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Mutations in *DYNC2H1*, the cytoplasmic dynein 2, heavy chain 1 motor protein gene, cause short-rib polydactyly type I, Saldino–Noonan type

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Abstract

The short-rib polydactyly syndromes (SRPS) are autosomal recessively inherited, genetically heterogeneous skeletal ciliopathies. SRPS phenotypes were historically categorized as types I–IV, with type I first delineated by Saldino and Noonan in 1972. Characteristic findings among all forms of SRP include short horizontal ribs, short limbs and polydactyly. The SRP type I phenotype is characterized by a very small thorax, extreme micromelia, very short, poorly mineralized long bones, and multiple organ system anomalies. To date, the molecular basis of this most severe type of SRP, also known as Saldino–Noonan syndrome, has not been determined. We identified three SRP cases that fit the original phenotypic description of SRP type I. In all three cases, exome sequence analysis revealed compound heterozygosity for mutations in *DYNC2H1*, which encodes the main component of the retrograde IFT A motor, cytoplasmic dynein 2 heavy chain 1. Thus

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Conflict of interest

None of the authors have any conflict of interests.

SRP type I, II, III and asphyxiating thoracic dystrophy (ATD), which also result from *DYNC2H1* mutations. Herein we describe the phenotypic features, radiographic findings, and molecular basis of SRP type I.

Keywords

DYNC2H1; dynein cytoplasmic 2 heavy chain 1; IFT-A; intraflagellar transport A; Saldino–Noonan syndrome; short rib polydactyly syndrome; short rib polydactyly syndrome type I

The short-rib polydactyly syndromes (SRPS) are a genetically heterogeneous group of autosomal recessive skeletal ciliopathies that fall with the larger group of inherited osteochondrodysplasias (1). Characteristic findings in SRPS include short horizontal ribs, short limbs and polydactyly. Historically, four distinct SRP types have been recognized: SRP I (Saldino–Noonan syndrome, MIM 263530); SRP II (Majewski syndrome, MIM 263520); SRP III (Verma–Naumoff syndrome, MIM 263510); and SRP IV (Beemer–Langer syndrome, MIM 269860). The current nosology groups SRPS within the ‘Ciliopathies with major skeletal involvement’ category and subcategorizes SRP types I and III together (1). The short-rib dysplasia group also includes chondroectodermal dysplasia (Ellis–van Creveld syndrome or EVC, MIM 225500), oral-facial-digital syndrome type 4 (Mohr–Majewski syndrome, MIM 258860), asphyxiating thoracic dystrophy (ATD or Jeune syndrome, MIM 208500), and thoracolumbar pelvic dysplasia (Barnes syndrome, MIM 187760). Although each SRP subtype was characterized by distinct features, significant phenotypic overlap among cases of different subtypes as well as transitional findings shared among the subtypes led to the suggestion as early as 1977 that SRPS should be considered a single group of disorders that presents along a spectrum of variable phenotypic expression (2–6).

Classification of SRPS cases had primarily been based on the clinical phenotype and radiographic findings in each individual case, with little molecular information available. The identification of mutations in genes that encode proteins involved in ciliary function has facilitated correlating the specific molecular defect with the SRPS subtypes. Mutation analysis has revealed extensive locus and allelic heterogeneity, identifying defects in multiple components of the ciliary apparatus including the dynein motor complex [*DYNC2H1* (OMIM 603297) and *DYNC2LI1* (OMIM 617083)], the IFT-A retrograde transport complex [*WDR19* (OMIM 608151), *WDR34* (OMIM 613363), *WDR35* (OMIM 613602), *WDR60* (OMIM 615462)], *IFT140* (OMIM 614620), *TTC21B* (OMIM 612014) and *TCTEX1D2*], the IFT-B anterograde transport complex [*IFT80* (OMIM 611177) and *IFT172* (OMIM 607386)], basal body and centrosomal proteins [*EVC1* (OMIM 604831), *EVC2* (OMIM 607261), *KIAA0586* (OMIM 610178) and *CEP120* (OMIM 613446)], a planar cell polarity component [*INTU* (OMIM 610621)], as well as kinases and related ciliary molecules [*ICK* (OMIM 612325), *NEK1* (OMIM 604588)], *C21ORF2* (OMIM 603191), among the skeletal ciliopathies. In addition, some related ciliopathies have also been shown to result from mutations in these genes; cranioectodermal dysplasia (OMIM 614378 and 613610) due to abnormalities in *WDR19* and *WDR35*, respectively, and Bardet–

Biedl syndrome (OMIM 209901) due to defects in IFT172. However, thus far no genes have been associated with SRP type I (7).

SRP type I was first characterized in 1972 (8) as a new syndrome delineated in two stillborn siblings with severe thoracic dystrophy, micromelia, hypoplastic long bones, and multiple internal congenital anomalies. Radiographic features included short, horizontal ribs forming a small, narrow chest (more severe than that seen in the other SRP types), polydactyly in most cases, poorly ossified bones in the hands, duplicated calcaneal ossification, marked shortening of the long bones with pointed metaphyses, absent fibulae, small pelvic bones, underdeveloped thoracic and cervical vertebral bodies, vertebral body clefts and advanced sternal bone age despite an overall picture of delayed ossification (4, 8–11). SRP types I and III share some radiographic features, although SRP III has less severe narrowing of the thorax and more rounded metaphyses than seen in SRP I (9).

Extraskelatal clinical manifestations in SRP I vary widely from case to case, but reported characteristics include a hydropic appearance, dolichocephaly, natal teeth, micrognathia, and internal organ anomalies in the genitourinary, cardiovascular, gastrointestinal, and pulmonary systems (8, 11). While multiple studies have reported a predominance of female cases in SRP I, compared with an equal sex distribution in SRP III (12), this was contradicted in a case review of published cases (3). While SRP types I and III are usually classified together, the features distinguishing type I from type III include overall more severe clinical and radiographic abnormalities in SRP type I, including greater organ system involvement.

Mutations in *DYNC2H1* are the most common cause of both SRP and ATD, accounting for about 50% of cases (McInerney–Leo 2014, Valerie, Amy, lots of refs). *DYNC2H1* encodes the 4307 amino acid heavy chain of the cytoplasmic dynein 2 complex. Loss-of-function or missense mutations in *DYNC2H1* occur throughout the length of the gene/protein and most cases have private mutations. The cytoplasmic dynein 2 complex serves as the motor for retrograde IFT-A transport, and thus is responsible for transport of cargo away from the tip of the cilia. The IFT complex serves as an adaptor between cargoes and IFT motors.

Skeletal ciliopathies result from mutations in the genes that encode proteins involved in intraflagellar transport, IFT, a specialized transport process that drives macromolecules from the base to the tip of the cilium (anterograde transport) and from the tip of the cilium back to the cell body (retrograde transport). Transport is mediated by multiprotein complexes that include IFT complexes, the BBSome, IFT motors, and associated cargoes (13).

Heterotrimeric kinesin-II and cytoplasmic dynein-2 are the IFT motors responsible for anterograde and retrograde transport, respectively. The dynein-2 motor complex consists of the heavy chain (DYNC2H1), an intermediate chain (D2IC), a light intermediate chain (D2LIC), and a light chain (LC). The IFT-A complex consists of proteins (IFT144, IFT140, IFT139, IFT122, IFT121, and IFT43) that act as adaptors between the dynein motor and associated cargoes (14). DYNC2H1 plays a direct role in contacting and translocating the dynein 2 complex along the microtubules and loss of the motor leads to accumulation of IFT-B (anterograde) particles that cannot be properly recycled (15, 16). In an analysis of a cohort of patients with skeletal ciliopathies we identified three cases of SRP type I and

describe the phenotypic and radiographic findings, and the molecular basis, of the phenotype, which in each case resulted from compound heterozygosity for *DYNC2H1* mutations.

Materials and methods

Under an approved UCLA human subjects protocol consent was obtained for all individuals and exome sequence analysis carried out at the University of Washington Center for Mendelian Genomics using published methods (17). Briefly, the samples were barcoded, captured using the NimbleGen SeqCap EZ Exome Library v2.0 probe library targeting 36.5 Mb of the genome, and sequenced on the Illumina GAIIX platform with 50 bp paired-end reads. Novoalign was used to align the sequence data to the human reference genome (NCBI build 37) and the Genome Analysis Toolkit (GATK) was used for post-processing and variant calling according to GATK Best Practices recommendations. For each sample, 96% of the targeted bases were covered by at least 10 independent reads and 90% of the targeted bases were covered by at least 20 independent reads. Variants were identified under an autosomal recessive model and filtered against dbSNP137, NIEHS EGP exome samples (v. 0.0.8), exomes from the NHLBI Exome Sequencing Project (ESP6500), 1000 genomes (release 3.20120430), and in-house exome samples. Variants were further compared with known disease-causing mutations in HGMD (2012.2), were annotated using VAX (18) and mutation pathogenicity was predicted using the programs Polyphen (19), Sift (20), Condel (21), and CADD (22). Allele frequencies were calculated based on the frequency of the allele in the 1000 Genomes Project and Exome Variant Server (ESP). Mutations were confirmed by bidirectional Sanger sequencing of amplified DNA from each proband. Sequence trace files were aligned and analyzed using Sequencer.

Results

Clinical presentation of SRP I cases

The three affected fetuses (International Skeletal Dysplasia Registry reference numbers R05-103, R96-212 and R00-264) all presented *in utero*. In two cases (R05-103 and R96-212), the affected fetuses were identified in the second trimester and underwent termination of pregnancy at 16 and 18 weeks, respectively. Postnatal evaluation showed a consistent phenotype that included a very narrow thorax, polydactyly of all limbs, and abdominal ascites. In addition, each affected individual had multiple craniofacial dysmorphisms (Table 1). Case R00-264 presented with the most extensive organ system involvement, which included cleft lip and palate, as well as anomalies of the cardiovascular, gastrointestinal, and genitourinary systems as summarized in Table 1. This individual also had hydrops fetalis. Similarly, case R05-103 also presented with hydrops fetalis, ascites and nuchal edema (Table 1) along with marked pulmonary hypoplasia (lung weight/body weight ratio of 0.001, with normal >0.03 (50th percentile at 18 weeks gestational age) (25). There was limited clinical information available for R96-212 because no autopsy was performed (Table 1). However, like the other cases, abdominal protuberance and ascites and a very narrow thorax were observed along with multiple craniofacial anomalies (Table 1).

Radiographic presentation of SRP I cases

All three cases shared radiographic features that are detailed in Table 2 and Figs. 1 and 2. Ossification of the calvarium, metacarpals, metatarsals, and all phalanges (feet and hands) was severely delayed in all three cases. In the vertebral column, hypoplastic vertebrae, mild platyspondyly with severely widened intervertebral disc spaces and coronal clefts were observed. There was micromelia with bilateral shortening of all long bones in both the upper and lower extremities. Each individual had only 11 ribs visible on X-ray, all of which were horizontal and extremely short. Pelvic abnormalities included ilia with decreased height and increased width. The lower extremities showed ovoid tibiae and underdeveloped or absent fibulae. Two cases exhibited bilateral polydactyly and one case had left hand and foot polydactyly. One case had calcaneal duplicate ossification.

DYNC2H1 mutations cause SRP I

The exome data were filtered to identify all variants in the genes known to be associated with the skeletal ciliopathies. In each of the three SRP I cases, the only skeletal ciliopathy gene in which there were two pathogenic variants found was *DYNC2H1* (NM_001080463), and the affected individuals were each compound heterozygotes for different mutations in the gene (Table 3 and Fig. 3). Case R00-264 was heterozygous for a mutation in exon 42 (c.6834G>T) predicted to cause the amino acid substitution p.Trp2278Cys and alter the third ATP binding and hydrolysis domain of the protein. The second mutation was in exon 74 (c.10886G>C) and is predicted to cause the amino acid substitution p.Arg3629Pro. Case R05-103B harbored mutations in exons 40 (c.6387G>T) and 52 (c.8339T>C), predicted to cause the amino acid substitutions p.Trp2129Cys and p.Leu2780Ser, respectively. Both missense mutations are located within ATP binding and hydrolysis domains (AAA1 and AAA4, respectively). Case R96-212 was heterozygous for a mutation in exon 28 (c.4267C>T) predicted to cause the amino acid substitution p.Arg1423Cys, located in the stem domain, and a nonsense mutation in exon 70 (c.10594C>T) introducing a stop at codon 3532. All six mutations occurred at sites that are highly conserved among species and have CADD scores over 15, predicting the changes to be highly deleterious (22).

Discussion

Shortened limbs, narrow thoraxes, and polydactyly are characteristic of all four types of SRP, which define the perinatal lethal end of the skeletal ciliopathy spectrum, the SRPs. SRP I represents the most severe form of SRP. On radiographic analysis, Saldino and Noonan reported pointed metaphyses in the long bones as characteristic of SRP I (8). We observed pointed metaphyses in one individual and there was variability in the shape of the metaphyseal ends, suggesting that differentiating SRPs based on whether the metaphyses are rounded or pointed may not be that helpful. Lachman and Taybi (11) reported absent fibulae as being characteristic of SRP I, an insight supported by the cohort presented here, in which there were absent fibulae bilaterally in one individual and very small, dysplastic fibulae in the two others. Other reported radiographic findings in SRP type I such as platyspondyly, coronal clefts, and poor mineralization of the cervical vertebral bodies were seen in these cases, suggesting that there are multiple radiographic findings that help to characterize SRP I (Table 2).

The three fetuses presented with significant ascites and hydrops fetalis at early gestational ages. In addition, cardiovascular, gastrointestinal, renal and genital anomalies were identified, showing the important role of *DYNC2H1* and cilia function during fetal development. These abnormalities included failure of proper organogenesis, particularly of the gastrointestinal and genitourinary systems (Table 1). Craniofacial abnormalities included cleft lip (overt cleft and midline notching of the superior lip/alveolar ridge) and palate in two individuals.

All three SRP I cases studied resulted from compound heterozygosity for mutations in *DYNC2H1*, the heavy chain of the cytoplasmic dynein 2 complex, the motor for retrograde IFT transport. All of the mutations are very rare, consistent with the low frequency of SRP in the population, with four of the mutations having not been observed either among SRP cases or reported in the public sequence databases. Five of the mutations are missense changes and all alter highly evolutionarily conserved residues, consistent with pathogenicity. All six mutations were predicted to be deleterious by multiple programs that estimate the effect on protein function. Three of the six mutations were missense changes affecting one of the six ATP binding and hydrolysis domains of the protein, indicating that disruption of the catalytic function of the dynein complex can have dramatic effects on skeletal and organ system development. The clearest evidence of pathogenicity was found in case R96-212, who was a compound heterozygote for a null mutation and a missense change (p.Arg1423Cys) that introduced an unpaired cysteine, and thus a structural alteration, into the stem domain of the dynein 2 complex. The latter change was previously identified in a case of SRP II (23). However, while finding two mutations in all three cases strongly supports pathogenicity in SRP I, it remains unclear from the genotypes why the particular mutation combinations observed had such a profound clinical outcome.

The molecular genetic data support the classification of SRP I and III together, which has been proposed previously based on radiographic and clinical phenotype. While mutations in at least 15 genes have been associated with SRPS, aside from their common involvement in cilia function, precisely how the specific alterations in each of these genes, many with non-overlapping roles in cilia biology, produce SRPS remains unresolved. Among SRPS-associated genes, *DYNC2H1* is the most commonly involved locus (24) and *DYNC2H1* mutations have been found in a broad spectrum of skeletal ciliopathies ranging from the perinatal lethal SRP types I, II, and III to non-lethal ATD cases. Particularly for the missense mutations, which alter residues throughout the protein and affect multiple functional domains, it remains challenging to understand how each mutation exerts its phenotypic effect. However the findings presented here now support *DYNC2H1* as the primary candidate gene to consider when counseling families in which SRP I has occurred.

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Fig. 1.

Anterior/posterior radiographs of three cases of short-rib polydactyly syndromes (SRP) type I (Saldino–Noonan syndrome). Radiographic features characteristic of SRP type I included poor ossification of the calvarium, hands and feet, very short horizontal ribs, platyspondyly, ilia with decreased height and increased width, bilateral shortening of all long bones with ovoid tibiae, under-developed or absent fibulae and polydactyly.

Fig. 2. Lateral radiographs of short-rib polydactyly syndromes (SRP) type I (Saldino–Noonan syndrome). Radiographic features included 11 pairs of very short horizontal ribs, enlarged intervertebral disc spaces, mild platyspondyly and coronal clefts.

Fig. 3.

Cartoon of the dynein-2 consists of a heavy chain (DYNC2H1) protein molecule depicting the locations of the predicted mutations. The scale indicates the length of the protein based on number of amino acids. AAA, ATP binding and hydrolysis domains. The two mutations found for each SRP I case are identified by color, green, R05-103B; blue, R00-264; purple, R96-212.

Table 1

Clinical findings

Case ID (ISDR ^a)	Gestational age at evaluation	Mutation	Extremities	Craniofacial	Pulmonary/thorax	Cardiac	Gastrointestinal	Hydrops	Genitourinary
R00-264	30 wks (stillborn)	c.6834G >T p.Trp2278Cys c.10886G >C, p.Arg3629Pro	Polydactyly left hand and foot	Cleft lip and palate	Pleural effusions	Common atrioventricular canal; enlarged pulmonary trunk; patent ductus arteriosus; small R/L pulmonary main arteries	Anorectal agenesis; incomplete intestinal rotation; gallbladder agenesis	Abdominal ascites; nuchal edema	Malformed urinary bladder; urethral agenesis; left renal agenesis; malformed right kidney; uterus didelphys; double hypoplastic vagina
R05-103B	TAB ^b , 18 weeks	c.6387G>T p.Trp2129Cys c.8339T>C p.Leu2780Ser	Bilateral polydactyly hands and feet	Dolichocephaly; hypertelorism; cleft palate; midline notching of superior lip/ alveolar ridge; low-set ears; multiple oral frenulae				Abdominal ascites; nuchal edema	Absent vaginal opening, clitoris, labia minora and urethral meatus; thick-walled, nodular bladder; bilateral renal cystic dysplasia
R96-212	TAB ^b , 16 weeks	c.4267C >T p.Arg1423Cys c.10594C >T p.Arg3532Ter	Bilateral polydactyly hands and feet	Broad face; flattened nasal bridge; posteriorly- located ears	Narrow thorax			Abdominal protuberance	Female genitalia

^aInternational Skeletal Dysplasia Registry.^bTherapeutic abortion.

Radiographic table

Table 2

Case ID (ISDR ^a)	Cranium	Vertebral column	Upper extremities	Thorax	Pelvis	Lower extremities
R00-264	Slightly delayed calvarial ossification	Delayed ossification of cervical vertebral bodies; coronal clefts; mild platyspondyly with significantly widened IVD spaces	Proportionate micromelia; bilateral shortening of humeri, radii and ulnae, with rounded metaphyses mixed with lateral spikes; lack of ossification of metacarpals and phalanges; unilateral hand polydactyly	Extremely short, horizontal ribs; 11 rib pairs; anterior rib cupping; handlebar clavicles	Decreased height and increased width of ileum, vertical inclination of pubis, horizontal acetabular roofs	Bilateral shortening of femora/hypoplastic ovoid tibiae; severely hypoplastic fibulae; delayed ossification of metatarsals and phalanges; bilateral polydactyly; duplicated calcaneal ossification
R05-103B	Delayed calvarial and facial bone ossification	Mild platyspondyly with widened IVD space, vertebral body hypoplasia, coronal clefts	Proportionate micromelia; bilateral shortening of humeri, radii and ulnae; pointed metaphyses; lack of ossification of metacarpals and phalanges; bilateral post-axial polydactyly	Extremely short, horizontal ribs; 11 rib pairs; mild anterior rib cupping; handlebar clavicles	Decreased height of ileum, hypoplastic acetabular roofs	Bilateral shortening of femora/hypoplastic tibiae/fibulae; cupped femoral metaphyses; lack of ossification of metatarsals and phalanges; bilateral post-axial polydactyly
R96-212	Delayed calvarial ossification, decreased anterior cranial fossa	Mild platyspondyly with significantly widened IVD ^b space	Proportionate micromelia; bilateral shortening of humeri, radii and ulnae; flattened metaphyses; lack of ossification of metacarpals and phalanges; bilateral post-axial polydactyly	Extremely short, horizontal ribs, 11 rib pairs, mild anterior cupping, handlebar clavicles	Trident acetabular roofs; decreased height/increased width of ileum; hypoplastic ischia with duplicated ossification centers	Bilateral shortened femora and tibiae/absent fibulae; cupped metaphyses; lack of ossification of metatarsals and phalanges; bilateral post-axial polydactyly

^aInternational Skeletal Dysplasia Registry.^bIntervertebral disc.

Table 3

DYNC2H1 mutations in SRP1 cohort

Sample ID	Genotype	Mutation	Location	Cons.	CADD	Allele freq.
R00-264	0/1	c.6834G>T, p.Trp2278Cys	Exon 42	4.25	21.8	
	0/1	c.10886G>C, p.Arg3629Pro	Exon 74	4.15	21.2	
R05-103B	0/1	c.6387G>T, p.Trp2129Cys	Exon 40	3.82	22.1	
	0/1	c.8339T>C, p.Leu2780Ser	Exon 52	4.03	17.08	
R96-212	0/1	c.4267C>T, p.Arg1423Cys	Exon 28	4.40	26	0.00003709
	0/1	c.10594C>T, p.Arg3532Ter	Exon 70	4.45	52	0.0001012