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Post-Zygotic *ACTB* Mutations Underlie Congenital Smooth Muscle Hamartomas

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Abstract

Background: Congenital smooth muscle hamartomas (CSMHs) are benign lesions that share clinical and histopathological features with Becker nevus, a mosaic disorder associated with post-zygotic *ACTB* mutations. Given the clinical and histopathological overlap between CSMH and Beckernevus, we hypothesized that post-zygotic mutations in *ACTB* may underlie CSMH.

Methods: Direct sequencing of *ACTB* gene in affected and unaffected tissue isolated from 1 case of hemihypertrichosis and hemihypertrophy corresponding to giant segmental CSMH and hemihypertrophy. This was followed by direct sequencing with and without enrichment assay for hotspot *ACTB* mutations in affected tissue from 12 samples of isolated CSMH from unrelated individuals.

Results: In total we identified somatic missense *ACTB* mutations in 9 out of 13 CSMHs (69%). Mutations were either novel or previously reported in Becker nevi and Becker nevus syndrome.

Conclusions: CSMHs result from post-zygotic *ACTB* mutations. This study proves that CSMHs and Becker nevi are nosologically related and expand the phenotypic spectrum of *ACTB* mutations.

Keywords

smooth muscle hamartoma; nevus; beta-actin; ACTB; Becker’s nevus

INTRODUCTION

Congenital smooth muscle hamartoma (CSMH) is a benign skin lesion affecting approximately 1:2600 live births.[1] It usually presents as an indurated, slightly pigmented or flesh-colored plaque with perifollicular papules or coarse hair.[1–3] Rubbing of the lesion frequently triggers transient firmness due to erection of the arrector pili (pseudo Darier’s

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sign). Multiple CSMHs and diffuse CSMH, often referred as Michelin tire baby syndrome have also been described.[3] Notably, a review of the literature revealed some cases of diffuse CSMH with segmental involvement of the skin with or without extracutaneous manifestation, [4–6] suggesting that multilineage post-zygotic mutations underlies this presentation. Histopathologically, there is excessive proliferation of ectopic smooth muscle within the dermis. Hair follicles are normal in number and hyperkeratosis, acanthosis and hyperpigmentation of the basal cell layer can sometimes be seen.[2]

Becker nevus is a cutaneous hamartoma that typically appears in peri-puberty as a unilateral pigmented patch and becomes more prominent with increased thickness, pigmentation, and hair growth after puberty.[7] In Becker nevus syndrome, Becker nevi are associated with other developmental defects such as unilateral breast hypoplasia and musculoskeletal anomalies including ipsilateral hypoplasia of muscles and scoliosis.[7] The main histopathologic features are slight hyperkeratosis with variable acanthosis and elongation of the rete ridges accompanied by basal hyperpigmentation, slightly increased number of melanocytes and pigment incontinence. Apart from the epidermal alterations, hamartomatous irregular enlarged smooth muscle bundles are often seen in the dermis.[8]

Some authors suggest that Becker nevus and CSMH may lie on the same spectrum of cutaneous hamartomas,[8,9] while other reports characterize these conditions as separate entities.[10,11] While Becker nevus is androgen dependent with peripubertal onset, and increased pigmentation, induration and hairiness after puberty,[12] CSMH typically presents at birth and becomes less apparent with diminished pigmentation and hypertrichosis over time. [1] However, both congenital cases of Becker nevus and acquired cases of smooth muscle hamartoma have been reported in the literature,[9,13] and there is significant overlap between the lesions both clinically and histopathologically.

Recently, post-zygotic *ACTB* R147C and R147S mutations were found to be associated with Becker nevus and Becker nevus syndrome.[14] Given the significant clinical and histopathological overlap between CSMH and Becker nevus, we hypothesized that post-zygotic mutations in *ACTB* may underlie CSMH. Through direct sequencing of *ACTB* gene in one subject with CSMH associated with hemihypertrophy and 12 CSMH samples, we characterized the genotypic spectrum of *ACTB* mutations in CSMHs.

MATERIAL AND METHODS

The study was approved by the Institutional Review Board and complied with the declaration of Helsinki principles. For research conducted on pre-existing paraffin embedded samples, signed consent was waived. Case MOS1 was referred to our tertiary dermatology practice. Parental permission was obtained in writing. Skin biopsy specimens were identified by searching the departmental dermatopathology database by diagnosis. Cases collected during 2009–2015 with available paraffin blocks that yielded sufficient DNA for subsequent analysis were included. To avoid including cases of Becker nevi, only cases diagnosed at the age of 2-year-old or younger were included. In total, 12 readily available blocks of CSMH from 12 unique patients were identified. All samples were de-identified.

Tissue culture:

Skin biopsy from affected skin of MOS1 was transported in DMEM (Invitrogen, Carlsbad, California) with 1% penicillin/streptomycin and incubated overnight in 1X dispase (ZenBio, Research Triangle Park, North Carolina). Primary keratinocytes were isolated from resulting epidermal sheet using 0.05% trypsin- 2% EDTA and were plated onto a feeder layer of mitomycin C treated 3T3 cells in DMEM/F12 medium. Cells were subsequently propagated in Epilife (Invitrogen, Carlsbad, California). The remaining affected dermis was chopped and plated in a dish with DMEM with 1% penicillin/streptomycin to grow fibroblasts. When fibroblasts colonies were well- established, cells were split using 0.25% trypsin and propagated in the same medium.

Genomic DNA isolation:

Genomic DNA (gDNA) was isolated from 1 mm cores obtained from formalin-fixed paraffin-embedded (FFPE) affected tissue or cells cultured from affected skin (MOS1), using the DNeasy Micro Kit (Qiagen, Hilden, Germany) with added deparaffinization performed for FFPE tissue. gDNA from saliva was isolated using the Saliva DNA Isolation Reagent Kit (Norgen Biotek, Thorold, Ontario, Canada).

Polymerase chain reaction and *ACTB* sequencing:

gDNA isolated from cores of FFPE affected tissue was amplified using Kapa 2G Fast polymerase (Kapa Biosystems, Woburn, Massachusetts). Resultant *ACTB* amplicons were directly sequenced. Primers for amplification and sequencing of *ACTB* exon 1–5 and exon-intron junctions are listed in Supplementary Material 1. Given that recurrent c.C439A and c.C439T mutations were found in Becker nevi, samples were screened for these specific mutations using an enrichment assay utilizing the fact that c.C439A and c.C439T mutations obliterate a HaeIII enzymatic digestion site. Of note, any mutation at positions c.436-c.439 (p.G146, p.R147) obliterate the enzymatic digestion site of HaeIII. As previously described, gDNA was amplified and the resulting *ACTB* amplicon was digested by HaeIII for 140 minutes at 37°c degrees. Following digestion, the DNA was subjected to a second round of PCR amplification. PCR products were Sanger sequenced.[14]

RESULTS

Our index case was a 2-year-old male who presented with complaints of growth and hairiness over the right side of his back. His growth and development had been normal. Physical examination revealed right-sided hypertrichosis of the upper back and posterior right arm, as well as hemihypertrophy of the right upper extremity (Figure 1A). Laboratory investigations including complete blood count, comprehensive metabolic panel, and alpha-fetoprotein, as well as abdominal ultrasound were found to be normal. A biopsy of the right upper arm showed increased numbers of small twigs of smooth muscle in the dermis, some associated with hair follicles and some in between the hair follicles. Deep dermis and subcutis were unremarkable (Figure 1A). Given the clinical and histopathological findings, he was diagnosed with giant segmental CSMH and hemihypertrophy. The large area of CSMH involvement along with the hemihypertrophy suggested a multilineage mesodermal mosaicism. Sanger sequencing of DNA isolated from fibroblasts cultured from affected skin

revealed a somatic c.C439A, p.R147S mutation in *ACTB*, while sequencing of keratinocytes from the same lesion as well as saliva did not show the mutation (Figure 1B). Sequencing of *ACTB* exons and exon-intron junctions in additional 12 FFPE samples of CSMHs revealed somatic *ACTB* mutations in 3 samples: c.C439T, p.R147C, c.411G>T, p.Q137H and c.331A>G, p. N111D (Table 1, Figure 2). These mutations are absent from the GnomAD healthy controls database and are predicted damaging by Polyphen2 scores (Table 1). Using the mutation enrichment assay, we identified recurrent novel *ACTB* p.G146 mutations in 5 samples predicted damaging by Polyphen2 scores (Table 1, Figure 2). In total we identified *ACTB* mutations in 9 out of 13 CSMHs (69%).

DISCUSSION

CSMH and Becker nevus are hamartomas with partial clinical and histopathological overlap. The finding that 9 lesions from 9 unrelated individuals show distinct, clustered mutations in *ACTB* provides strong evidence that CSMHs derive from post-zygotic missense *ACTB* mutations. We identified novel *ACTB* mutations as well as *ACTB* mutations that were previously reported in Becker nevi. The dissimilarities between Becker nevi and CSMHs suggest that the path to developing either lesion in the presence of an *ACTB* mutation could be determined by intra-uterine environmental factors, mutation lineage or timing, as well as modifier genes.

Notably, our index case presented with giant segmental CSMH and hemihypertrophy, and the *ACTB* p.R147S mutation was detected in affected fibroblasts suggesting that multilineage *ACTB* mutation underlies his peculiar presentation. Subjects with Becker nevus syndrome often present with hypoplasia (e.g of the breast, shoulder girdle or limb) rather than hypertrophy,[7] and Cai et al. demonstrated by laser capture microdissection that the p.R147C mutation in one case of Becker nevus syndrome was localized to the pillar muscle and absent in the surrounding dermis.[14] The difference in phenotype (hypertrophy versus hypoplasia) could be explained by different cell lineages involved in each presentation. However, one should bear in mind that inability to demonstrate mesenchymal involvement in Becker nevus syndrome could be attributed to the difficulties in capturing the mutant cell population by dissection. In our case, culturing fibroblasts from affected skin enriched the mutant fibroblasts allowing the detection of the mutant signal.

Hemihypertrophy is a rare congenital presentation frequently associated with tumor predisposition and other developmental defects such as vascular and lymphatic malformations. It can result from either multilineage mosaic mutation (e.g. *PIK3CA* related overgrowth syndrome, Proteus syndrome),[15] or germline imprinting disorder (Beckwith-Wiedemann and Silver-Russell syndromes).[16] Only five cases of isolated hemihypertrophy with hemihypertrichosis were previously reported in the literature.[17–21] While evaluation for internal defects and tumors is a common practice in hemihypertrophies, histopathologic evaluation is not. The index case emphasizes the importance of histopathologic examination in establishing diagnosis and adds *ACTB* to the growing list of genes that underlie congenital hemihypertrophy.

ACTB encodes cytoplasmic β-actin, a ubiquitous cytoskeletal housekeeping protein that supports fundamental cellular processes including cell growth and migration, cell morphology, cytokinesis and maintenance of cell polarity.[22,23] Distinct gain-of-function germline mutations cause Baraitser-Winter syndrome (BRWS), an autosomal dominant disorder that features typical craniofacial features including trigonocephaly, hypertelorism, ptosis and arched eyebrows along with intellectual disability, cortical malformations, coloboma and sensorineural hearing loss.[24] Reduction of shoulder girdle muscle bulk and progressive joint stiffness is common.[25] Other missense mutations result in juvenile-onset dystonia associated with malformation and hearing loss.[26,27] Heterozygous loss of function mutations were recently described in a spectrum of developmental disorders with partial overlap with BRWS, suggesting that the underlying mechanism of BRWS is not just due to a gain of function, but might also include some effects resulting from loss-of-function or dominant-negative effects.[28] While some of the presumably gain-of-function *ACTB* mutations in BRWS and juvenile dystonia were shown to alter F-actin polymer dynamics leading to abnormal cytoskeletal organization and cell adhesion,[26,27,29] in *ACTB* p.R147C and p.R147S mutation expressing myocytes no gross abnormality in cytoskeleton was noted. However, *ACTB* p.R147C mutation expressing myocytes stimulated with Smoothened showed increased hedgehog signaling.[14] CSMHs as Becker nevi manifest abnormal smooth muscle development and hair growth. Hedgehog signaling controls hair and muscle development and homeostasis and is tightly influenced by the dynamic of actin cytoskeleton, [30–32] supporting the hypothesis that p.R147S mutation can potentiate Hedgehog signaling through disruption of the actin cytoskeleton.

Together our data prove that CSMHs result from somatic *ACTB* mutations and provide evidence for phenotypic expansion in which different mutations in the same gene can have different underlying genetic effects and thus result in different phenotypes. Further studies are warranted to explain how *ACTB* p.R147 mutations associated with both Becker nevi and CSMHs present with distinguishable features.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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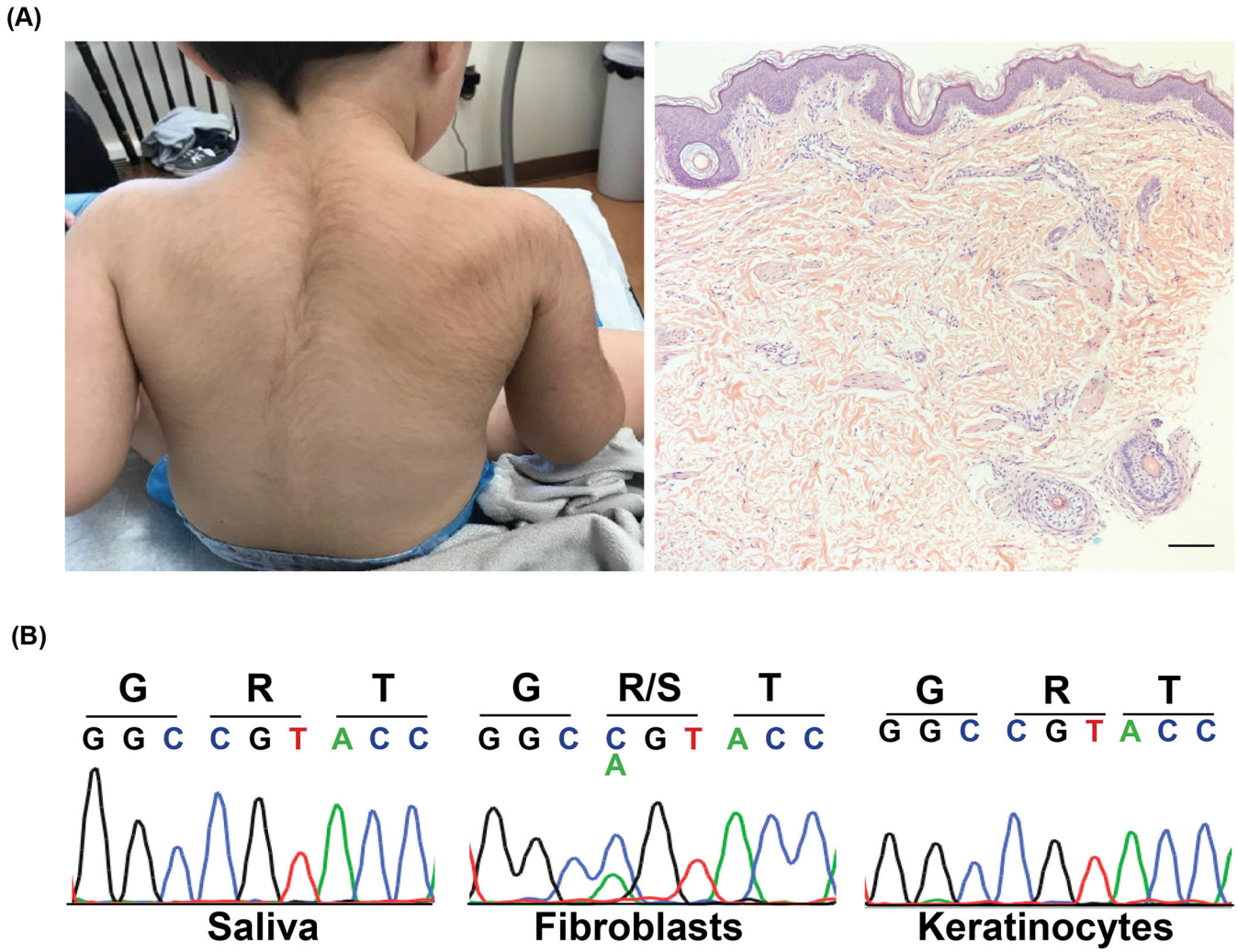

Figure 1: Giant segmental congenital smooth muscle hamartoma with hemihypertrophy results from post-zygotic *ACTB* p.R147S mutation.

A) The index case presented with right sided block-like hypertrichosis overlying hemihypertrophy of the right upper back and limb. Histopathologic examination of affected skin revealed increased numbers of small bundles of smooth muscle in the dermis. Scale bar=100 μm. B) Sanger sequencing of fibroblasts grown from affected skin showed *ACTB* c.439C>A, p.R147S mutation that was absent in affected keratinocytes as well as in saliva.

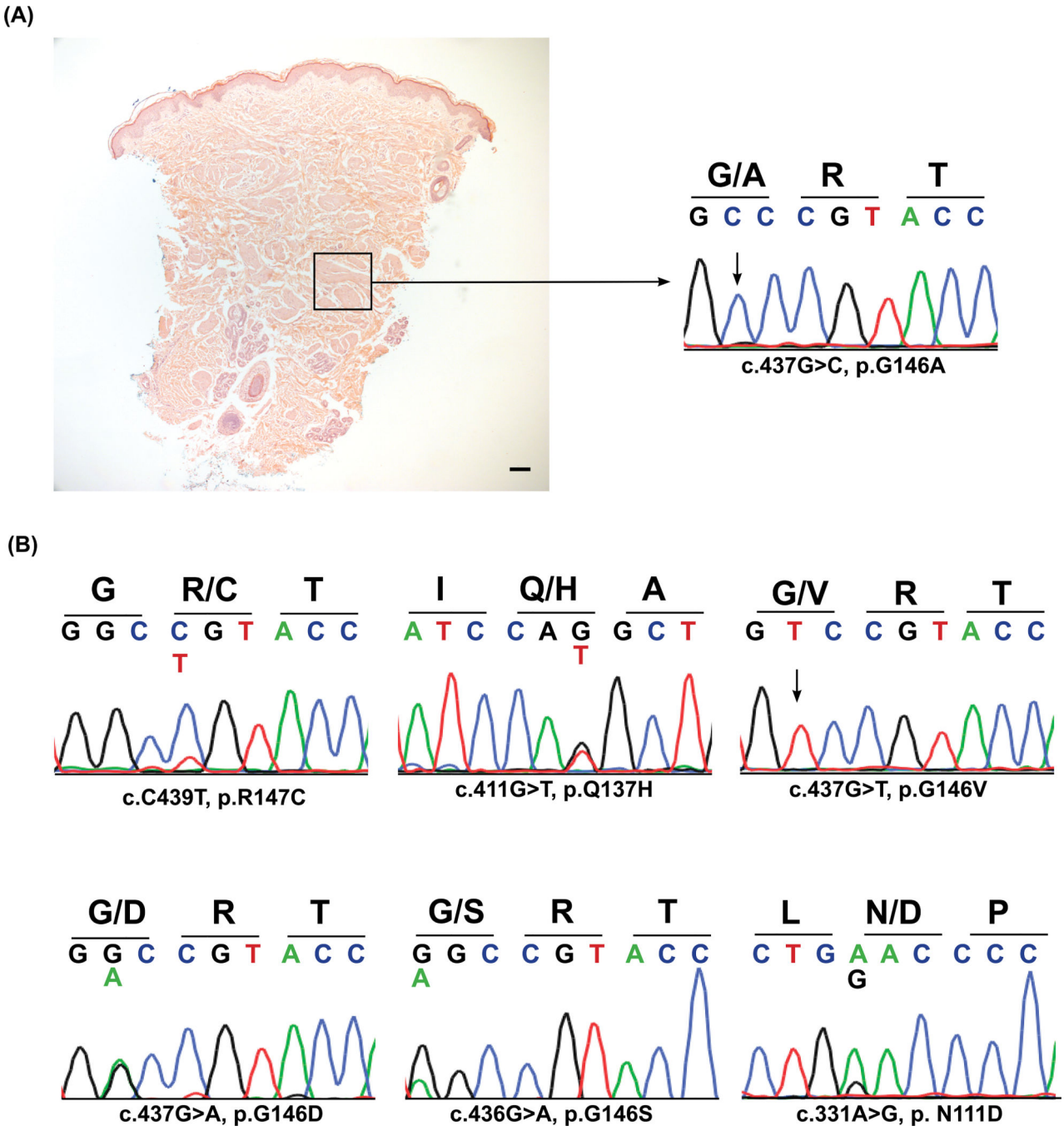

Figure 2: Histopathological features and genetic analysis of isolated congenital smooth muscle hamartomas.

A) Typical histopathological features of smooth muscle hamartoma in one of the included samples in the cohort. Scale bar=100 μm. Sanger sequencing of DNA isolates from smooth muscle twigs within the dermis shows *ACTB* p.G146A mutation. Mutant allele is enriched due to enrichment assay. B) Other *ACTB* mutations that were identified in the cohort. Arrows indicate enriched variant due to the use of enrichment assay.

Table 1:

Clinical characteristics and genetic analysis of the study cohort:

Sample ID	Age	Sex	Anatomic location	identified *ACTB* Mutation	Polyphen2 HumDiv/ Polyphen2 HumVar
MOS1[¶]	2 y.o	M	Right upper limb and back	c.439C>A p.R147S	0.959/0.967[#]
MOS2	2 y.o	F	Right lateral thigh	None	
MOS3	1 y.o	F	Right abdomen	None	
MOS4	1 y.o	F	Left lower back	c.C439T, p.R147C	0.998/0.981[#]
MOS5	1 y.o	F	Right upper buttocks	None	
MOS6	7 y.o	F	Mid abdomen	c.411G>T, p.Q137H	0.895/0.962[#]
MOS7	5 m.o	M	Left upper back	c.437G>C, p.G146A	0.999/1[#]
MOS8	2 y.o	M	Left back	c.437G>T, p.G146V	1/1[#]
MOS9	1 y.o	M	Right upper arm	c.437G>A, p.G146D	1/1[#]
MOS10	1 y.o	F	Right thigh	c.436G>A, p.G146S	0.999/1[#]
MOS11	6 m.o	M	Right upper thigh	None	
MOS12	3 m.o	M	Left back	c.331A>G, p. N111D	0.999/1[#]
MOS13	8 m.o	M	Right back	c.437G>C, p.G146A	0.999/1[#]

[¶]The index case with giant congenital smooth muscle hamartoma and hemihypertrophy is indicated as MOS1.

[#]Polyphen2 scores correspond to predicted damaging mutations.