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ARTICLE

Protein-altering *MYH3* variants are associated with a spectrum of phenotypes extending to spondylocarpotarsal synostosis syndrome

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Spondylocarpotarsal synostosis syndrome (SCT) is a rare Mendelian disorder (OMIM #272460) characterized by prenatal vertebral fusion, scoliosis, short stature and carpal and tarsal synostosis. SCT is typically known as an autosomal recessive disease caused by variants in the *FLNB* gene. The genetic basis of the rarer cases of vertical transmissions remains unknown. In two independent families with symptoms related to autosomal dominant SCT, we identified – by exome sequencing – two protein-altering variants in the *embryonic myosin heavy chain 3* (*MYH3*) gene. As *MYH3* variants are also associated with distal arthrogryposis (DA1, DA2A, DA2B) and autosomal dominant multiple pterygium syndromes (MPS), the present study expands the phenotypic spectrum of *MYH3* variants to autosomal dominant SCT. Vertebral, carpal and tarsal fusions observed in both families further confirm that *MYH3* plays a key role in skeletal development.
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INTRODUCTION

Spondylocarpotarsal synostosis syndrome (SCT; OMIM #272460) is a rare Mendelian disorder characterized by vertebral fusion, scoliosis, short stature and carpal and tarsal synostosis. Several additional manifestations have been described including cleft palate, sensorineural or mixed hearing loss, joint limitations, clinodactyly and enamel hypoplasia. The disease was first recognized more than 40 years ago.[1] To date, however, only 30 cases have been reported (reviewed by one of us in 2008).[2–5] The vast majority of patients show autosomal recessive inheritance with variants in the gene, *FLNB*, encoding the cytoskeleton protein filamin B.[6] We identified two families with dominant disease transmission: one with typical SCT previously reported by one of us[2] and the second with multiple pterygium syndrome (MPS; DA8) associated with skeletal manifestations. The present genetic study reveals that *embryonic myosin heavy chain 3* (*MYH3*) mutations are associated with a spectrum of phenotypes including distal arthrogryposis (DA), MPS and SCT.

MYH3 encodes the heavy chain of embryonic myosin. The gene is known to be involved in DA) syndromes that constitute a group of disorders characterized by multiple congenital contractures of the upper and lower limbs.[7–10] Variants in *MYH3* cause DA1 (OMIM #108120),[11,12] DA2A (Freeman–Sheldon syndrome; OMIM #193700)[13–15] and DA2B (Sheldon–Hall syndrome; OMIM #601680).[16,17] Interestingly, recent findings from Chong *et al*[18] indicate that *MYH3* is also involved in autosomal dominant MPS that, in addition to congenital contractures of the limbs, is characterized by multiple pterygia and skeletal anomalies including fusion of vertebra and scoliosis. The latter report further suggests for embryonic myosin to play a key role in skeletal development.

The present study expands the spectrum of *MYH3* variant to SCT that is characterized by a predominant bone phenotype and therefore confirms the role of *MYH3* in skeletal development.

SUBJECTS AND METHODS

Subjects and exome sequencing

Subjects reported in this study are members of two unrelated families, both presenting vertical transmission of SCT. The following individuals were exome sequenced: in the first family, the proband (a boy) 1-III1, his affected mother (1-II2) and his healthy grandparents (1-I1 and 1-I2); in the second family, the proband (girl) (2-II1) and her affected mother (2-I2) (Figure 1). All subjects gave written informed consent for genetic analyses.

Genomic DNA was extracted and purified from peripheral blood using standard protocols. Exome capture was performed with the SureSelect 50MB Target Enrichment System v3 (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol. Prepared libraries were subjected to paired-end sequencing on a SOLiD 5500 platform (Life Technologies, Carlsbad, CA, USA) with 75 bases forward reads and 35 bases reverse reads. An average of 7.9 Gb of mappable sequence data per individual was obtained and mapped to the hg19 reference genome using Lifescope software v2.5.1 (Life Technologies). The mean coverage was 142-fold (median of 142-fold) and 84.1% of the target sequence was covered at a minimum of 10×. Variants were called using the GATK software (Broad Institute, Boston, MA, USA) and LifeScope (Life Technologies) algorithms with medium-stringency calling settings. Only variants called by both methods and covered by more than five variant reads were considered. Annotation was performed with the KGGSeq software package (Hong Kong University, Hong Kong, China). As SCT is extremely

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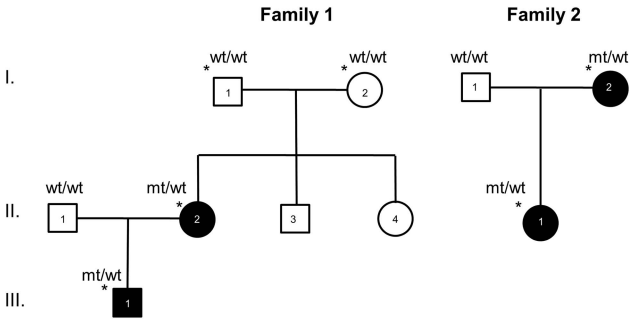

Figure 1 Pedigrees of families 1 and 2. Black icons denote affected individuals. Asterisks indicate exome-sequenced subjects.

rare (30 cases reported to date), we focused only on protein-altering variants (missense, nonsense, splice-site variants and coding indels) with alternative allele frequency <0.0001 in dbSNP (build 135), 1000 Genomes Project data and an in-house database including 200 exomes. To identify potential causal variants in family 1, we focused on *de novo* variants of patient 1-II.2 (ie, absent in 1-I1 and 1-I2) that were also present in 1-III.1. In the second family, all variants common to 2-I2 and 2-II1 were analyzed.

Sanger sequencing

Candidate variants were checked using conventional capillary Sanger sequencing. Briefly, 344 and 318 bp DNA stretches of *MYH3* exons 11 and 30 were amplified using the Expand Long Template PCR System (Roche, Meylan, France), following the manufacturer's recommendations. The PCR primer pairs were 5′-GGTGAAGCCCAGGATGTC-3′ (forward) and 5′-GGTAAAAGTTC CAGCCCAGG-3′ (reverse) for exon 11 and 5′-AGTATTTTGTTAGAAACC TAGAGGAGAGCTC-3′ (forward) and 5′-CTGAGTGATGAAGCCACACCCA -3′ (reverse) for exon 30. After purification with the Exostar kit (GE Healthcare, Little Chalfont, UK), PCR products were sequenced bidirectionally with the same amplification primers using Big Dye Terminator Kit v3.1 (Life Technologies). Sequence reactions were run on an ABI PRISM 3730xl sequencer (Life Technologies). The identified variants have been submitted to the *MYH3*-specific Leiden Open Variation Database (www.lovd.nl/MYH3) with individual IDs 00060260 (1-II.2), 00060275 (1-III.1), 00060276 (2-I.2) and 00060277 (2-II.1).

RESULTS

Clinical examination

Table 1 summarizes the clinical findings in the two cases of SCT (family 1) and the two cases of MPS (family 2). In each family two cases concordant with an autosomal dominant inheritance of the disease were studied.

Family 1: patient 1-II.2

This presently 37-year-old woman was first described by some of us in Isidor *et al*.[2] As described in that report, at her last medical examination (age 26 years) she presented the following symptoms: a short neck with limited mobility, low hairline, limited extension of both elbows, moderate hearing deficit, a cleft palate, bilateral single palmar crease and an absence of the flexion crease of the third phalange of all fingers except the thumb. Hands further showed bilateral fusion of trapezium and scaphoid, lunate–triquetrum and the right-hand fusion of capitate–hamate. Standard X-rays (see Isidor *et al*[2] – cited above – for radiographs) showed progressive thoracic scoliosis, lumbar lordosis, lack of normal segmentation of T7–11 vertebral bodies with progressive vertebral fusion extending to T2, fusions of C1–C4 vertebral bodies and partial tarsal fusion between the scaphoid and the cuneiform. For further clinical phenotyping see Isidor *et al*.[2]

Table 1 Comparison of clinical features in the four patients

	Family 1		*Family 2*	
	II.2	*III.1*	*I.2*	*II.1*
Sex (M/F)	F	M	F	F
Short stature	+	+	−	+
Spine anomalies				
Short trunk	+	+	−	+
Thoracolumbar fusions	+	+	−	+
Scoliosis	+	+	−	+
Cervical fusion	+	−	+	+
Short neck	+	+	+	+
Sacral anomaly	+	+	−	+
Limb anomalies				
Carpal fusion	+	[a]	+	+
Tarsal fusion	+	[a]	+	+
Joint mobility limitation	+	+	+	+
Clinodactyly 5th	+	+	+	−
Elbow, knee and shoulder pterygium	−	−	−	+
Single palmar crease	−	−	+	−
ORL				
Hearing loss	+	+	−	−
High arched/cleft palate	+	+	+	+
Inguinal hernia	−	+	−	+
Other clinical features				
Facial dysmorphy	+	+	+	+
Pterygium colli	−	−	+	+
Respiratory failure	−	−	−	+

[a]Evaluation of this feature was impossible because of severe bone age delay.

Family 1: patient 1-III.1 (proband)

This 16-year-old boy is the unique child of patient II.2 described above. He was also part of the original clinical report by some of us.[2] At his last visit (9 years old) he showed a posterior cleft palate, a short neck, low set and posteriorly rotated ears, positional plagiocephaly without torticollis, short hands, bilateral single palmar crease and clinodactyly of both fifth fingers. He had a mild hearing deficit. X-rays revealed narrowing of the intervertebral space T8–T11, progressive scoliosis and fusion of T5–8 vertebral bodies. For further clinical phenotyping see Isidor *et al*.[2]

Family 2: patient 2-I.2

Patient 2-I.2 was seen at 27 years of age for genetic counseling. She presented a Klippel–Feil syndrome (C1–C2 and C6–C7 fusions) along with feet malformations, both diagnosed in childhood. Bilateral pes cavus was also recorded at birth. Her left foot became painful at 11 years of age and required surgery. A bilateral tarsal coalition between navicular and first cuneiform was also documented. Psychomotor development was normal.

Clinical examination at 36 years of age showed normal stature (159 cm), pterygium colli, low posterior hair line, high arched palate and dysmorphic facial features with down-turned palpebral fissures and low set ears. Feet examination showed pes cavus but normal toes. Neurological examination and muscular tone were normal, as were the hands, except for bilateral fifth-digit camptodactyly and single palmar crease. X-rays showed fusions of C1–C2 and C6–C7 vertebral bodies (Figure 2a) and decreased height of the intervertebral discs T9–T10 and T11–T12 (Figure 2b). Hand and feet X-rays confirmed

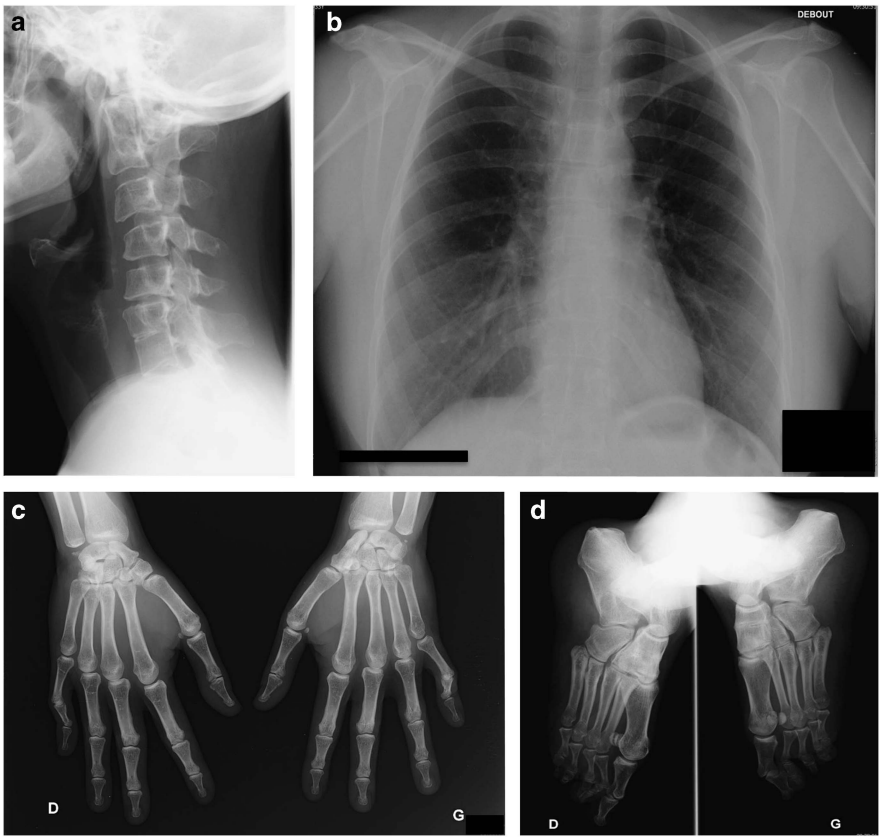

Figure 2 X-ray imaging of patient 2-I.2. (**a**) Lateral X-ray of cervical spine showing C1–C2 and C6–C7 anterior fusion associated with C1–C3 and C5–C7 posterior fusion. (**b**) AP pulmonary X-ray showing narrowing of intervertebral space T9–T10 and T11–T12, suggesting anterior fusions. Note the absence of spine or rib cage deformation. (**c**) AP X-rays of hands showing capitate–hamate and lunate–triquetrum coalitions of both hands associated with scaphoid–trapezium coalition of the left hand. (**d**) Oblique X-rays of feet showing a coalition between the navicular and the three cuneiforms of both feet.

carpal and tarsal synostosis (Figures 2c and d). Renal and cardiac ultrasounds were normal as was audition and standard karyotyping.

Family 2: patient 2-II.1

This 6-year-old girl is the unique child of patient 2-I.2, described above. Increased nuchal translucency and abnormal rachis were uncovered during pregnancy by ultrasound at 10 WG. Standard prenatal karyotyping was normal. Prenatal sonographic follow-up showed diffuse severe vertebral fusions. Termination of pregnancy was considered but the mother chose not to do so. The girl was born at 41 weeks, and she presented a short stature (43.6 cm; below 3rd percentile) but normal weight (3.08 kg 10th–50th percentile). Neonatal examination revealed low spontaneous mobility, torticollis, a large and short neck and severe joint limitations with pterygia of knees, ankles, shoulders and open hands with unmarked palmar creases. She also carried a posterior cleft palate and neonatal failure to thrive that led to gastrostomy. Facial features included ptosis, low set ears and short neck. A simple sacral dimple was noted, and spinal cord was low set (L2–L3; Figure 3).

Diffuse vertebral segmentation anomalies were confirmed, involving thoracic, cervical and lumbar segments with fusions of vertebral bodies, posterior arches and spinous process, leading to several block vertebra and severe scoliosis (Figure 4).

Soon after birth, she showed motor development delays because of these orthopedic defects; walking was acquired at 2 and a half years. She kept a very short stature (height 4 SD, weight 2 SD and OFC 1 SD) with severe disproportion because of the impaired growth of her thoracic cage. A bicuspid aortic valve with grade 1 aortic insufficiency, slight aortic supravalvular stenosis and a patent ductus arteriosus (which required surgery) were also diagnosed. Furthermore, she presented a left inguinal hernia that required surgery as well. A few episodes of hypercalcemia and hypercalciuria were observed but remained unexplained. She had a respiratory failure episode following severe bronchiolitis with an aspergillus superinfection and her restrictive lung disease. Muscular examination was normal. Renal and spinal cord sonography as well as brain MRI were normal as was cognitive development. Surgery was necessary on the popliteal pterygium. At 4 years of age, C4–C5 dislocation with spinal cord involvement was diagnosed and an anterior epiphysiodesis was performed. Carpotarsal fusions were revealed by radiographic examinations.

Exome sequencing and identification of the candidate gene

Before exome sequencing, CGH array analysis and direct sequencing of the plausible causal genes – *FLNB*, *NOG* and *GDF6* – failed to identify any chromosomal rearrangements or variants. The exome of a total of six subjects (family 1: index patient 1-III1, his healthy maternal grandparents and his affected mother; family 2: index patient 2-II1 and her affected mother (2-I2)) were sequenced. On average, 49 529 SNPs (median of 49 481) and 1861 indels (median of 1926) were identified for each individual. For SNPs, we focused only on the protein-altering events, represented by an average of 8251 variants (median of 8252) and corresponding to missense, frameshift, non-

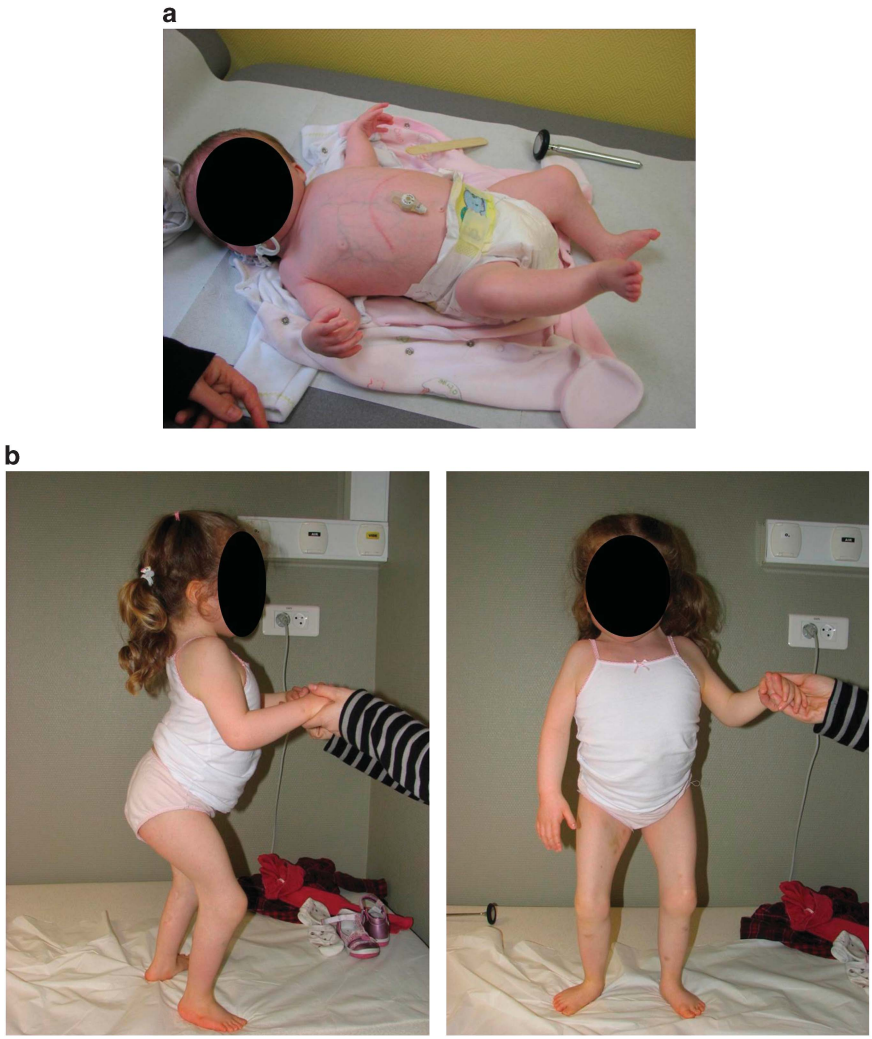

Figure 3 Phenotypic characteristics of patient 2-II.1. (**a**) Photograph of patient 2-II.1 at 6 months of age. (**b**) Photographs of the patient 2-II.1 at 4 years of age. Note the short stature, short trunk and neck, scoliosis, knee pterygium and neck webbing. A detailed description of the phenotype of the patient is presented in Table 1.

frameshift, splicing, stoploss and stopgain SNPs. In the case of indels, only frameshift, non-frameshift, stoploss, stopgain and splicing events were taken into account, corresponding to an average number of 168 (median of 187).

After filtering out variants with an alternative allele frequency >0.0001 in dbSNP (build 135), 1000 Genome Project data and an in-house database from each proband (1-III1 and 2-II1), a dominant heredity model was applied to further reduce the number of candidates to 2 in family 1 (*MYH3* and *SYT14*, each with one *de novo* variant) and 30 in family 2 (Supplementary Table 1 and Supplementary Figure 1). The unique mutated gene in both families was *MYH3* (NM_002470.3, NG_011537.1). In family 1, a *de novo* variant located in exon 11, which belongs to the coding region of the head domain (c.998C>G; p.(Thr333Arg)), was identified. In family 2, we uncovered a variant located in exon 30 that is part of the coding region of the protein tail (c.4031T>C; p.(Leu1344Pro)). Both missense variants were validated by Sanger sequencing and were shown to segregate with the disease in each family (Supplementary Figure 2). Both variants have deleterious predictive values using various tools such as Sift, Polyphen or GERP (Supplementary Table 1). In addition, neither variant was found in ESP6500, 1000 Genomes, the ExAC Browser (60 706 unrelated individuals) and an internal exome database (200 exomes).

DISCUSSION

The two families reported here show both vertical disease transmission with *MYH3* mutations and present phenotypic spectra going from pure SCT (family 1) to MPS (family 2). In family 2, the phenotype is characterized by a strong intrafamilial variability in terms of severity of vertebral fusions and orthopedic deformities. The child is severely disabled with short stature, major orthopedic deformations, persistent feeding difficulties and cardiac disease, although the mother is not disabled and shows normal stature and normal mobility except for that of the cervical spine. Carpotarsal and vertebral fusions are rare and appear in different clinical entities in which SCT is well characterized. Other rare entities include carpotarsal and vertebral coalition in different clinical and radiologic context (spondylocostal dysplasia, Robinow syndrome, multiple synostosis, proximal symphalangism). Two cases with a form of syndromic carpotarsal and

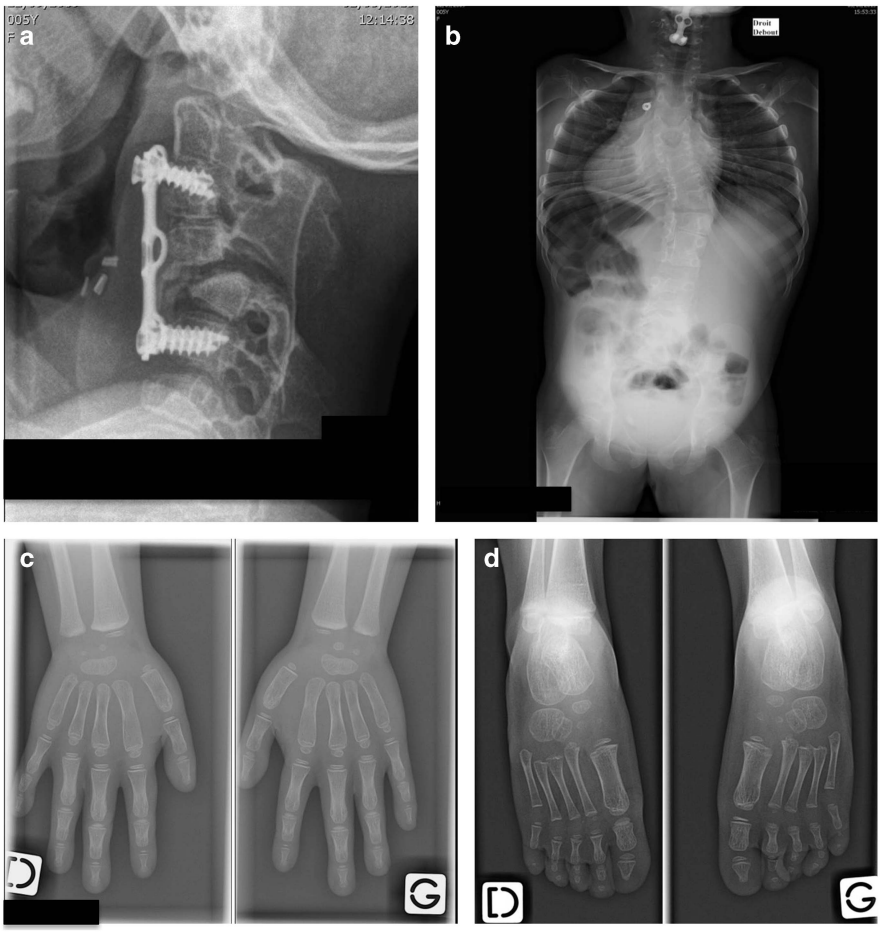

Figure 4 Radiographic examination of patient 2-II.1 at 5 years and 6 months of age (**a**) and at 3 years and 8 months of age (**b**, **c**, **d**). (**a**) Lateral X-ray of cervical spine 3 months after a C3–C6 anterior epiphysiodesis with tibial bone graft and plate fixation showing an incomplete reduction of a C4–C5 dislocation and posterior spinal fusions. (**b**) PA X-ray of thoracolumbar spine showing thoracic vertebral fusions without rib fusions responsible for growth disturbance with a short trunk and a right thoracolumbar curve. (**c**) AP X-rays of hands showing capitate–hamate coalition in both hands. (**d**) Dorsal plantar X-rays of feet where no coalition is observed.

vertebral fusion with cardiac disease and severe feeding difficulties may overlap with our cases,[19] but cardiac findings seem different.

To date, *MYH3* variants have been associated only with autosomal dominant DA) syndromes, including DA1, DA2A, DA2B and autosomal dominant MPS (DA8). Autosomal dominant MPS is a rare form of distal arthrogryposis quite different from the others because of the absence of myotonic facies and severe pterygia possibly associated with skeletal abnormalities reported as scoliosis and vertebral fusions.[18,20–23] In the description of Chong *et al*,[18] vertebral fusions and scoliosis seem very similar to those observed in the present report and the patients share many clinical features including camptodactyly, hypoplastic flexion creases, short stature, ptosis and neck webbing. Although family 2 presents with a carpotarsal coalition that has not been described by Chong *et al*,[18] the important overlapping symptomatology and the fact that both carpal and tarsal fusions have been reported previously in families affected by dominantly inherited MPS indicate that this family has MPS rather than SCT.[20–22] Although hearing loss has been reported in several studies related to DA2A[14,24] and MPS,[25] to our knowledge this feature was never associated with underlying *MYH3* mutations. Altogether, these observations indicate that *MYH3* mutations cause a spectrum of diseases including DA, MPS and SCT. The extensive phenotypic variability of *MYH3*-related diseases may be due to modifier genes or additional mutations. Identification of additional cases would be helpful to clarify the extent of the associated phenotypes.

The *MYH3* gene encodes the skeletal muscle myosin MYH3, composed of a myosin head-like domain (amino acids 1–779), a neck domain (amino acids 779–840) and a long coiled-coil rod domain (amino acids 840–1940), for a majority corresponding to the protein tail (amino acids 1070–1940). In DA1, DA2A and DA2B, all variants described so far are located within the head and neck domains (Figure 5). Variants in the tail domain have so far exclusively been observed in association with bone abnormalities. Such domain-specific phenotypes have previously been shown for *MYH9*, where variants in the motor domain coding region were associated with more severe clinical phenotypes than those in the tail domain coding part.[26] Localization of variants in *MYH7* have also various phenotypic impacts: those associated with myosin storage myopathy modify the distal rod region of the protein, whereas those associated with distal myopathy are located either in the rod or in the globular myosin head coding regions.[27]

Our present data confirm the conclusions of the recent report by Chong *et al*,[18] suggesting that MYH3 plays a role in skeletal development and variants in this protein may perturb this

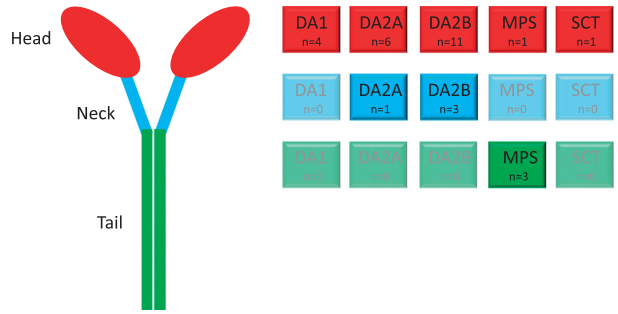

Figure 5 Protein localization of variants that cause *MYH3*-related diseases. The numbers of known variants in each of the three main protein domains (head, neck and tail) are indicated in the boxes of the right panel. DA1, distal arthrogryposis type 1; DA2A, distal arthrogryposis type 2A; DA2B, distal arthrogryposis type 2B; MPS, multiple pterygium syndrome; SCT, spondylocarpotarsal synostosis; *n*, number of variants observed.

development and *in fine* cause MPS or in our case SCT. Several further observations support this hypothesis: (1) *MYH3* transcripts have been detected in bones of 17-week-old fetuses;[18] (2) another skeletal muscle protein – troponin I type 2 (TNNI2) – has a proven role in skeletal development;[28] (3) expression of myosins 2, 4 and 7 is associated with osteoblast activity and differentiation;[29] and (4) severe deformation and failure of maturation of cranial skeletogenesis have been observed in zebrafish mutated in the *myod* gene, a key player in cranial myogenesis.[30]

In conclusion, the present report broadens the phenotype associated with *MYH3* variants to autosomal dominant SCT and confirms the role of MYH3 in bone development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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