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Identification of novel *DYNC2H1* mutations associated with short rib-polydactyly syndrome type III using next-generation panel sequencing

L.S. Chen*, S.J. Shi*, P.S. Zou, M. Ma, X.H. Chen and D.H. Cao

Aristogenesis Center, Hospital of PLA, Shenyang, China

*These authors contributed equally to this study.
Corresponding author: D.H. Cao
E-mail: dhcao427@sina.com

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ABSTRACT. Short rib-polydactyly syndrome type III (SRPS3) is a perinatal lethal skeletal disorder with polydactyly and multisystem organ abnormalities. While ultrasound of the fetus can detect skeletal abnormalities characteristic of SRPS3, the syndrome is often difficult to diagnose before birth. As SRPS3 is an autosomal recessive disorder, identification of the gene mutations involved could lead to the development of prenatal genetic testing as an accurate method of diagnosis. In this study, we describe genetic screening approaches to identify potential abnormalities associated with SRPS3. Karyotype analysis, array comparative genomic hybridization (aCGH), and next-generation panel sequencing were each performed on a fetus showing signs of the disorder, as well as on the mother and father. Karyotype and aCGH results revealed no abnormalities. However, next-generation panel sequencing identified novel mutations in the *DYNC2H1* gene. The fetus was compound heterozygous for both a missense mutation c.8313A > T and a frameshift mutation c.10711_10714delTTTA in the

DYNC2H1 gene, which were inherited from the mother and father, respectively. These variants were further confirmed using Sanger sequencing and have not been previously reported. Our study indicates the utility of using next-generation panel sequencing in screening for novel disease-associated mutations.

Key words: Short rib-polydactyly syndrome type III; *DYNC2H1* gene; Next-generation panel sequencing

INTRODUCTION

Short rib-polydactyly syndrome type III (SRPS3) is a perinatal lethal skeletal disorder with polydactyly and multisystem organ abnormalities. It belongs to a group of syndromes called SRPS of which there are five different types: SRPS I [Saldino-Noonan syndrome (MIM 263530)], SRPS II [Majewski syndrome (MIM 263520)], SRPS III [Verma-Naumoff syndrome (MIM 263510)], SRPS IV [Beemer-Langer syndrome (MIM 269860)], and SRPS V (MIM 614091). SRPS3 is an autosomal recessive disease characterized by a constricted thoracic cage, short ribs, shortened tubular bones, and a 'trident' appearance of the acetabular roof. Polydactyly is variably present. Non-skeletal involvement can include cleft lip/palate as well as anomalies of major organs such as the brain, eye, heart, kidneys, liver, pancreas, intestines, and genitalia. Some forms of the disease are lethal in the neonatal period due to respiratory insufficiency secondary to a severely restricted thoracic cage, whereas other forms are compatible with life. Recently some studies have found that mutations in the genes *WDR34*, *WDR60*, *IFT80*, *NEK1*, and *DYNC2H1* could cause SRPS3 (Table 1).

Here we present a case study of a fetus with short limbs identified during ultrasound examination. We describe the application of molecular screening methods to identify genetic abnormalities with a particular focus on genes associated with the development of short limbs.

Table 1. Mutations of different genes identified in families with short rib-polydactyly syndrome type III (SRPS3).

Origin	Age of affected individual	Gene	Nucleotide change	Amino acid change	Location	Reference
Arabic	Carried to term	*WDR34*	c.1061C > T	p.Thr345Met	Exon 7	Huber et al., 2013
India	Tp at 19 wg	*WDR34*	c.1339C > T	p.Arg447Gln	Exon 8	Huber et al., 2013
Algeria	Tp at 26 wg	*WDR34*	c.1022C > T	p.Ala341Val	Exon 7	Huber et al., 2013
France	Tp at 25 wg	*DYNC2H1*	c.[4610A > G] + [7382G > T]	p.[Gln1537Arg] + [Gly2461Val]	Exon 30 + exon 45	Dagoneau et al., 2009
Madagascar	Tp at 24 wg	*DYNC2H1*	c.[5959A > G] + [10130delT]	p.[Thr1987Ala] + [Leu3377CysfsX34]	Exon 38 + exon 67	Dagoneau et al., 2009
Australia	Tp at 17 wg	*WDR60*	c.[2246C > T] + [1891C > T]	p.[Thr749Met] + [Gln631*]	Exon 15 + exon 14	McInerney-Leo et al., 2013
Brazil	Carried to term	*IFT80*	c.1213G > C	p.Gly241Arg	Exon 8	Cavalcanti et al., 2011
China	Tp at 22 wg	*NEK1*	IVS4-1A > G	Unknown	Unknown	Chen et al., 2012

Tp: terminated preganancy; wg: week of gestation.

MATERIAL AND METHODS

Case subjects

The case subject was a 31-year-old woman at four months of pregnancy. Upon

ultrasound examination, her fetus was observed to have a shortened bilateral long bone and femur, the shaft of the femur was bent, the end of the shaft of the femur was bulky, and the ribs were short and small. The skeletal system of the fetus was abnormal. The woman was previously pregnant with a fetus that exhibited the same ultrasonic detection patterns, so both mother and father (33 years old) were referred to a prenatal diagnosis clinic and chromosomal analysis was recommended. Neither the mother nor father had a history of consanguinity or genetic disease. They did not smoke or drink, and had not been exposed to radiation or chemical insult. Routine blood, urine, liver, and renal function analyses were normal for both, and electrocardiography and chest X-rays showed no abnormalities in the heart and lungs. The pregnancy was later terminated and the aborted fetus was found to have four shortened limbs.

Informed consent was obtained from both parents for participation in this study, and both were made aware that their information may be used in future research. Participant information was not anonymized, and all authors had access to identifying information. The study was approved by the No. 202 Hospital of People's Liberation Army (PLA) Ethics Committee.

Karyotyping

Karyotyping was performed on G-banded metaphases obtained from peripheral blood of the parents and cultured using standard procedures. Karyotyping of amniotic fluid was performed at 18 weeks of pregnancy according to standard techniques (Chandler and Yunis, 1978). Results are described in accordance with the 2013 International System for Human Cytogenetic Nomenclature (ISCN).

Array comparative genomic hybridization analysis

Genomic DNA was extracted from peripheral blood (parents) and amniocytes (fetus) using a DNA extraction kit (Tiangen, China) according to the manufacturer instructions. Array comparative genomic hybridization (aCGH) analysis was performed with oligonucleotide-based custom arrays (Agilent Technologies, Santa Clara, CA, USA) using standard protocols. Briefly, equal amounts of test DNA and normal sex-matched DNA, which is from 100 unrelated health controls, were digested with *Alu*I and *Rsa*I. Next, they were differentially labeled with cyanine-5 and cyanine-3 fluorescent dyes using a SureTag Complete DNA Labeling Kit (Agilent Technologies). Hybridizations were carried out at 65°C for 24 h. After washing, slides were scanned using an Agilent SureScan Microarray Scanner, and the images were extracted and analyzed using Feature Extraction v11.5 and Cytogenomics v2.5 software (Agilent Technologies), respectively.

Next-generation panel sequencing

Targeted sequence capture and next generation sequencing

Targeted sequence capture was performed using a protocol developed at the Beijing Genomics Institute (BGI). In brief, 1 μg genomic DNA was fragmented into lengths of 200 bp followed in sequence by end-repair, A-tailing and adaptor ligation, a 4-cycle pre-capture polymerase chain reaction (PCR) amplification, targeted sequence capture, and 15-cycle post-capture PCR. Targeted sequence capture was performed using custom oligonucleotide

probe sets (NimbleGen, Roche, Basel, Switzerland) which cover the exons and immediately adjacent intron region of 463 genetic short-limbed genes (Table 2). Sequencing was performed on a HiSeq 2000 platform (Illumina, San Diego, CA, USA) as paired end 90 bp according to the manufacturer instructions. The targeted regions were covered by a mean depth of over 190-fold, with 99.45% of the targeted region covered by at least one read and 97.71% by at least 20 reads.

Read mapping, variant detection, annotation, and interpretation

Image analyses, error estimation and base calling were performed using the Illumina Pipeline (version 1.3.4) to generate primary sequence data. Low-quality reads and potential adaptor contaminations were removed from the primary data using a local algorithm. The remaining short-reads were mapped to the human genome (hg18) using Burrows-Wheeler Aligner software (http://sourceforge.net/projects/bio-bwa/). Single nucleotide variants (SNVs) were detected using Short Oligonucleotide Analysis Package single nucleotide polymorphism software (http: //soap.genomics.org.cn/) and small insertions and deletions (InDels) were detected using sequence alignment/maps tools Pileup software (http://sourceforge.net/projects/samtools/). Variants were annotated using a BGI in-house developed annotation pipeline and were interpreted according to the American College of Medical Genetics and Genomics recommended standards (Richards et al., 2008).

Confirmation by Sanger sequencing

Potential mutations identified by next-generation panel sequencing were further confirmed by PCR and Sanger sequencing in the proband and the unaffected parents. The specific PCR primers were designed using Primer5 software (Premier Biosoft International, Palo Alto, CA). The products were directly sequenced with an ABI PRISM BigDye kit on an ABI 3730 DNA sequencer (Applied Biosystems, Carlsbad, CA). Sequencing results were analyzed using the DNASTAR package (DNASTAR, Madison, WI).The novel identified variants were subsequently verified and screened in 100 unrelated healthy controls with DNA sequencing

RESULTS

Karyotyping of G-banded metaphases showed that the karyotype of both parents and the fetus were normal (data not shown). Array-CGH analysis also did not reveal any abnormalities, with no copy number changes associated with known microdeletion or microduplication syndromes observed (data not shown).

However, using next-generation panel sequencing, more than 800 SNVs and InDels were identified in each of the three subjects across the 463 short-limbed related genes analyzed. After filtering for potential polymorphism variants (> 0.5% in the 1000 Genomes database (http://www.1000genomes.org/home) and > 0.5% in BGI in-house database) and non-functional relevance, there were between 16 and 17 non-synonymous variants and splicing site/coding InDels remaining (Tables 3 and 4). Two of these mapped to the *DYNC2H1* gene and displayed a typical disease gene inheritance pattern. The mother had a heterozygous c.8313A > T missense mutation, the father had a heterozygous c.10711_10714delTTTA frameshift mutation, and the fetus was compound heterozygous for both. These variants were further confirmed using Sanger sequencing (Figure 1).

Table 2. 463 short limb genes targeted for next-generation panel sequencing.

Classification of disease	Disease	Targeted Genes	No.	Total No.
Metabolic system	Rickets	*PHEX*, *ENPP1*, *FGF23*, *CLCN5*, *SLC34A3*, *VDR*, *CYP2R1*, *CYP27B1*	8	53
	Lysosomal storage disease	*SLC37A4*, *AGL*, *PFKM*, *PHKA1*, *PHKB*, *PHKA2*, *ALDOA*, *PGAM2*, *G6PC*, *GALNS*, *IDUA*, *IDS*, *ARSB*, *HYAL1*, *GUSB*, *GLB1*, *GNPTG*, *GNPTAB*, *MAN2B1*	19	
	Congenital disorder of glycosylation	*MGAT2*, *SLC35C1*, *TMEM165*	3	
	Others	*GPD1*, *GBA*, *SMPD1*, *MLYCD*, *CTSA*, *UROC1*, *FUCA1*, *PIGO*, *AGA*, *GK*, *CTNS*, *PHGDH*, *HGD*, *ATP7A*, *SLC17A5*, *MPO*, *MVK*, *ADA*, *PCCB*, *PLOD3*, *SLC6A19*, *ACADS*, *SUMF1*	23	
Endocrine system	Growth hormone deficiency	*SOX3*, *GHSR*, *GHRHR*, *GHR*, *BTK*, *GH1*, *SHH*, *SLC29A3*, *NDN*, *CEP57*, *ALMS1*, *SNRPN*	12	46
	Hypothyroidism	*NKX2-5*, *TSHB*, *THRA*, *PAX8*, *TSHR*, *IYD*, *TPO*, *TG*, *DUOXA2*, *DUOX2*, *SLC26A4*, *SLC5A5*, *GCM2*, *SECISBP2*, *THRB*, *TRHR*, *TRH*	17	
	Hyperthyroidism	*TSHR*	1	
	Pituitary hormone deficiency	*PROP1*, *OTX2*, *POU1F1*, *HESX1*, *LHX4*, *LHX3*	6	
	Microcephalic osteodysplastic primordial dwarfism	*PCNT*, *RNU4ATAC*	2	
	Others	*IGF1R*, *GNAS*, *HSD11B2*, *B3GALTL*, *INSR*, *CYP11B1*, *CASR*	7	
Skeletal system	Achondrogenesis	*TRIP11*, *COL2A1*	2	114
	Hypochondroplasia	*FGFR3*	1	
	Chondrodysplasia	*ARSE*, *PTH1R*, *IMPAD1*, *EBP*, *GNPAT*, *PEX7*, *AGPS*, *BMPR1B*, *RMRP*	9	
	Chondrosarcoma	*EXT1*	1	
	Acrodysostosis	*PDE4D*, *PRKAR1A*	2	
	Acromesomelic dysplasia	*GDF5*, *NPR2*	2	
	Brachydactyly	*GDF5*, *ROR2*, *NOG*, *PTHLH*, *HOXD13*, *IHH*, *BMPR1B*, *HDAC4*, *SOX9*	9	
	Polydactyly	*NEK1*, *WDR35*, *DYNC2H1*, *PIK3R2*, *AKT3*	5	
	Epiphyseal dysplasia	*COL9A3*, *COL9A2*, *COL9A1*, *COMP*, *MATN3*, *SLC26A2*	6	
	Metaphyseal anadysplasia	*MMP13*, *COL10A1*, *MMP9*	3	
	Osteogenesis imperfecta	*SERPINH1*, *CRTAP*, *BMP1*, *FKBP10*, *COL1A2*, *LEPRE1*, *IFITM5*, *SERPINF1*, *PPIB*, *COL1A1*, *SP7*	11	
	Spondyloepiphyseal dysplasia	*MMP13*, *PAPSS2*, *MATN3*, *TRAPPC2*, *KIF22*, *ACP5*, *DDR2*, *ACAN*, *COL2A1*, *CHST3*	10	
	Spondylocostal dysostosis	*HES7*, *DLL3*	2	
	Microcephaly	*MCPH1*, *ASPM*	2	
	Arthrogryposis	*MYH3*, *TPM2*, *TNNT3*	3	
	Craniodiaphyseal dysplasia	*IFT43*, *SOST*, *WDR35*, *WDR19*	4	
	Stickler syndrome	*COL2A1*, *COL9A2*, *COL9A1*, *COL11A1*	4	
	Others	*SEC23A*, *MMP2*, *ESCO2*, *RIN2*, *LMX1B*, *GHR*, *SLC35D1*, *EFNB1*, *DYM*, *LIFR*, *GORAB*, *SH3BP2*, *FGFR1*, *TBX15*, *COMP*, *CANT1*, *TFAP2A*, *RUNX2*, *TMCO1*, *CHRNA1*, *CTSK*, *FLNB*, *EVC*, *FBN1*, *B3GAT3*, *EFTUD2*, *EIF2AK3*, *SLC26A2*, *EVC2*, *CHRNG*, *CHRND*, *SMAD4*, *POC1A*, *TBX3*, *WNT7A*, *ERCC6*, *ERCC2*, *FBN1*, *ADAMTSL2*, *EXT2*, *TRPV4*, *SF3B4*, *CA2*, *WDR19*, *GDF6*, *FAM123B*, *TNFRSF11B*, *TNNI2*	48	
Digestive system	Cholestasis	*ATP8B1*, *ABCB11*	2	5
	Colorectal cancer	*BUB1B*	1	
	Hepatocellular carcinoma, somatic	*CASP8*	1	
	Hennekam lymphangiectasia-lymphedema syndrome	*CCBE1*	1	
Skin	Cutis laxa	*ATP6V0A2*, *LTBP4*, *PYCR1*, *ALDH18A1*	4	17
	Dyskeratosis congenit	*DKC1*, *TERT*, *TINF2*	3	
	Trichothiodystrophy	*ERCC3*, *GTF2H5*	2	
	Trichorhinophalangeal syndrome	*EXT1*, *TRPS1*	2	
	Epidermolysis bullosa simplex	*PLEC1*	1	
	Focal dermal hypoplasia	*PORCN*	1	
	Dermatitis	*SLC39A4*	1	
	Poikiloderma	*C16orf57*	1	
	Incontinentia pigmenti	*IKBKG*	1	
	Restrictive dermopathy	*ZMPSTE24*	1	

Continued on next page

Table 2. Continued.

Classification of disease	Disease	Targeted Genes	No.	Total No.
Neuromuscular	Mental retardation	*SLC9A6*, *SMS*, *HCFC1*, *NSUN2*, *RAB40AL*, *CASK*, *KDM5C*, *RPS6KA3*, *CUL4B*	9	36
	Pontocerebellar hypoplasia	*CHMP1A*, *EXOSC3*	2	
	Spastic paraplegia	*AP4S1*, *AP4E1*, *AP4B1*	3	
	Spinocerebellar ataxia	*GRM1*, *ZNF592*	2	
	Muscular dystrophy	*SEPN1*, *LARGE*, *SEPT9*	3	
	Myasthenic syndrome	*RAPSN*	1	
	Myosclerosis	*COL6A2*	1	
	Polymicrogyria	*RTTN*	1	
	Mental retardation	*SOX3*	1	
	Others	*NAA10*, *NF1*, *LRP5*, *ATM*, *SHROOM4*, *SIL1*, *SLC6A8*, *IGBP1*, *SHH*, *NDE1*, *IKBKAP*, *KIF1A*, *VLDLR*	13	
ENT (ear-nose-throat) facial features	Microphthalmia	*BCOR*, *STRA6*, *OTX2*, *SMOC1*, *HCCS*, *SOX2*	6	11
	Enlarged vestibular aqueduct	*FOXI1*, *KCNJ10*	2	
	Deafness with enlarged vestibular aqueduct	*SLC26A4*	1	
	Tooth agenesis	*LTBP3*	1	
	Dent disease	*CLCN5*	1	
Hematological system	Fanconi anemia	*FANCE*, *FANCC*, *FANCA*, *FANCG*, *FANCF*, *RAD51C*, *FANCD2*, *SLX4*	8	19
	Heinz body anemia	*HBB*, *HBA1*	2	
	Anemia	*SLC19A2*, *RPS19*, *KLF1*, *GATA1*	4	
	Leukemia	*RARA*	1	
	Others	*LBR*, *ATRX*, *RBM8A*, *UROS*	4	
Immune system	Immunodeficiency	*LAMTOR2*, *LRBA*	2	6
	Agammaglobulinemia	*BTK*	1	
	Arthropathy, progressive pseudorheumatoid	*WISP3*	1	
	Autoimmune disease	*ITCH*	1	
	others	*SMARCAL1*	1	
Other syndromes	3-M syndrome	*CUL7*, *CCDC8*	2	141
	Bartter syndrome	*KCNJ1*, *SLC12A2*	2	
	Cockayne syndrome	*ERCC8*, *ERCC6*	2	
	Cornelia de Lange syndrome	*SMC1A*, *NIPBL*, *SMC3*, *HDAC8*, *RAD21*	5	
	Ehlers-Danlos syndrome	*ADAMTS2*, *SLC39A13*, *COL1A2*, *B4GALT7*, *COL3A1*, *COL1A1*, *COL5A2*, *COL5A1*	8	
	Feingold syndrome	*MYCN*, *MIR17HG*	2	
	Meier-Gorlin syndrome	*ORC6*, *ORC1*, *CDT1*, *CDC6*, *ORC4*	5	
	Noonan syndrome	*KRAS*, *RAF1*, *BRAF*, *SOS1*, *PTPN11*	5	
	Oral-facial-digital syndrome	*OFD1*, *TCTN3*	2	
	Robinow syndrome	*WNT5A*, *ROR3*	2	
	Rubinstein-Taybi syndrome	*CREBBP*, *EP300*	2	
	Schwartz-Jampel syndrome	*LIFR*, *HSPG2*	2	
	Seckel syndrome	*ATR*, *RBBP8*, *CEP152*, *NIN*, *CEP63*, *CENPJ*	6	
	Weill-Marchesani syndrome	*ADAMTS17*, *FBN1*, *LTBP2*, *ADAMTS10*	4	
	Warburg micro syndrome	*RAB3GAP1*, *RAB3GAP2*, *RAB18*	3	
	Rett syndrome	*FOXG1*, *MECP2*	2	
	Others	*LRP2*, *NKX2-1*, *RAB3GAP2*, *FGD1*, *KCNJ2*, *SBDS*, *L1CAM*, *AAAS*, *BRAF*, *TWIST1*, *NBAS*, *SEMA3E*, *PQBP1*, *SPG20*, *G6PC3*, *HYLS1*, *NBN*, *RAI1*, *IGF2*, *UBR1*, *OCRL*, *CHD7*, *GLA*, *CD96*, *LMNA*, *PHF6*, *HRAS*, *IFT140*, *SRCAP*, *CTC1*, *OTX2*, *ASXL1*, *FTO*, *ANKRD11*, *HSD17B4*, *MED12*, *KIF7*, *DHCR7*, *CTDP1*, *MYH8*, *DDX11*, *POR*, *KAT6B*, *ALDH3A2*, *TP63*, *SDHA*, *ALPL*, *MLL*, *WRN*, *MAP2K1*, *NSDHL*, *FAM20C*, *NSD1*, *H19*, *RECQL4*, *TRIM37*, *ZEB2*, *FOXE1*, *KANSL1*, *BANF1*, *BLM*, *VPS13B*, *RAB23*, *TBX1*, *HPRT1*, *SLC16A2*, *SMARCA2*, *COX4I2*, *MAP2K2*, *NOTCH2*, *CRLF1*, *WFS1*, *ZBTB16*, *ARX*, *DLX5*, *MGP*, *FGFR2*, *TAZ*, *PITX2*, *ACTB*, *PLOD2*, *SLC34A1*, *MBTPS2*, *TMEM237*, *TBCE*, *MKS1*, *GPC6*, *RIPK4*, *FLNA*, *GJB6*, *ERCC3*, *MPV17*, *ATPAF2*, *AQP2*	94	

Neither of these mutations has been previously reported, nor were they observed in the approximately 6000 controls in the 1000 Genomes Project and the BGI in-house database.

Table 3. Mother with 17 mutational sites of non-synonymous variants and splicing site/coding InDels remaining.

Gene	NM_ID	Mut_name	Function	Mut_type
ASPM	NM_018136.4	c.1732G > A	missense	Het
PLOD2	NM_000935.2	c.805G > A	missense	Het
NSD1	NM_172349.2	c.2380A > G	missense	Het
SLC34A1	NM_003052.4	c.533T > A	nonsense	Het
PLEC	NM_000445.3	c.12728G > A	missense	Het
SLC34A3	NM_001177316.1	c.1612C > T	missense	Het
KAT6B	NM_012330.3	c.3270_3278delTGAAGAGGA	cds-del	Het
RAPSN	NM_032645.4	c.457G > T	missense	Het
DYNC2H1	NM_001080463.1	c.8313A > T	missense	Het
PTHLH	NM_198965.1	c.447C > G	missense	Het
FBN1	NM_000138.4	c.3455C > T	missense	Het
SRCAP	NM_006662.2	c.8018C > T	missense	Het
ATP8B1	NM_005603.4	c.1151G > A	missense	Het
GDF5	NM_000557.2	c.979C > G	missense	Het
CTSA	NM_001127695.1	c.1036G > A	missense	Het
PCNT	NM_006031.5	c.6150+3G > A	intron	Het
EP300	NM_001429.3	c.2237A > C	missense	Het

Table 4. Father with 16 mutational sites of non-synonymous variants and splicing site/coding InDels remaining.

Gene	NM_ID	Mut_name	Function	Mut_type
LEPRE1	NM_022356.3	c.1057A > G	missense	Het
NBAS	NM_015909.3	c.656C > G	missense	Het
MPV17	NM_002437.4	c.373C > T	missense	Het
FLNB	NM_001164319.1	c.3583G > A	missense	Het
EVC	NM_153717.2	c.884C > G	missense	Het
GHR	NM_000163.4	c.206C > T	missense	Het
FANCE	NM_021922.2	c.266G > T	missense	Het
GUSB	NM_000181.3	c.10G > A	missense	Het
SLC34A3	NM_001177316.1	c.1612C > T	missense	Het
DYNC2H1	NM_001080463.1	c.10711_10714delTTTA	frameshift	Het
MLL2	NM_003482.3	c.15403A > G	missense	Het
STRA6	NM_001142619.1	c.380-9A > T	intron	Het
SRCAP	NM_006662.2	c.6610-5G > A	intron	Het
CCBE1	NM_133459.3	c.654+5G > C	intron	Het
PCNT	NM_006031.5	c.3557G > A	missense	Het
PCNT	NM_006031.5	c.5582C > T	missense	Het

Het = heterozygous.

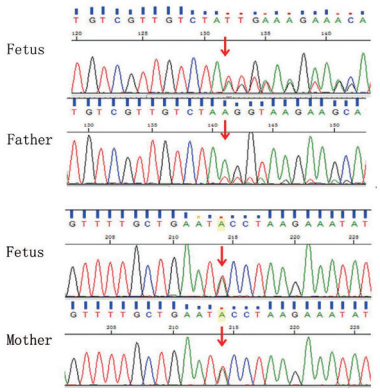

Figure 1. Mutations detected in the *DYNC2H1* gene in the mother, father and fetus. A comparison between all three individuals is shown.

DISCUSSION

The aim of this study was to determine whether a four-month-old fetus with short limbs harbored any genetic abnormalities. Given that the karyotype analysis and aCGH levels were normal in both the fetus and the parents, we suspected a single gene defect. Among a panel of 463 genes associated with short limb development, we identified mutations in the *DYNC2H1* gene which is associated with SRPS3.

The *DYNC2H1* gene is located at chromosome 11q22.3. It encodes a large cytoplasmic dynein protein involved in the structure and function of cilia. Cilia are highly conserved microtubule-based organelles that project from the cell surface into the extracellular environment and play diverse roles in cellular motility, sensory transduction, and signaling. In humans, ciliary dysfunction is associated with a large spectrum of disorders (Bisgrove and Yost, 2006; Eggenschwiler and Anderson, 2007; Tran et al., 2008), including defects in a variety of proteins necessary for intraflagellar transport as well as in components of the primary cilia, basal body, and centrosome. Many of these phenotypes include polydactyly and some include abnormal skeletogenesis. Mutations in *DYNC2H1* have been associated with a heterogeneous spectrum of conditions related to altered primary cilium function that often involve polydactyly, abnormal skeletogenesis, and polycystic kidneys.

To date, there are several reports of *DYNC2H1* mutations being associated with SRPS3. Dagoneau et al. (2009) identified two cases with SRPS3. The first case had compound heterozygosity for mutations c.4610A > G and c.7382G > T in exons 30 and 45, respectively, of the *DYNC2H1* gene. The second case had compound heterozygosity for mutations c.5959A > G and c.10130delT in exons 38 and 67, respectively, of the *DYNC2H1* gene. Merrill et al. (2009) identified three cases with SRPS3. The first case had homozygosity for the mutations c.1759C > T in exon 12 of the *DYNC2H1* gene. The second case had compound heterozygosity for mutations c.6614G > A and c.8512C > T in exons 41 and 53, respectively, of *DYNC2H1*. The third case had compound heterozygosity for mutations c.624_625GT > AA and c.IVS33 + 1G > T in exons 5 and 33, respectively, of *DYNC2H1*. Okamoto et al. (2015) identified compound heterozygosity for mutations c.5682_5683delAA and c.9070C > T in exons 37 and 57, respectively, of the *DYNC2H1* gene in a fetus with SRPS3. Mei et al. (2015) identified compound heterozygosity for mutations c.1151C > T and c.4351C > T in exons 8 and 28, respectively, of the *DYNC2H1* gene in a fetus with SRPS3. All cases showed similar radiographic findings, such as shortened long bones and a narrow thorax.

In our study, the *DYNC2H1* mutations identified have not been previously reported. The c.8313A > T missense mutation results in an arginine to serine substitution at amino acid position 2771. The c.10711_10714delTTTA mutation causes a frameshift that introduces a stop codon at amino acid position 3571, resulting in a truncated protein and possible protein instability. The presence of both of these mutations in the fetus, but only one copy in each of the unaffected parents, follows the pattern of an autosomal recessive disease gene. At the same time, we found the skeletal system of the fetus showed similar characteristic to the reported cases. With the known association between *DYNC2H1* mutations and SRPS3, we conclude that these novel mutations are the cause of SRPS3 in the fetus.

We have identified novel mutations in *DYNC2H1* that appear to be associated with SRPS3. This was achieved using the innovative approach of next generation sequencing to screen for mutations in a panel of genes potentially associated with the disease under study. Similar application of this new technology to other disorders may enhance our ability to

identify disease-associated gene mutations. Furthermore, use of this methodology in prenatal genetic testing could help strengthen our understanding of the genetic causes of birth defects, as well as improve the prospects for early detection.

Conflicts of interest

The authors declare no conflict of interest.

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