Exome Sequencing Identifies a Dominant *TNNT3* Mutation in a Large Family with Distal Arthrogryposis

Sarah B. Daly^{a, b} Hitesh Shah^c James O'Sullivan^{a, b} Beverley Anderson^{a, b}
Sanjeev Bhaskar^{a, b} Simon Williams^{a, b} Nada Al-Sheqaih^{a, b}
Abdul Mueed Bidchol^d Siddharth Banka^{a, b} William G. Newman^{a, b}
Katta M. Girisha^d

^aManchester Centre for Genomic Medicine, University of Manchester, and ^bManchester Centre for Genomic Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK; ^cPediatric Orthopedics Services, Department of Orthopedics, and ^dDepartment of Medical Genetics, Kasturba Medical College, Manipal University, Manipal, India

Key Words

Distal arthrogryposis · Sheldon-Hall syndrome · *TNNT3*

Abstract

Distal arthrogryposis (DA) is a group of rare, clinically and genetically heterogeneous disorders primarily characterized by congenital contractures of the distal limb joints without a neuromuscular disease. Mutations in at least 8 different genes have been shown to cause DA. Here, we report a 4-generation Indian family with 18 affected members presenting variable features of camptodactyly, brachydactyly, syndactyly, decreased flexion palmar creases, ulnar deviation of the hands, sandal gaps and club feet. We undertook exome sequencing of 3 distantly related affected individuals. Bioinformatics filtering revealed a known pathogenic missense mutation c.188G>A (p.Arg63His) in *TNNT3* in all 3 affected individuals that segregated with the phenotype. The affected individuals exhibit significant phenotypic variability. This study demonstrates the value of exome sequencing helping to define the causative variant in genetically heterogeneous disorders.

© 2014 S. Karger AG, Basel

The distal arthrogryposis (DA) syndrome comprises a group of clinically and genetically heterogeneous disorders characterized by congenital contractures of the distal limb joints without neurological and/or muscular disease [Bamshad et al., 1996]. DA is clinically classified into different subtypes (DA1–DA10) [Bamshad et al., 1996; Hall, 1997; Krakowiak et al., 1998] with several overlapping features. The identification of genes causing DA make it apparent that these are overlapping phenotypes caused by many genes involved in the contractile apparatus in skeletal muscle fibers [Beck et al., 2013].

To date, 8 genes have been reported to cause DA: DA1A (OMIM 108120, *TPM2*) [Sung et al., 2003a], DA1B (OMIM 614335, *MYBPC1*) [Gurnett et al., 2010], DA2A (OMIM 193700, Freeman-Sheldon syndrome, *MYH3*) [Toydemir et al., 2006], DA2B (OMIM 601680, Sheldon-Hall syndrome, *TNNI2*, *TNNT3*, *TPM2* and *MYH3*) [Sung et al.,

W.G.N. and K.M.G. contributed equally to this work.

Table 1. Heterozygous variants identified by whole-exome sequencing

Gene	HGVS_cDNA	Amino acid change	Variation name	Protein effect
<i>SDK1</i>	c.5540G>A	p.Arg1847Gln	–	SIFT = tolerated (0.82)/PolyPhen = benign (0.078)
<i>NOD1</i>	c.676G>A	p.Ala226Thr	–	SIFT = tolerated (0.76)/PolyPhen = benign (0.008)
<i>C8orf40</i>	c.125A>G	p.Tyr42Cys	–	SIFT = deleterious (0)/PolyPhen = probably damaging (0.997)
<i>OR52N4</i>	c.323C>G	p.Thr108Ser	–	SIFT = tolerated (0.54)/PolyPhen = benign (0.014)
<i>DNHD1</i>	c.100C>T	p.Gln34Ter	–	not predictable with SIFT/PolyPhen
<i>C14orf135</i>	c.538C>T	p.His180Tyr	–	SIFT = deleterious (0.02)/PolyPhen = probably damaging (0.87)
<i>YLPM1</i>	c.704C>T	p.Pro235Leu	–	SIFT = deleterious (0)/PolyPhen = probably damaging (0.996)
<i>TNNT3</i>	c.188G>A	p.Arg63His	CM031754	SIFT = deleterious (0)/PolyPhen = probably damaging (1)

439 heterozygous variants (with an allele frequency of <1% on the Exome Variant Server) were present in the 3 affected individuals. Eight heterozygous variants were present in 3 individuals and had not been seen in our in-house database of 300 exomes. Of these, one (in *TNNT3*) had a CM reference code indicating that it had been reported in the Human Gene Mutation Database (HGMD). – = Not available.

2003a, b], DA5 (OMIM 615065, *ECEL1*) [McMillin et al., 2013], DA7 (OMIM 158300, trismus-pseudocamptodactyly syndrome, *MYH8*) [Veugelers et al., 2004], and DA9 (OMIM 121050, *FBN2*). Most of the genes are known to encode a contractile protein involved in the formation of fast-twitch muscle contractile myofibers, and mutations in these genes are found to enhance the contractile function in muscles [Robinson et al., 2007].

Here, we describe a large Indian family with DA. We performed whole-exome sequencing in 3 individuals and identified a previously reported pathogenic mutation in all affected family members. In this study, we discuss the clinical features that demonstrate a mild and variable phenotype associated with a *TNNT3* mutation.

Material and Methods

Patients

We ascertained a large family comprising 18 affected individuals (fig. 1). The inheritance pattern is autosomal dominant with a variable expression of DA. On examination, all patients had normal cognitive levels and no physical abnormalities. Blood samples were obtained from 10 affected individuals and 2 healthy family members (III:7 and III:17). Detailed clinical information is available for 4 patients (IV:21, IV:10, IV:7, and IV:3). Photographs are available for 5 additionally affected individuals (II:3, III:1, III:8, III:16, and IV:9). Clinical data and samples were obtained with informed consent for all patients. The study was approved by the ethics committee of the institute. Given that DA is genetically heterogeneous and the phenotypic differentiation is often difficult, we performed whole-exome sequencing in 3 affected individuals.

Exome Sequencing

In order to define a common variant explaining the phenotype, we performed whole-exome capture (Agilent SureSelect 38 Mb) followed by massively parallel sequencing (Illumina HiSeq2000) in 3 most distantly related affected individuals (IV:3, IV:7 and IV:9). Over 3 Gb of sequence was generated for each patient. Reads were aligned

with the human reference genome version GRCh37/Hg19, and >93% of the targeted exome was represented by at least 10-fold coverage. Single-nucleotide substitutions and small insertion and/or deletion variants were identified with our in-house variant-calling pipeline, and exome variant profiles were analyzed with a model of a rare autosomal dominant disorder. We identified a previously reported heterozygous missense mutation in *TNNT3* (NM_006757.3): c.188G>A (p.Arg63His) in the 3 affected sequenced individuals (table 1). The mutation was confirmed by Sanger sequencing to segregate in 7 further clinically affected individuals (II:3, III:1, III:8, III:16, IV:6, IV:10, and IV:21). The mutation prediction tools SIFT (score = 0) and PolyPhen-2 (score = 1) predicted them to be damaging.

Results

Clinical Descriptions

Patient IV:21 was first evaluated at 2 years of age because of limping, secondary to a developmental dysplasia of her hips. Her weight was 10 kg (3rd centile) and her height was 84 cm (25–50th centile). She underwent open reduction of both hips sequentially at the age of 2 years 4 months. She was also noted to have joint laxity.

The girl achieved head control at 3 months, rolled over at 4 months, was able to sit without support at 7 months, stand alone at 1 year and started walking at 1 year and 4 months. She had an unremarkable antenatal, natal and postnatal history. Her birth weight was 2.6 kg (3–10th centile). The girls's cardiorespiratory system was clinically normal. Her complete blood count and urine examination were normal.

At 3 and a half years of age, her height was 122 cm (10–25th centile) and weight 26 kg (50th centile). Her hands showed mild cutaneous syndactyly of F2–5 (fig. 2b). She had mild camptodactyly of the right middle finger, mild clinodactyly, and decreased flexion palmar creases (fig. 2d). Her feet were normal (fig. 2c).

Fig. 1. Pedigree of the family. The three individuals selected for exome sequencing are indicated by red arrows.

Fig. 2. Patient IV:21 has a triangular face (a) and mild cutaneous syndactyly (arrows) of F2–4 (b). Feet are normal (c). Mild camptodactyly of the right middle finger, mild clinodactyly, and decreased flexion creases are also seen (d). Radiograph of the hips show bilateral dislocation of the hip joints (e).

Fig. 3. Patient IV:10 has proximally positioned thumbs, mild cutaneous syndactyly of F2–5 (a), decreased palmar creases and tapering fingers (b). Feet had short big toes, wide sandal gaps and forefoot adduction (c). Radiographs of the patient's hands show contractures of thumbs at metacarpophalangeal joints, partial cutaneous syndactyly (arrows) and clinodactyly (d), and radiographs of the feet show metatarsus varus (e).

Fig. 4. Patient IV:7 has a triangular face (a). The boy has surgical scars due to the correction of his club feet and small toes (b). Mild brachydactyly and mild cutaneous syndactyly with decreased palmar creases are shown (c, d). Radiographs of both feet (before surgery) show bilateral equinus and varus deformities (e, f).

Fig. 5. Clinical photographs of patient IV:3 show a triangular face (a) and surgical scars following correction of club feet and short toes (b). The girl's hands show mild brachydactyly and mild cutaneous syndactyly of F2–5 (c, d). Ulnar deviation of the wrist and

all fingers with camptodactyly and decreased flexion creases of the hands are also seen (d). Lateral radiographs of both feet (before surgery) revealed bilateral congenital vertical talus (e, f).

Patient IV:10 first presented at 12 years of age. She was born to nonconsanguineous parents after an uneventful pregnancy. Her height was 143 cm (10th centile) and weight 36.8 kg (25th centile). She apparently had a normal head size (not measured). Both hands showed partial cutaneous syndactyly, camptodactyly and bilateral clinodactyly (fig. 3a, d). She also had decreased palmar creases and tapering fingers (fig. 3b). Radiographs of her hands revealed contractures of the thumbs at the metacarpophalangeal joints (fig. 3d). Short big toes, wide sandal gaps, and forefoot adductions were also observed (fig. 3c). Radiographs of her feet revealed metatarsus varus (fig. 3e). She apparently had normal intellect and unremarkable cardiac, respiratory and abdominal examination results. Her muscle tone, power and deep tendon reflexes also were normal.

Patient IV:7 had a birth weight of 2.5 kg (3–10th centile). He was born at 8.5 months' gestation by Caesarean section in view of oligohydramnios. He attained head control at 3 months, rolled over at 4 months, sat with

support at 8 months and sat independently at 10 months. He had bilateral club feet. Forefoot and midfoot deformities improved with manipulation (fig. 4b). His height was 73 cm (50th centile) at 10 months and his weight was 9.96 kg (50–75th centile). He had a café au lait spot over the left groin and a fading hemangioma over his forehead. He had an allergic reaction to plaster cast. His cardiorespiratory system was normal. His bone development was without any noticeable problems. His urine and complete blood count were unremarkable. He underwent posteromedial soft tissue release on each foot for correction of residual equinovarus at 1 year 8 months of age. At 4.5 years, he did not have any residual deformities. His face was triangular (fig. 4a). Both hands had proximally placed thumbs, mild brachydactyly and cutaneous syndactyly (fig. 4c). He was also noted to have decreased palmar creases and simian creases bilaterally (fig. 4d). His feet showed brachydactyly, and before correction, his radiographs revealed bilateral equinus and varus deformities (fig. 4e, f).

6

7

Fig. 6. Hands of patient II:3 reveal brachydactyly, camptodactyly and tapering fingers (**a**, **b**), with decreased palmar creases (**b**). Feet show brachydactyly and increased sandal gaps (**c**).

Fig. 7. Patient III:1 almost has normal hands and feet (**a-c**); however, she carries a mutation.

Fig. 8. Clinical photographs of patient III:8 show subtle brachydactyly in hands and feet (a–c).

Patient IV:3 was first examined at 15 months of age. Her height was 72 cm (3–10th centile), weight 7.5 kg (<3rd centile) and her head circumference was 43.5 cm (<–3 SD). She was noted to have contractures of her fingers and toes at birth that improved with physical therapy. She was born by normal delivery with an uncomplicated pregnancy. The child had a triangular face, small deep-set eyes, a bulbous nose and a long philtrum (fig. 5a). She had bilateral congenital vertical talus (fig. 5e, f). Bilateral soft tissue release and realignment of talonavicular joints were done for correction of vertical talus (fig. 5b). Her hands showed mild camptodactyly and brachydactyly (fig. 5c), tapering fingers, and mild cutaneous syndactyly with decreased flexion creases (fig. 5d). She also had ulnar deviation of her wrists (fig. 5d); however, she had itchy (allergic) erythematous scaling lesions due to splinting at 2.5 years. At 3 years, her development was age appropriate, her height was 80.5 cm (<3rd centile) and she weighed 9.5 kg (<3rd centile). The complete blood count and urine examination were normal. At 4 years of

age, her height was 89 cm (<3rd centile) and her weight 11.5 kg (<3rd centile).

Patient II:3 was a 60-year-old-male and had brachydactyly, camptodactyly and tapering fingers (fig. 6a, b). He was also noted to have decreased palmar creases (fig. 6b). His feet had broad big toes, wide sandal gaps and mild brachydactyly (fig. 6c). Patient III:1 was a 35-year-old female. Phenotypically, she had normal hands and feet (fig. 7a–c), but Sanger sequencing revealed the same mutation c.188G>A (p.Arg63His). Patient III:8 presented at age 42 years. She had subtle brachydactyly in hands and feet (fig. 8a–c). Patient III:16 was 35 years old and had mild brachydactyly and decreased palmar creases (fig. 9a, b). He had normal feet (fig. 9c). Patient IV:9, a 22-year-old male, had brachydactyly, clinodactyly and tapering fingers (fig. 10a). His thumbs were short and proximally placed. He also had decreased palmar creases (fig. 10b). Camptodactyly was obvious in his right little finger (fig. 10a, b). His feet were normal (fig. 10c).

Fig. 9. Patient III:16 has very mild brachydactyly and decreased palmar creases (a–c).

Discussion

The family described here has DA with autosomal dominant inheritance. The condition shows variable expression and is almost nonpenetrant in patients III:1, III:8 and III:16. If we had not examined the other affected family members, it would have been impossible to detect the very subtle brachydactyly and decreased palmar creases and attribute this to the diagnosis of DA. The hands in affected members show variable partial cutaneous syndactyly, brachydactyly, camptodactyly, proximally placed thumbs, decreased palmar creases, ulnar deviation of the wrists and tapering fingers. The feet are either normal or show mild brachydactyly and increased sandal gaps. Dislocations of the hips, talipes equinovarus, vertical talus and forefoot adduction (metatarsus varus) are occasion-

ally observed. All affected individuals had a triangular face and normal intellect. Clinically, heart, lungs, abdomen, muscle power and tone were normal. Motor and sensory functions were also normal.

The family fulfils the criteria for diagnosis of DA with ulnar deviation, camptodactyly and hypoplastic flexion creases in the upper limbs and talipes equinovarus, metatarsus varus and vertical talus in the lower limbs. However, absence of a 'whistling face', cleft palate, scoliosis, ophthalmic manifestations, sensori-neural hearing loss, trismus, pseudocamptodactyly, multiple pterygium and crumpled ears in any of the family members clinically assign this family to DA1. Consistent with DA, the family exhibits a wide variability of clinical expression. The mildest end of the spectrum is compatible with reduced penetrance of the condition.

Fig. 10. Hands of patient IV:9 show brachydactyly, clinodactyly and tapering fingers with decreased palmar creases. Thumbs are short and proximally placed. Camptodactyly is more pronounced in the right little finger (**a, b**). Feet are normal (**c**).

We observed severe hip involvement in one affected individual (IV:21). Patient IV:21 demonstrated subtle facial dysmorphism, which would not exclude a diagnosis of mild DA2B. As of now, *TPM2* is the only gene associated with DA1 [Sung et al., 2003a]. As previously defined [Bamshad et al., 1996], the boundaries between the different subtypes of DA are indistinct but have been used to aid management, counseling and investigation of the etiology of these genetically and phenotypically related conditions.

Mutations in *TNNT3* have been reported to cause DA [Sung et al., 2003b; Gurnett et al., 2009; Zhao et al., 2011; Beck et al., 2013]. The phenotypes were described as DA2B (Sheldon-Hall syndrome/Freeman-Sheldon variant) [Sung et al., 2003b; Zhao et al., 2011; Beck et al., 2013] and DA1 [Gurnett et al., 2009; Beck et al., 2013]. Hence, it appears that mutations in *TNNT3* are likely to cause both DA1 and DA2B. As facial features distinguish DA1 from DA2, DA1 can be viewed to represent DA2 with a normal or mild facial phenotype.

Only 8 kindreds have been documented carrying a *TNNT3* mutation. All mutations involved the amino acid position 63 (c.187C>A: p.Arg63Ser [Beck et al., 2013]; c.187C>T: p.Arg63Cys [Zhao et al., 2011; Beck et al., 2013]; c.188G>A: p.Arg63His [Sung et al., 2003b; Gurnett et al., 2009; Beck et al., 2013]). This highlights the critical role of arginine at position 63 of the tropomyosin T3 protein. Alteration of the arginine at this position disturbs the interaction with tropomyosin, which in turn increases the calcium ions in the skeletal muscle and the contractility of the muscles and may cause the development of contractures and limb deformities [Robinson et al., 2007].

We compared the clinical features of patients with a *TNNT3* mutation at codon 63 (table 2). Only Zhao et al. [2011] have described clinical features of 5 of their patients. We could note that brachydactyly, partial cutaneous syndactyly, broad big toes and sandal gaps are newly recognized features of this condition.

Table 2. Comparison of phenotypes of individuals with a mutation at codon 63 of *TNNT3*

Patient No.	Zhao et al. [2011]: mutation c.187C>T; p.Arg63Cys					Our study: mutation c.188G>A; p.Arg63His			
	III:1	I:1	II:1	II:3	III:2	IV:21	IV:10	IV:7	IV:3
<i>Craniofacial features</i>									
Triangular face	+	+	+	+	–	+	+	+	+
Long philtrum	ND	ND	ND	ND	ND	+	+	+	+
Micrognathia	ND	ND	ND	ND	ND	+	+	+	+
Small mouth	+	+	+	+	–	–	–	–	+
Down-slanting palpebral fissures	+	+	+	+	–	–	–	–	–
Preauricular tags	+	ND	ND	ND	ND	–	–	–	–
Broad nasal root	ND	ND	ND	ND	ND	–	–	+	+
<i>Skeletal features</i>									
Ulnar deviation of fingers	+	ND	ND	ND	+	–	–	–	+
Camptodactyly	+	+	+	–	–	+	+	–	+
Brachydactyly (fingers and toes)	–	–	–	–	–	+	+	+	+
Cutaneous syndactyly	–	–	–	–	–	+	+	+	+
Clinodactyly	–	–	–	–	–	+	+	–	–
Overlapping fingers	+	ND	ND	ND	ND	–	–	–	–
Decreased flexion creases	+	ND	ND	ND	ND	+	+	+	+
Adducted thumbs	+	ND	ND	ND	ND	–	+	–	+
Talipes equinovarus (club foot)	+	+	+	+	+	–	+	+	+
Vertical talus	+	+	+	+	+	–	–	–	+
Broad big toes	–	–	–	–	–	+	+	+	+
Sandal gaps	–	–	–	–	–	+	+	–	–
<i>Other features</i>	–	–	–	*	–	**	***	–	–

* Short middle fingers on both hands, which were the same length as the index and ring fingers; ** bilateral dislocation of hip joints; *** tapering fingers.
 + = Observed; – = not observed; ND = not determined.

This family also highlights that exome sequencing can be a useful diagnostic tool for investigating heterogeneous conditions such as DA, where a definitive clinical diagnosis can be extremely difficult.

To summarize, we report a large Indian family with DA (DA1 or a milder variant of DA2B), demonstrating a wide intra-familial variability of the phenotype. This is the ninth kindred carrying a mutation in *TNNT3*. Arginine at position 63 was disturbed in all 9 families, highlighting its crucial role in maintaining joint mobility in distal limbs in humans.

Acknowledgements

We thank all the family members for participating in this study. K.M.G. is grateful to the Manipal University for supporting this work through Dr. T.M.A. Pai Endowment Chair in Mendelian Genetics. We also acknowledge the support of the Manchester Biomedical Research Centre.

References

- Bamshad M, Jorde LB, Carey JC: A revised and extended classification of the distal arthrogryposes. *Am J Med Genet* 65:277–281 (1996).
- Beck AE, McMillin MJ, Gildersleeve HI, Kezele PR, Shively KM, et al: Spectrum of mutations that cause distal arthrogryposis types 1 and 2B. *Am J Med Genet A* 161A:550–555 (2013).
- Gurnett CA, Alaei F, Desruisseau D, Boehm S, Dobbs MB: Skeletal muscle contractile gene (*TNNT3*, *MYH3*, *TPM2*) mutations not found in vertical talus or clubfoot. *Clin Orthop Relat Res* 467:1195–1200 (2009).
- Gurnett CA, Desruisseau DM, McCall K, Choi R, Meyer ZI, et al: Myosin binding protein C1: a novel gene for autosomal dominant distal arthrogryposis type 1. *Hum Mol Genet* 19:1165–1173 (2010).
- Hall JG: Arthrogryposis multiplex congenita: etiology, genetics, classification, diagnostic approach, and general aspects. *J Pediatr Orthop B* 6:159–166 (1997).
- Krakowiak PA, Bohnsack JF, Carey JC, Bamshad M: Clinical analysis of a variant of Freeman-Sheldon syndrome (DA2B). *Am J Med Genet* 76:93–98 (1998).

- McMillin MJ, Below JE, Shively KM, Beck AE, Gildersleeve HI, et al: Mutations in *ECEL1* cause distal arthrogryposis type 5D. *Am J Hum Genet* 92:150–156 (2013).
- Robinson P, Lipscomb S, Preston LC, Altin E, Watkins H, et al: Mutations in fast skeletal troponin I, troponin T, and beta-tropomyosin that cause distal arthrogryposis all increase contractile function. *FASEB J* 21:896–905 (2007).
- Sung SS, Brassington AM, Grannatt K, Rutherford A, Whitby FG, et al: Mutations in genes encoding fast-twitch contractile proteins cause distal arthrogryposis syndromes. *Am J Hum Genet* 72:681–690 (2003a).
- Sung SS, Brassington AM, Krakowiak PA, Carey JC, Jorde LB, Bamshad M: Mutations in *TNNT3* cause multiple congenital contractures: a second locus for distal arthrogryposis type 2B. *Am J Hum Genet* 73:212–214 (2003b).
- Toydemir RM, Rutherford A, Whitby FG, Jorde LB, Carey JC, Bamshad M: Mutations in embryonic myosin heavy chain (*MYH3*) cause Freeman-Sheldon syndrome and Sheldon-Hall syndrome. *Nat Genet* 38:561–565 (2006).
- Veugelers M, Bressan M, McDermott DA, Wermowicz S, Morton CC, et al: Mutation of perinatal myosin heavy chain associated with a Carney complex variant. *N Engl J Med* 351:460–469 (2004).
- Zhao N, Jiang M, Han W, Bian C, Li X, et al: A novel mutation in *TNNT3* associated with Sheldon-Hall syndrome in a Chinese family with vertical talus. *Eur J Med Genet* 54:351–353 (2011).