Second reported case of Short-Rib-Polydactyly type III in China with a novel mutation of *DYNC2H1* gene detected by Next Generation Sequencing

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Abstract— Short ribs polydactyly syndrome is part of a spectrum of ciliopathies with overlapping syndrome but distinct genes A 31-years old pregnant woman at 17 weeks gestation was referred to our Genetic and Prenatal diagnosis center after having abnormal antenatal ultrasound findings including extremely narrow fetal thorax. Thoracic –to- abdominal circumference ratio was less than 0.6. The upper limbs and lower limbs bones were micromelic ,14 weeks and 15 weeks gestation age respectively, all with polydactyly. Bilateral club feet were noted. Fetal echocardiography revealed a ventricular septal defect. The fetus was aborted and autopsy revealed 11 pairs of ribs that were short, a narrow thorax, short upper limb bones and short lower limb bones. Targeted Next Generation Sequencing identified one heterozygous missense mutation c4625C>A (p.A1542E) for mother and a heterozygous deletion mutation c.11568delT (p.E3857Sfs*82) for father and a compound heterozygous mutations c4625C>A (p.A1542E) and c.11568delT (p.E3857Sfs*82) in fetus This case of SRPS type 3 is the eighth case being reported worldwide and second case in china.

Index Terms— Short ribs polydactyly syndrome, DYNC2H1 gene, next generation sequencing,

1 Introduction

Short rib-polydactyly syndrome (SRPS) is a rare inherited, autosomal recessive, lethal skeletal disorder characterized by a constricted thoracic cage, short ribs, frequent pre- and postaxial polydactyly and multisystem organ abnormalities including the brain, heart, kidneys, gastrointestinal and genitalia. There are four major recognized types namely type 1 (Saldino-Noonan syndrome), type 2 (Majewski syndrome), type 3 (Verma-Naumoff syndrome), and type 4 (Beemar-Langer syndrome) of more or less same lethality in the neonatal period due to respiratory insufficiency secondary to a severely restricted thoracic cage. Provisional diagnosis of SPRS can be obtained by prenatal ultrasonography, postnatal radiography and fetal autopsy. However classification of SRPS (I-V) is definite only after identification of distinct genes specific to each type by Targeted Next Generation Sequencing (NGS). Here we described a couple whose fetus was noted to have abnormally short upper and lower limbs and thoracoabdominal ratio on prenatal ultrasonography and later diagnosed as SPRS III by NGS

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2 Case Report

2.1 Review Stage

A 31-year-old pregnant woman at 17 weeks gestation was referred to our prenatal and genetic diagnosis center after her fetus was observed to have shortened bilateral femur, the shaft of the femur was bent and the ribs were short and small on antenatal ultrasound imaging (USG). Because the woman was previously pregnant with a fetus that exhibited same USG patterns, both woman and her husband (33 years old) were referred to our prenatal diagnosis clinic for chromosomal analysis. Neither the mother nor father had a history of consanguinity or genetic disease. They denied smoking or drinking and had not been exposed to radiation or chemical insult. Routine blood test, urine analysis, liver and renal function were normal for both. Electrocardiography and chest X-rays showed no abnormalities of the heart and lungs. Since this is a lethal disorder, we advised elective termination of this pregnancy followed by fetal autopsy and genetic testing. Targeted Next-generation sequencing was performed using standard protocol developed at the Beijing Genomics Institute (BGI). 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.

2.2 Karyotyping

Peripheral venous blood for parents and amniocytes for fetus were source for chromosome extraction of parents and fetus respectively. Karyotyping was performed on G-banded metaphases chromosomes obtained from peripheral blood of the parents and cultured using standard procedures. Karyotyping of amniotic fluid was performed at 17 weeks of pregnancy according to standard techniques.

2.3 Targeted Next Generation Sequencing and Sanger Sequencing

Peripheral venous blood samples were extracted from the mother and father and amniocytes for fetus by Tiangen Extraction kit (Beijing, China) the manufacturer's instruction (MyGenostics, Beijing, China). A panel of genes for fetal osteopathy was used and High throughput sequencing was used to detect and analyze the mutation of 225 exons coding regions for fetal osteopathy. PCR and Sanger sequencing were performed to confirm the candidate mutation using an ABI 3130XL automatic genetic analyzer (Applied Biosystems, Foster City, CA).
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3 Results

3.1 Ultrasound and autopsy findings

A single live intrauterine fetus of 17 weeks gestation (as assessed by biparietal diameter (44mm) and head circumference (168mm)) without polyhydramnios. The fetal thorax was extremely narrow. Abdominal circumference was 135mm. Thoracic to abdominal circumference ratio was less than 0.6. USG of the fetal face showed no anomaly and abdomen revealed normal kidneys, liver, stomach bubble and bladder. The upper limb bones were micromelic (14 weeks gestation) and there was polydactyly. The lower limb bones were micromelic (16mm=15w0d). Bilateral club feet with polydactyly was also noted. There were no neural tube defects. Fetal echocardiography revealed a ventricular septal defect. The umbilical cord was normal three-vessel cord. Labor was induced and the mother delivered a 192g male stillborn baby vaginally. Autopsy of stillborn baby revealed 11 pairs of rib that were short, a narrow thorax, short upper limb bones and short lower limb bones. Within lethal chondrodysplasias, these findings with a dominant pattern of narrow thorax and short ribs with polydactyly, the most likely diagnosis is Short ribs polydactyly syndrome.

3.2 Mutation identification by targeted NGS and Sanger Sequencing

One heterozygous missense mutation *c4625C>A (p.A1542E)* for the mother and a heterozygous deletion *mutation c.11568delT (p.E3857Sfs*82)* for the father were detected in the coding region of the *DYNC2H1* gene by sequencing 225 known exons for fetal osteopathy. The results were verified by Sanger sequencing. The fetus had a compound heterozygosity for both mutations *c4625C>A (p.A1542E)* and *c.11568delT (p.E3857Sfs*82)* corresponding to short rib thoracic dysplasia with or without polydactyly syndrome type 3.
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4 Discussion

The USG findings, shortened long bones and a narrow thorax and polydactyly, are crucial findings for SRPS but molecular analysis results are diagnostic. Both the mother and father were carriers of heterozygous mutations suggesting an autosomal recessive pattern of inheritance in the fetus. Compound heterozygosity for mutations *c4625C>A (p.A1542E)* and *c.11568delT (p.E3857Sfs*82*) in the fetus have not been documented in previous literature. To the best of our knowledge this is the eight SRPS III case in family reported worldwide and second case in China[1]. The *c4625C>A* missense mutation results in an Arginine to Glutamic acid substitution at amino acid position 1542. The *c.11568delT (p.E3857Sfs*82)* mutation causes a frameshift mutation at amino acid position 3857, resulting in a truncated protein and possible protein instability. These mutations alter the protein structure encoded by *DYNC2H1* genes resulting in functionally abnormal proteins. Phenotypically SRPS III characteristics are the outcome of *DYNC2H1* genes mutation.

Located at chromosome 11q22.3, the *DYNC2H1* genes encode for a cytoplasmic dynein-2 complex that is involved in retrograde transport in the cilium Cilia are projections from cell surface into the rich extracellular matrix environment that includes growth factors and signaling molecules and play diverse roles in cellular motility, sensory transduction, and signaling, including Sonic Hedgehog pathway responsible for proliferation, development and maturation of a properly patterned skeleton capable of promoting linear growth. Elongation and maintenance of the cilia is dependent on the dynamic bidirectional process of intraflagellular transport (IFT) where retrograde transport from the distal tip to the basal body is accomplished by the cytoplasmic dynein-2 complex[2]. *DYNC2H1* plays an essential role in cilia function in cartilage and bone growth and mutation of *DYNC2H1* results in altered dynein-2 protein structure and function, hence failure of material transport within cilia[3]. Ciliopathies collectively affect all major organ systems, including the skeleton. This ciliopathy consequently cause the chondrodysplasia phenotypes in SRPS characterized by constricted thoracic cage, short ribs, shortened tubular bones, polydactyly as well as anomalies of major organs such as the brain, eye, heart, kidneys, liver, pancreas, intestines and genitalia.

Diagnosing SRPSs is a difficult task due to overlaping clinical and radiological features of the four major types; type 1 (Saldino-Noonan syndrome), type 2 (Majewski syndrome),

type 3 (Verma-Naumoff syndrome), and type 4 (Beemar-Langer syndrome) which has led to difficulties in distinguishing between them [4, 5, 6]. Clinical, radiological, ultrasound and autopsy findings described in previous literatures have been essential for diagnosis to date [6, 7, 8]. However molecular analysis has provided the cutting edge in giving accurate diagnosis by identifying causative gene(s). The different types are results of several mutations of different genes and secondary intrauterine modification of the phenotype. Molecular genetic analysis remains the gold standard for identifying the causative genes for a definite diagnosis. Next generation sequencing will certainly become routinely available in clinical diagnosis or screening for discovery of novel mutations in rare genetic disorders. In prenatal diagnosis this helps to provide accurate information to prepare parents to be for arrival of an affected baby and therefore a choice to proceed or terminate pregnancy.

5 Conclusion

Due to its lethality, an early detection or diagnosis of SRPS is important. Clinically USG is crucial and highly suggestive of SPRS while NGS is gold standard for definitive diagnosis. A novel compound mutation was identified using NGS which emphasizes its importance in prenatal diagnosis of rare disesase.

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Conflict of Interest

The author has no conflict of interest.

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