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Spectrum of Mutations that cause Distal Arthrogryposis Types 1 and 2B

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Abstract

The distal arthrogryposis (DA) syndromes are a group of disorders characterized by non-progressive congenital contractures of the limbs. Mutations that cause distal arthrogryposis syndromes have been reported in six genes, each of which encodes a component of the contractile apparatus of skeletal myofibers. However, these reports have usually emanated from gene discovery efforts and thus potentially bias estimates of the frequency of pathogenic mutations at each locus. We characterized the spectrum of pathogenic variants in a cohort of 153 cases of DA1 (n = 48) and DA2B (n = 105). Disease-causing mutations in 56/153 (37%) kindreds including 14/48 (29%) with DA1 and 42/105 (40%) with DA2B were distributed nearly equally across *TNNI2*, *TNNT3*, *TPM2*, and *MYH3*. In *TNNI2*, *TNNT3*, and *TPM2* the same mutation caused DA1 in some families and DA2B in others. We found no significant differences among the clinical characteristics of DA by locus or between each locus and DA1 or DA2B. Collectively, the substantial overlap between phenotypic characteristics and spectrum of mutations suggest that DA1 and DA2B should be considered phenotypic extremes of the same disorder.

Keywords

arthrogryposis; distal arthrogryposis; distal arthrogryposis type 2B; distal arthrogryposis type 1; contracture; clubfoot; congenital vertical talus; congenital limb deformities; congenital foot deformities; congenital hand deformities; congenital upper extremity deformities; congenital lower extremity deformities; musculoskeletal abnormalities; human *TNNI2* protein; human *TNNT3* protein; human *TPM2* protein; human *MYH3* polypeptide; troponin I; troponin T; myosin heavy chains; muscle; skeletal muscle

INTRODUCTION

The distal arthrogryposis (DA) syndromes are a group of ten autosomal dominant disorders (DA1-DA10) characterized mainly by non-progressive congenital contractures of the upper

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and lower limbs [Bamshad et al., 1996b; Krakowiak et al., 1998]. These different distal arthrogryposes were delineated in large part as a heuristic to define phenotypically homogenous subsets of families for positional cloning efforts. To this end, gene discovery studies suggested that the two most common DAs, DA1 (OMIM 108120 <http://www.omim.org>) and DA2B (Sheldon-Hall syndrome [SHS], OMIM 601680 <http://www.omim.org>) were etiologically distinct conditions: DA1 caused by mutations in *TPM2* while mutations in *TNNI2* and *TNNT3* caused DA2B. However, more recently, we and others have identified mutations in *TPM2* that cause DA2B, and found that DA1 can also be caused by mutations in *TNNI2* or *TNNT3*. These results suggest that DA1 and DA2B, rather than distinct conditions, represent instead the phenotypic extremes of the same phenotypically variable and genetically heterogeneous condition. To better understand the extent of genetic heterogeneity underlying DA1 and DA2B, we performed Sanger sequencing of all coding exons of *TNNI2*, *TNNT3*, *TPM2*, and *MYH3* in a cohort of 153 probands with DA1 (n = 48) or DA2B (n = 105) [Sung et al., 2003a; Sung et al., 2003b; Toydemir et al., 2006].

MATERIALS AND METHODS

Patients

All studies were approved by the Institutional Review Board at the University of Washington and at the Seattle Children's Research Institute. Assent was obtained for all children ages 7 to 17 years of age, and informed consent was obtained from all other participants. Cases were ascertained from general genetics clinics and by direct referral from clinical geneticists and other health-care providers in the United States, Europe and elsewhere and by self-referral. Phenotypic data were collected from a self-administered questionnaire, review of medical records, phone interviews and photographs. The questionnaire was designed to solicit information about family history, prenatal history, physical characteristics, psychosocial development, and medical/surgical interventions (see supplemental information). The diagnosis of DA1 or DA2B was made by review of the phenotypic data and photographs using established diagnostic criteria [Bamshad et al., 1996a; Bamshad et al., 1996b; Krakowiak et al., 1997; Krakowiak et al., 1998].

DA1 and DA2B share some clinical characteristics including overlapping fingers at birth, camptodactyly, ulnar deviation, hypoplastic and/or absent flexion creases and positional foot deformities such as talipes equinovarus, calcaneovalgus deformities, a vertical talus, metatarsus varus, and/or camptodactyly. However, DA1 and DA2B can be, in some cases, distinguished from one another based on diagnostic criteria that we developed previously. These criteria include the absence of facial contractures in most individuals with DA1 and the presence of mild to moderate facial contractures in DA2B. Additionally, calcaneovalgus deformities appear to be more common in DA2B than DA1. Nevertheless, making the distinction between DA1 and DA2B based on clinical criteria alone is often challenging.

Mutation Analysis

DNA was extracted from blood or buccal samples with the Gentra PureGene kit (Qiagen Inc., Valencia, CA). Primers were designed for all coding regions of *TNNI2*, *TNNT3*, and *TPM2* using Primer3 (<http://frodo.wi.mit.edu/primer3/>). Primers used for sequencing *TNNI2*, *TNNT3* and *TPM2* are in eTable SI (See Supporting Information online). Primers for *MYH3* have been described previously [Veugelers et al., 2004; Toydemir et al., 2006]. Each coding exon and exon-intron boundary was amplified with Taq polymerase (Life Technologies Corp., Carlsbad, CA) and standard protocols. Amplicons were cleaned using exonuclease I (New England Biolabs, Ipswich, MA) and shrimp alkaline phosphatase (USB Corp., Cleveland, OH). Cleaned amplicons were Sanger sequenced using BigDye

Terminator v3.1 (Applied Biosystems, Inc.). Sequencing fragments were purified on a Sephadex G-50 (Sigma-Aldrich, St. Louis, MO) column in a 96-well format using dye terminator removal plates (EMD Millipore, Billerica, MA). Purified fragments were run on an automated sequencer (ABI 3130xl, Life Technologies Corp.). Electropherograms of both forward and reverse strands were manually reviewed with CodonCode Aligner version 3.5.6 (CodonCode Corp., Dedham, MA).

Sequencing was performed on the proband of each family. If a putative mutation was identified, all additional family members from whom DNA was available were tested to determine whether the variant had arisen *de novo* in putatively sporadic cases or whether it segregated only in all the affected individuals in multiplex families. Pedigrees for newly identified families with mutations in *TNNI2*, *TNNT3*, *TPM2*, and *MYH3* are found in eFigures S1–S4 respectively, and chromatograms of novel mutations are shown in eFigure S5 (See Supporting Information online). Each proband in whom a mutation was not identified in *TNNI2*, *TNNT3*, *TPM2*, and *MYH3* was also screened by Sanger sequencing for mutations in *TPM1* and *TNNC2*.

RESULTS

All protein-coding exons and intron-exon boundaries of *TNNI2*, *TNNT3*, *TPM2*, and *MYH3* were sequenced in a cohort of 153 probands with DA1 (n = 48) and DA2B (n = 105) (eTable SII). This cohort included 86 simplex cases and 67 familial (i.e., parent-child) cases. Variants that were inferred to be disease-causing were identified in 56/153 (37%) kindreds (Table I) including 14/48 (29%) with DA1 and 42/105 (40%) with DA2B. None of these variants were found in dbSNP (build 132, <http://www.ncbi.nlm.nih.gov/projects/SNP/>), the 1000 Genomes Project pilot data, or >10,000 chromosomes sequenced as part of the NHLBI Exome Sequencing Project (ESP Exome Variant Server, <http://evs.gs.washington.edu/EVS/>). In total, pathogenic variants were found at 30 sites. Additionally, there were 7 sites at which recurrent mutations were observed.

Distal arthrogryposis type 1

Fourteen mutations at 11 different sites that cause DA1 were found in *TNNI2* (3/48), *TPM2* (3/48), *TNNT3* (4/48), and *MYH3* (4/48) (Tables I and II, eFigure S6). All three of the mutations found in *TNNI2* (p.E167del, p.R174Q and p.K176del) occurred in exon 7, the terminal exon, and were found in familial cases. All three of these mutations have previously been reported only in families with DA2B [Sung et al., 2003a; Jiang et al., 2006; Kimber et al., 2006; Shrimpton and Hoo, 2006]. Both of the mutations found in *TNNT3* that caused DA1 (p.R63C and p.R63H) have been reported previously [Sung et al., 2003b; Zhao et al., 2011], but only in cases with DA2B. Specifically, the p.R63H variant was found in a sporadic DA1 case and a multiplex kindred with seven affected members in four generations and the p.R63C variant was identified in two families in each of which affected individuals with DA1 spanned three-generations. All three mutations found in *TPM2* (p.R91G, p.E97K and p.R133W) were missense variants that occurred in familial DA1 cases. The p.E97K missense variant was novel, while p.R91G had been previously reported in a multiplex kindred with DA1 [Sung et al., 2003a], and p.R133W in a mother and her daughter with DA2B [Tajsharghi et al., 2007]. Two families with DA1 were found to have the same novel p.G184A variant in *MYH3*, and another family had a novel p.K504N variant in *MYH3*. These two novel *MYH3* variants were each found in familial DA1 cases. The p.A234T variant has previously been reported in a family with DA2B [Tajsharghi et al., 2008]. Consistent with the observations that mutations in *MYH3* can cause DA1 or DA2B, a missense variant in *MYH3*, p.F437I, has also been described previously in a family with DA1 [Alvarado et al., 2011]. However, the p.A234T variant is the only mutation thus far observed in both DA1 and DA2B.

Distal arthrogryposis type 2B

Mutations that cause DA2B were found in 42/105 (40%) families and in four different genes including *TNNI2* (11/105), *TNNT3* (8/105), *TPM2* (11/105), and *MYH3* (12/105) (Tables I and II, eFigure S6). All mutations in *TNNI2* were found in the terminal exon and included five in familial cases and six in sporadic cases. The R156* mutation was recurrent in two familial and three sporadic cases, while the p.R174Q mutation was recurrent in two familial and one sporadic case. These two recurrent mutations in *TNNI2* (p.R156* and p.R174Q) were reported previously in families with DA2B [Sung et al., 2003a; Shrimpton and Hoo, 2006]. Seven DA2B families had either the p.R63H or p.R63C variant in *TNNT3* that have been observed in DA1. In addition, a novel amino acid substitution at the same arginine residue (p.R63S) was found to cause DA2B in a multiplex family. In one family with a p.R63C mutation, an apparently unaffected sister of the proband shared the same putatively pathogenic variant even though it was present in neither parent (eFigure S3). Gonadal mosaicism has been previously postulated to explain the occurrence of multiple affected sibs born to unaffected, mutation-negative parents in DA2B [Sung et al., 2003a]. In this instance, the unaffected sister would also have to be non-penetrant. Of 11 families with mutations in *TPM2*, five had the same p.R133W variant that has been reported previously in DA2B [Tajsharghi et al., 2007]. Six of the mutations in *TPM2* were each observed just once, including three in sporadic cases with *de novo* missense mutations (p.D100G, p.E145K, and p.Y261C), one in a sporadic case with an in-frame nine basepair deletion (p.Q47_K49del), and two in familial cases with inherited missense mutations (p.D20H, p.R105P). Mutations in *MYH3* have previously been reported to be the most common cause of DA2B [Toydemir et al., 2006], but we discovered an almost equal number of cases caused by mutations in *TNNI2* and *TPM2*. Four families with DA2B had novel mutations in *MYH3* (p.G246A, p.L340Q, p.F466C, p.F835del). The p.G246A and p.F466C *MYH3* missense mutations and the in-frame single amino acid deletion p.F835del arose *de novo*.

Genotype-Phenotype Relationships

Mutations in *TNNI2*, *TNNT3*, *TPM2* and *MYH3* were found to cause both DA1 and DA2B. While the fraction of DA1 vs. DA2B families explained by mutations in each gene differed (Table I and Figure S6), these differences were not significant ($p > 0.35$; Fisher's exact test). Furthermore, in *TNNI2*, *TNNT3*, and *TPM2*, the same mutation caused DA1 in some families and DA2B in others (Table II).

To more objectively compare clinical characteristics and the overall severity of DA1 and DA2B among affected individuals with different disease-causing mutations, we developed a quantitative phenotypic score summarizing the physical findings and natural history (Table S3). Because the diagnosis of DA2B depends on the presence of non-limb findings, we also distinguished phenotypic scores based only on limb vs. only on non-limb findings. As expected, the mean phenotypic score for probands with DA1 vs. probands with DA2B was significantly different ($p = 0.007$; Student's *t*-test) as were the mean phenotypic scores for limb traits ($p = 0.02$; Student's *t*-test) and non-limb traits ($p = 0.02$; Student's *t*-test) suggesting that the lower mean phenotypic score for DA1 vs. DA2B is explained by differences in both limb and non-limb findings (Figure S7).

We first tested whether phenotype scores varied between DA1 and DA2B probands by locus (eFigure S8) and found no significant differences among DA outcomes caused by mutations in *TNNI2*, *TPM2*, *TNNT3* and *MYH3* ($p = 0.44$; ANOVA). We repeated the analysis for DA1 and DA2B separately, and again, observed no significant differences among phenotypic scores by locus for either DA1 or DA2B. These analyses were repeated using only all affected family members both inclusive and exclusive of probands with similar results (eFigure S9 and eTable S4). Finally, we decomposed the phenotypic scores by

clinical feature and found no significant difference in the observation of any individual clinical finding by locus in individuals with DA1 and DA2B (eFigure S10) in probands only or in all affected individuals. Accordingly, we found no compelling empirical evidence that disease locus is predictive of phenotypic outcome—at least as quantified herein.

DISCUSSION

We have expanded the spectrum of mutations in *TNNI2*, *TNNT3*, *TPM2* and *MYH3* that cause DA1 and DA2B and explored the relationship between each causative locus and some of the major, objective phenotypic characteristics of the distal arthrogryposes. We found no significant differences among the clinical characteristics of DA by locus or between each locus and DA1 or DA2B. Given the substantial overlap between phenotypic characteristics and the spectrum of mutations that cause DA1 and DA2B, it is plausible that DA1 and DA2B be considered phenotypic extremes of the same disorder. Nevertheless, delineation as separate disorders could continue to be useful for clinical management, particularly if there was a substantial genotype phenotype relationship consistent with differences in clinical characteristics, natural history, and/or outcomes. Additionally, since the genetic basis can be explained in less than 50% of DA1 and DA2B cases it is certainly plausible that genotype – phenotype relationships exist but have yet to be discovered.

The majority of variants found to cause DA1 and DA2B are novel missense mutations, and appear to arise *de novo*. While mutations that cause DA1 and DA2B are found throughout *TPM2* and *MYH3*, mutations in *TNNI2* and *TNNT3* are limited to a single exon or a single amino acid, respectively. A stepwise approach in which these regions are the first screened might be a reasonable approach to diagnostic testing. However, capture of all exons followed by next-generation sequencing would be more comprehensive and likely to be less costly over the long term.

Comparison of the objective clinical characteristics among mutation-positive cases allowed us to explore the relationship between each causal locus and DA1/DA2B phenotype. Overall, the clinical characteristics of mutation-positive cases did not differ by locus for limb traits, non-limb traits, or overall phenotype. However, the phenotypic data available to us for analysis were limited and, for many cases, we lacked specific information about each contracture that was present. Analysis of genotype-phenotype relationships using both a larger set of DA1/DA2B cases, and with access to more comprehensive phenotypic information would be valuable.

No causal mutation could be identified in 97 of the cases referred to us with a diagnosis of DA1/DA2B. As expected a higher fraction of cases in this subset are sporadic (64%) rather than familial (36%) cases. In these mutation-negative cases, we screened by Sanger sequencing all the exons and exon-intron boundaries of *TPM1* and *TNNC2* as well as exons of *MYH1*, *MYH2*, *MYH4*, *MYH7*, and *MYH8* homologous to those in *MYH3* in which mutations causing DA2B are found. No additional pathogenic changes were found (data not shown).

The proteins encoded by the genes discovered to cause DA are perhaps both qualitatively and quantitatively the most important molecules in skeletal muscle, as they bring about the production of force. The mechanism(s) by which the function of these proteins is impaired to cause congenital contractures is unknown. Nearly all of the different mutations that cause DA1 (i.e., 12/12) and DA2B (28/29) are missense mutations or a short in-frame deletion (of one to three amino acids) consistent with the hypothesis that the contractile apparatus of skeletal myofibers is disrupted by either a gain of function or dominant-negative impairment of function.

Unlike conventional myopathies/muscular dystrophies caused by defects of sarcomeric proteins: (1) weakness, hypotonia, and muscle wasting are not characteristic of DA, (2) histological exam of skeletal muscle in individuals with DA is typically normal, (3) the course of disease in DA is not typically progressive and (4) most persons with conventional myopathies/muscular dystrophies do not exhibit congenital contractures. For these reasons, we strongly advocate that DA syndromes are unconventional myopathies and may be the largest group of known primary disorders of skeletal muscle contractile performance. Characterizing the spectrum of mutations that cause DA1 and DA2B is a small but important first step toward understanding the mechanism(s) that underlies congenital contractures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Summary of Genes Mutated in DA1 or DA2B

DA type	Number of kindreds screened (n =)	TNNI2	TNNT3	TPM2	MYH3	Total mutation positive	Percent mutation positive	Total mutation negative
DA1	48	3	4	3	4	14	29%	34
DA2B (SHS)	105	11	8	11	12	42	40%	63
Total =	153	14	12	14	16	56	37%	97

Table II

Mutations Found in *TNNI2*, *TNNT3*, *TPM2*, and *MYH3* in DA1 and DA2B (SHS)

DA type	Gene	cDNA alteration ^a	predicted protein alteration	Total # kindreds	Published kindreds	New kindreds	References/Notes	
DA1	TNNI2	c.496_498delGAG	p.E167del	1	-	1	Reported in DA2B only	
		c.521G>A	p.R174Q	1	-	1	Reported in DA2B only	
		c.523_525delAAG	p.K176del	1	-	1	Reported in DA2B only	
	TNNT3 ^b	c.187C>T	p.R63C	2	-	2	Reported in DA2B only	
		c.188G>A	p.R63H	2	-	2	Reported in DA2B only	
	TPM2 ^c	c.271C>G	p.R91G	1	1	-	Sung et al. 2003a	
		c.289G>A	p.E97K	1	-	1	novel, <i>de novo</i>	
		c.397C>T	p.R133W	1	-	1	Reported in DA2B only	
	MYH3	c.551G>C	p.G184A	2	-	2	novel	
		c.700G>A	p.A234T	1	-	1	Reported in DA2B only	
c.1309T>A		p.F437I	1	1	-	Alvarado et al. 2011		
c.1512G>T		p.K504N	1	-	1	novel		
Total =				15	2	13		
DA1/DA2B	TNNI2	c.523_525delAAG	p.K176del	2	2		Jiang et al. 2006; Kimber et al. 2006	
		Total =				2	2	0
		c.466C>T	p.R156* ^d	5	2	-	Sung et al. 2003a	
		c.484A>G	p.R162G	1	-	1	novel	
		c.496_498delGAG	p.E167del	1	1	-	Shrimpton & Hoo 2006	
	TNNI2	c.502A>G	p.K168E	1	-	1	novel	
		c.521G>A	p.R174Q	3	2	-	Sung et al. 2003a	
	DA2B		c.525G>T	p.K175N	1	-	1	novel, <i>de novo</i>
			c.187C>A	p.R63S	1	-	1	novel, same aa as R63C/R63H
		TNNT3 ^b	c.187C>T	p.R63C	2	-	2	Zhao et al. 2011
c.188G>A			p.R63H	5	1	-	Sung et al. 2003b	
					-	4		

DA type	Gene	cDNA alteration ^a	predicted protein alteration	Total # kindreds	Published kindreds	New kindreds	References/Notes
		c.58G>C	p.D20H	1	-	1	novel
		c.140_148del9	p.Q47_K49del	1	-	1	novel, <i>de novo</i>
		c.299A>G	p.D100G	1	-	1	novel, <i>de novo</i>
		c.314G>C	p.R105P	1	-	1	novel
	<i>TPM2</i>	c.397C>T	p.R133W	6	1	-	Tajsharghi et al. 2007
					-	5	
		c.433G>A	p.E145K	1	-	1	novel, <i>de novo</i>
		c.782A>G	p.Y261C	1	-	1	novel, <i>de novo</i>
		c.700G>A	p.A234T	1	1	-	Tajsharghi et al. 2008
		c.737G>C	p.G246A	1	-	1	novel, <i>de novo</i>
		c.782C>T	p.S261F	1	1	-	Toydemir et al. 2006
		c.1019T>A	p.L340Q	1	-	1	novel
		c.1123G>A	p.E375K	1	1	-	Toydemir et al. 2006
		c.1385A>G	p.D462G	1	1	-	Tajsharghi et al. 2008
		c.1397T>G	p.F466C	1	-	1	novel, <i>de novo</i>
	<i>MYH3</i>	c.1549G>T	p.D517Y	1	1	-	Toydemir et al. 2006
		c.2306G>T	p.G769V	1	1	-	Toydemir et al. 2006
		c.2503_2505delTTC	p.F835del	1	-	1	novel; <i>de novo</i> in one generation, inherited in the next generation
		c.2512A>G	p.K838E	1	1	-	Toydemir et al. 2006
		c.2521_2523delCTC	p.L841del	2	2	-	Toydemir et al. 2006
		c.4865A>C	p.D1622A	1	1	-	Toydemir et al. 2006
Total =				46	17	29	
Overall totals =				63	21	42	

^aNumbered with the A of the ATG start codon = 1

^bMultiple isoforms, following the convention of Sung et al. (2003b) NM_006757

^cMultiple isoforms, using NM_003289

^dp.R156* was previously named R156ter

^cT178I was originally reported in Toydemir et al. in both DA2A and DA2B kindreds. However, upon collecting additional longitudinal clinical information, it is now clear that two of the cases originally classified as DA2B fit more appropriately in the DA2A classification.