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BRIEF COMMUNICATION

De novo AFF3 variant in a patient with mesomelic dysplasia with foot malformation

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Abstract

Mesomelic dysplasia (MD) encompasses a heterogeneous group of disorders characterized by shortening of the middle segments of the limbs. Previous studies have revealed the development of Nievergelt type-like MD accompanied by postaxial toe reduction in a patient with a ~500 kb microdeletion at 2q11.2 involving *AFF3* alone, and the occurrence of Nievergelt type-like MD in mice with a ~353 kb deletion involving *Aff3*, together with strong expression of mouse *Aff3* in the developing limbs and zeugopod. We encountered a 2 6/12-year-old Japanese girl with an unclassifiable MD associated with hypoplasia of postaxial toes, and identified a *de novo* likely pathogenic variant of *AFF3* (NM_002285.2:c.697 G > A, p.(Ala233Thr)) by whole exome sequencing. The results provide further evidence for *AFF3* being the causative gene for MD with foot malformation which may be termed "*AFF3*-related MD" or "Steichen-Gersdorf type MD".

Introduction

Mesomelic dysplasia (MD) encompasses a heterogeneous group of disorders characterized by shortening of the middle segments of the limbs. To date, 11 types of MD and rhizo-MD are listed in the Nosology and Classification of Genetic Skeletal Disorders (NCGSD), and molecular studies have identified monoallelic *SHOX* abnormalities in Léri-Weill dyschondrosteosis, biallelic *SHOX* abnormalities in Langer MD, biallelic *GPC6* abnormalities and monoallelic *FZD2* abnormalities in omodysplasia, and biallelic *ROR2* abnormalities and monoallelic *WNT5A* or *DVL1* abnormalities in Robinow syndrome [1]. Furthermore, genetic studies have also revealed heterozygous duplication at chromosome 2q31 involving the *HOXD* cluster in Kantaputra type MD [2], heterozygous deletions at chromosome 8p13 encompassing *SULF1* and *CLCO5A1* in Verloes-David-Pfeiffer type MD [3], and heterozygous deletions at chromosome 6p22.3 including *MBOAT1*, *E2F3*, *CDKAL1*, and *SOX4* in Savarirayan type MD [4]. However, causative genes or underlying genetic factors have not been revealed in autosomal dominant Nievergelt type MD and autosomal recessive Kozlowski–Readon type MD, although it has been suggested that Savarirayan type and Nievergelt type MDs may represent allelic disorders because of their clinical similarity [5].

In addition to MDs listed in NCGSD, Steichen-Gersdorf et al. have reported Nievergelt type-like MD with triangular tibia and fibular aplasia as well as foot malformation in a female infant with a ~500 kb heterozygous deletion at chromosome 2q11.2 involving *AFF3* (alias, *LAF4*) as the sole deleted gene [6]. Furthermore, mouse *Aff3* is strongly expressed in the developing limbs [6], and mice with a ~353 kb heterozygous deletion involving *Aff3* exhibit skeletal phenotype reminiscent of Nievergelt type MD [7]. These findings imply a critical role of *AFF3* deletion in the

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development of Nievergelt type-like MD of the patient reported by Steichen-Gersdorf et al. [6].

Here, we report a likely pathogenic *de novo AFF3* variant identified in a patient with an unclassifiable MD with bilateral hypoplasia of the postaxial toes. The results provide further evidence for *AFF3* being involved in the development of MD with foot malformation.

Case report

This Japanese female patient was born at 38 weeks of gestation after an uncomplicated pregnancy and delivery. The parents were non-consanguineous and clinically normal (paternal height 175 cm, +0.7 SD; maternal height 154 cm, –0.8 SD). There was no family history of skeletal dysplasias. At birth, her length was 45.0 cm (–1.6 SD), her weight 2422 g (–1.4 SD), and her occipitofrontal circumference 32.0 cm (–0.7 SD).

At 7 months of age, she was referred to Kumamoto University hospital because of growth failure and developmental delay. At that time, her length was 61.5 cm (–2.6 SD), and her weight 6.470 g (–1.7 SD). Physical examination revealed mesomelic appearance of the upper and lower limbs, syndactyly of the left 4–5 toes, and a sacral dimple. She could not control her head. Routine laboratory tests were normal, as were endocrine studies for short stature. Radiological examinations showed MD with broad and bowed radii and ulnae, hypoplastic fibulae, syndactyly of the left 4th and 5th toes, rudimentary left 4th metatarsal, hypoplastic right 5th metatarsal, and 11 pairs of ribs (Fig. 1). Brain and whole spine magnetic resonance imaging and echocardiography showed no abnormalities, whereas abdominal ultrasonography revealed hypoplasia of bilateral kidneys. Chromosome analysis showed a 46,XX karyotype. On the latest examination at 2 years and 6 months of age, she showed failure to thrive and severe developmental delay. At that time, she measured 73.1 cm (–4.9 SD), weighed 7,360 g (–3.5 SD), and could neither control her head nor speak single words.

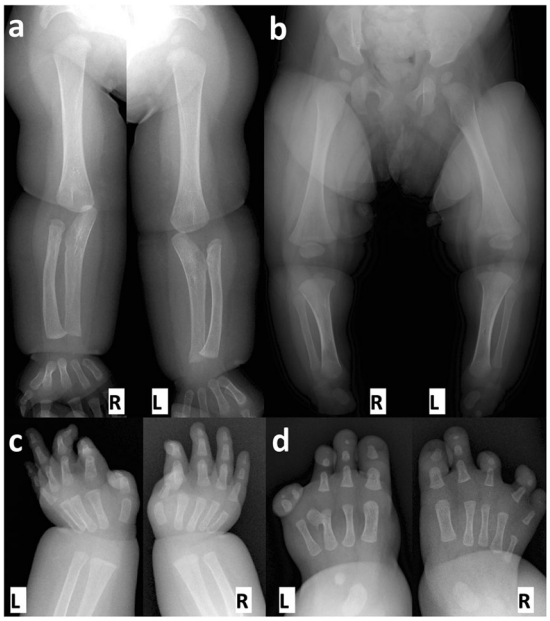

Fig. 1 Roentgenographic findings of this patient at seven months of age. **a** Arms, **b** Legs, **c** Hands, and **d** Feet. R, right; and L, left. Mesomelic shortening of the limbs is evident. The proximal ulnae are thick and the proximal radii are relatively narrow. The left proximal radius is subluxed. The proximal tibiae are broad, and fibulae are hypoplastic. The short tubular bones of the hands are unremarkable. The left foot shows syndactyly of the 4th and 5th toes with hypoplasia of the 4th metatarsal. The right foot shows hypoplasia of the 5th metatarsal

Genetic studies

This study was approved by the Institutional Review Board Committee at Hamamatsu University School of Medicine, and performed after obtaining written informed consent. Whole exome sequencing was carried out with SureSelect Human All Exon V6 (Agilent Technologies), using leukocyte genomic DNA samples of the patient and the parents. Captured libraries were sequenced by NextSeq 500 (Illumina) with 150 bp paired-end reads. Exome data processing, variant calling, and variant annotation were performed by the previously described methods [8], using Human GRCh37 as the reference genome. We extracted rare variants with minor allele frequencies of ≤0.01 in all the public and in-house databases utilized in this study, and performed *in silico* pathogenicity predictions for extracted rare variants by several methods. The databases and *in silico* pathogenicity prediction methods are shown in Fig. 2 and Supplemental Table 1, and their URLs are described in the footnotes of Supplemental Table 1.

Consequently, we identified a *de novo* heterozygous missense variant at exon 6 encoding the ALF domain of *AFF3* (NM_002285.2:c.697 G > A, p.(Ala233Thr)) (Fig. 2). This variant was confirmed by Sanger sequencing. It was completely absent from the public and in-house databases, and was assessed to have high pathogenicity. According to the ACMG Standards and Guidelines [9], this variant was evaluated as a "likely pathogenic variant", because this variant was positive for PS2 (*de novo* variant with confirmed maternity and paternity), PM2 (absent from controls), and PP3 (multiple lines of computational evidence in support of a deleterious effect). While rare compound heterozygous and homozygous variants were identified in *GOLGA4*, *PDL1M7*, and *TLN2*, there was no data in support of the relevance of such variants to the development of MD (Supplementary Table 1).

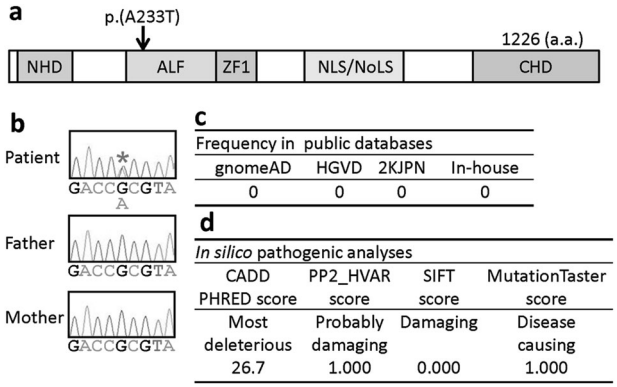

Fig. 2 Summary of molecular and *in silico* analyses. **a** Structure of the AFF3 protein and the position of the p.(A233T) variant. **b** Electrochromatograms showing the *de novo* c.697 G > A substitution of this patient (marked with a red asterisk). The primers used are: forward, 5′-CACAACAGGGCTCTCTCAGG-3′; and reverse, 5′-GAACTTG GAGAGCTTGGCCT-3′. **c** The absence of p.(A233T) in the public and in-house databases. For details, see Supplementary Table 1 and its footnotes. **d** High pathogenicity of p.(A233T). For details, see Supplementary Table 1 and its footnotes

Discussion

We identified a *de novo* likely pathogenic *AFF3* variant in this Japanese patient with an unclassifiable MD accompanied by hypoplasia of postaxial toes. The results, in conjunction with the development of Nievergelt type-like MD in a patient with a ~500 kb microdeletion at 2q11.2 involving *AFF3* alone [6], strong expression of mouse *Aff3* in the developing limbs [6], and the occurrence of skeletal phenotype reminiscent of Nievergelt type MD (a short zeugopod in the upper limbs and lower limb abnormalities including small triangular ossification center of the tibia and severe hypoplasia of the fibula) in mice with a ~353 kb deletion involving *Aff3* [7], imply that the *AFF3* variant is the causative factor for the development of MD in this patient.

Clinical findings of this patient with *AFF3* variant are summarized in Table 1, together with those of the patient with a deletion involving *AFF3* alone reported by Steichen-Gersdorf et al. [6]. (according to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), the same *AFF3* variant (NM_002285.2:c.697 G > A, p.(Ala233Thr)) has been found in a patient with skeletal disorder, short stature, global developmental delay, and seizures, but detailed skeletal findings have not been reported). Both patients had MD with foot malformation, with the skeletal phenotype being apparently severer in the deletion-positive patient. In addition, both patients also manifested developmental delay and renal anomalies, and the deletion-positive patient further exhibited complex visceral anomalies and intractable seizure. These findings would suggest that *AFF3* abnormalities are associated with a relatively wide phenotypic spectrum including MD with foot malformation, which may be termed "*AFF3*-related MD" or "Steichen–Gersdorf type MD", with variable expressivity and reduced penetrance. Such phenotypic variability would be explained by the property of the *AFF3* abnormalities (possibly hypomorphic missense variants *vs*. amorphic deletion) and the relevance of multiple genetic and environmental factors other than the *AFF3* abnormalities, although the deletion involving *AFF3* might also have perturbed expression of neighboring genes.

Notably, both patients had obvious psychomotor developmental delay (Table 1). In this regard, *de novo AFF3* missense variants have been identified in patients with severe developmental disorders and intellectual disabilities who are apparently free from skeletal disorders [10–12]. In addition, mouse *Aff3* is strongly expressed in the developing hindbrain as well as in the limb buds [6] and is required for normal cellular migration in the developing cortex [13]. Thus, psychomotor developmental delay would be regarded as a characteristic feature in ″*AFF3*-related MD″ or ″Steichen-Gersdorf type MD″.

AFF3 may also underlie the development of autosomal dominant MDs of unknown cause. In particular, skeletal

Table 1 Clinical findings of two patients with *AFF3* abnormalities

	This patient	Steichen-Gersdorf et al.
Genetic cause	p.(Ala233Thr)	Deletion
Skeletal findings		
Mesomelic shortening	+	+
Radial head subluxation	+[a]	+
Abnormal hands	–	–
Shortening of lower legs	+	+
Triangular tibia	–	+
Fibular aplasia	–	+
Fibular hypoplasia	+[b]	–
Foot deformity	+[c]	+[d]
Rib anomaly	+	+
Hip dislocation	–	+
Other features		
Colon malformation	–	+
Renal malformation	+	+
Genital malformation	–	+
Seizure	–	+
Developmental delay	+	+
Recurrent apnea	–	+
Death in infancy	–	+
Reference	This study	6

[a]Unilateral

[b]Mild

[c]Bilateral asymmetric postaxial hypoplasia

[d]Unilateral postaxial aplasia

features of "*AFF3*-related MD"or "Steichen–Gersdorf type MD" are similar to those of Savarirayan type and Nievergelt type MDs [14, 15] which may represent allelic disorders [5], and the *Aff3* deleted mice have exhibited skeletal phenotype reminiscent of Nievergelt type MD [7]. In addition, Savarirayan type, but not Nievergelt type, MD is often associated with developmental delay, although both types of MD are apparently free from visceral anomalies [4, 14–16]. Thus, *AFF3* would be worth analyzing in patients with underlying cause-unknown MDs, especially those accompanied by developmental delay (e.g., Savarirayan type MD of unknown underlying cause). By contrast, the relevance of *AFF3* abnormalities to an autosomal recessive Kozlowski–Readon type MD would be unlikely.

In summary, this study indicates that *AFF3* constitutes a causative gene for MD with foot malformation. Further studies will permit to clarify the phenotypic spectrum of "*AFF3*-related MD" or "Steichen–Gersdorf type MD", and the relevance of *AFF3* abnormalities to the development of other types of MDs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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