, not a diagnosis. J Invest Dermatol 103:141S-143S. https://doi.org/10.1111/1523-1747.ep12399466

Thomas IT, Frias JL, Cantu ES, Lafer CZ, Flannery DB, Graham JG Jr (1989) Association of pigmentary anomalies with chromo-somal and genetic mosaicism and chimerism. Am J Hum Genet 45:193–205

Tsuchida N, Nakashima M, Kato M, Heyman E, Inui T, Haginoya K, Watanabe S, Chiyonobu T, Morimoto M, Ohta M, Kumakura A, Kubota M, Kumagai Y, Hamano SI, Lourenco CM, Yahaya NA, Ch'ng GS, Ngu LH, Fattal-Valevski A, Weisz Hubshman M, Orenstein N, Marom D, Cohen L, Goldberg-Stern H, Uchiyama Y, Imagawa E, Mizuguchi T, Takata A, Miyake N, Nakajima H, Saitsu H, Miyatake S, Matsumoto N (2018) Detection of copy number variations in epilepsy using exome data. Clin Genet 93:577–587. https://doi.org/10.1111/cge.13144

Uchiyama Y, Yamaguchi D, Iwama K, Miyatake S, Hamanaka K, Tsuchida N, Aoi H, Azuma Y, Itai T, Saida K, Fukuda H, Seki-guchi F, Sakaguchi T, Lei M, Ohori S, Sakamoto M, Kato M, Koike T, Takahashi Y, Tanda K, Hyodo Y, Honjo RS, Bertola DR, Kim CA, Goto M, Okazaki T, Yamada H, Maegaki Y, Osaka H, Ngu LH, Siew CG, Teik KW, Akasaka M, Doi H, Tanaka F, Goto T, Guo L, Ikegawa S, Haginoya K, Haniffa M, Hiraishi N, Hiraki Y, Ikemoto S, Daida A, Hamano SI, Miura M, Ishiyama A, Kawano O, Kondo A, Matsumoto H, Okamoto N, Okanishi T, Oyoshi Y, Takeshita E, Suzuki T, Ogawa Y, Handa H, Miya-zono Y, Koshimizu E, Fujita A, Takata A, Miyake N, Mizugu-chi T, Matsumoto N (2021) Efficient detection of copy-number variations using exome data: batch- and sex-based analyses. Hum Mutat 42:50–65. https://doi.org/10.1002/humu.24129

Vabres P, Sorlin A, Kholmanskikh SS, Demeer B, St-Onge J, Duf-fourd Y, Kuentz P, Courcet JB, Carmignac V, Garret P, Bessis D, Boute O, Bron A, Captier G, Carmi E, Devauchelle B, Genevieve D, Gondry-Jouet C, Guibaud L, Lafon A, Mathieu-Dramard M, Thevenon J, Dobyns WB, Bernard G, Polubothu S, Faravelli F, Kinsler VA, Thauvin C, Faivre L, Ross ME, Riviere JB (2019) Postzygotic inactivating mutations of RHOA cause a mosaic neu-roectodermal syndrome. Nat Genet 51:1438–1441. https://doi.org/10.1038/s41588-019-0498-4

Yigit G, Saida K, DeMarzo D, Miyake N, Fujita A, Yang Tan T, White SM, Wadley A, Toliat MR, Motameny S, Franitza M, Stutterd CA, Chong PF, Kira R, Sengoku T, Ogata K, Guillen Sacoto MJ, Fresen C, Beck BB, Nurnberg P, Dieterich C, Wollnik B, Matsu-moto N, Altmuller J (2020) The recurrent postzygotic pathogenic variant p.Glu47Lys in RHOA causes a novel recognizable neu-roectodermal phenotype. Hum Mutat 41:591–599. https://doi.org/10.1002/humu.23964

Zhang X, Fan Y, Liu X, Zhu MA, Sun Y, Yan H, He Y, Ye X, Gu X, Yu Y (2019) A novel nonsense mutation of PHF6 in a female with extended phenotypes of Borjeson-Forssman-Lehmann syn-drome. J Clin Res Pediatr Endocrinol 11:419–425. https://doi.org/10.4274/jcrpe.galenos.2019.2018.0220

Zou Z, Tao T, Li H, Zhu X (2020) mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges. Cell Biosci 10:31. https://doi.org/10.1186/s13578-020-00396-1

Zweier C, Kraus C, Brueton L, Cole T, Degenhardt F, Engels H, Gillessen-Kaesbach G, Graul-Neumann L, Horn D, Hoyer J, Just W, Rauch A, Reis A, Wollnik B, Zeschnigk M, Ludecke HJ, Wieczorek D (2013) A new face of Borjeson-Forssman-Lehmann syndrome? De novo mutations in PHF6 in seven females with a distinct phenotype. J Med Genet 50:838–847. https://doi.org/10.1136/jmedgenet-2013-101918

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.