A novel WDR60 mutation contributes to a delayed diagnosis of Jeune asphyxiating thoracic dystrophy in a chinese patient: A case report

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Abstract

We reported a delayed diagnosised Chinese JATD case with mild skeletal phenotype, and presented with renal insufficiency as the initial symptom of disease onset. Novel bilateral c.2789C>T (p.S930L) mutations in WDR60 gene were identified. Our report will help to improve our awareness and diagnosibility for this disease in China.

TITLE PAGE

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Abstract

We reported a delayed diagnosised Chinese JATD case with mild skeletal phenotype, and presented with renal insufficiency as the initial symptom of disease onset. Novel bilateral c.2789C>T (p.S930L) mutations in WDR60 gene were identified. Our report will help to improve our awareness and diagnosibility for this disease in China.

Key clinical message

This JATD case presented with atypical skeletal anomalies , chronic renal failure was the initial symptom of the disease onset, easily resulting in an error diagnosis .

Key words: Jeune asphyxiating thoracic dystrophy; WDR60 gene; ciliopathyies; skeletal ciliopathyies; renal failure

Introduction

Primary cilium is an immotile microtubule-based structure that protrudes from the cell surface and distributes in almost all vertebrate cell types. In cell, cilium acts as an antenna and plays a pivotal role in chemical sensation, signal transduction, and control of organogenesis[1, 2, 3, 4].

Defects in the assembly and functions of cilia result in a variety of congenital disorders, known as ciliopathyies, characterized by the high heterogeneity of clinical manifestations. Ciliopathyies could involve most major tissues and organs and cause a broad spectrum of phenotypes including skeletal malformations and retinal degeneration, polycystic kidney, polydactyly, infertility, morbid obesity and mental retardation[5, 6, 7, 8].

Jeune asphyxiating thoracic dystrophy (JATD; MIM 208500) was first described in 1955[9], and characterized by skeletal anomalies, primarily shortened ribs and limbs, brachydactyly and variable polydactyly. The estimated incidence of this disease is about 1 in 100,000 live births[10]. JATD belongs to the ciliopathy diseases spectrum- skeletal ciliopathies. In addition to JATD, skeletal ciliopathies also encompass short-rib polydactyly syndromes (SRPS; MIM 611263, MIM613091, MIM 263520, MIM 269860, MIM 614091), Mainzer-Saldino syndrome (MZSDS; MIM 266920)[11], Sensenbrenner syndrome or cranio-ectodermal dysplasia (CED; MIM 218330)[12], oral-facial-digital syndrome 4 (OFD4; MIM 258860) and Ellis-van Creveld syndrome (EVC; MIM 225500)[10, 12, 13]. Apart from distinctive skeletal changes, these diseases often give rise to the involvement of extraskeletal organs, and cause extraskeletal phenotypes including polycystic kidney disease, retinal degeneration, and cardiac, liver, and brain anomalies[6, 14].

By far, it has been established a causative link between JATD and mutations in dozens of genes that all involved in the assembly and transport of cilia[13, 15, 16]. The association of WDR60 gene with ciliopathies was recognized for the first time in 2013[17]. WD60 protein acts as a dynein intermediate chain required for retrograde intraflagellar transport in cilia[18, 19]. mutations of WDR60 can cause both SRPS and JATD phenotypes, So far, 5 mutations have been identified and no case has been report in Chinese population [13, 17, 20]. Here, we describe a delayed diagnosis Chinese JATD case with moderate degree of skeletal phenotype and renal insufficiency as the initial symptom of disease onset. Novel bilateral c.2789C>T (p.S930L) mutations in WDR60 gene were identified from the patient and confirm the mutation co-segregation in the family. This report will expand the phenotypic spectrum caused by WDR60 mutations and contribute to improving our awareness and diagnosability for this disease in China.

Case presentation

The proband was a 46 -year-old male patient from healthy consanguineous parents. At about 38 years old, he was admitted to local hospital for dizziness. Examination and laboratory data on admission showed the blood pressure was 200mmHg/110mmHg, Serum Cr (1200μmol/L), respectively. The diagnoses of hypertension and chronic kidney disease (stage 5) were made according to his clinical features and biochemical data at that time. Then an antihypertensive therapy was taken by administration of antihypertensive drugs to control the blood pressure within normal range (about 140-150 mmHg/90 mmHg), and regular hemodialysis was adopted to improve the survival status and prognosis. 1 year ago, he was hospitalized to our hospital because of dizziness, headache and alalia, brain CT demonstrated hemorrhage in his left brain. Notably, physical examination on admission in our hospital showed the patient displayed disproportional short stature, with normal height (170cm), while presented conspicuous small chest and short extremities with brachydactyly and accompanied with deformity teeth (Figure 1). In addition, the routine fundoscopy revealed the concurrent existence of bilateral retinitis pigmentosa in this patient (Figure 2). According to the above-mentioned clinical characters and the history of kidney failure, the patient was reassessment and suggested the diagnosis of JATD. Clinical features and representative biochemical data are shown in table 1. The pedigree tree was shown in figure 3.

To confirm the diagnosis, genetic analysis was performed after the patient and his family members gave informed consent. The study protocol was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. Genomic DNA was extracted from the peripheral blood leucocytes using Blood genome DNA Extraction kit (Promega, USA). High-throughput sequencing was used to analyze all the exon regions and adjacent intronic regions of JATD/SRPS associated genes that have been reported previously including CEP120, DYNC2H1, EVC, EVC2, IFT43, IFT80, IFT122, IFT140, IFT172, NEK1, TTC21B, WDR19, WDR34, WDR35, and WDR60[14, 15, 17, 21, 22, 23]. After raw data processing, reads that passed were then mapped to the human reference genome (UCSC hg19) using the Burrows Wheeler Aligner (University of California, Santa Cruz, CA, USA). The variant call file (VCF) containing the detected variants was annotated with Variant Effect Predictor v83 and the dbNSFP (Database for Nonsynonymous SNPs' Functional Predictions) v3.1. After the selection process, a novel homozygous variant c.2789C>T (p.S930L) in exon 24 of WDR60 gene was found in the proband and the heterozygous variant was detected in his parents and his daughter. The variant was then confirmed by sanger sequencing verification (Figure 4). No mutation was found in any other pathogenic genes and no c.2789C>T (p.S930L) variant was found in one-hundred unrelated healthy subjects. According to guidelines from the American College of Medical Genetics and Genomics (ACMG, 2015), the variant was preliminarily determined as pathogenic (PM2 + PP3). PM2: the frequency in normal population database is 0.0003, which is low-frequency variation; PP3: protein function predicted as harmful, benign, harmful and harmful, by prediction software SIFT, polyphen-2, Mutationtaster and GERP + +, respectively.

Discussion and conclusion

Ciliopathy is a genetically heterogeneous group of diseases, which are caused by an underlying dysfunction of the primary cilium. Skeletal ciliopathies are the common forms of ciliopathies, which can be classified into different subtypes according to the clinical manifestations. Besides the common characters of skeletal development abnormalities, each subtype has its own unique feature different from others[12, 24].

As the important subtypes of skeletal ciliopathies, the clinical manifestations of JATD and SRPS are highly similar. They both manifested as short ribs, narrow chest, short fingers (toes), with or without polydactyl[6, 17]. And usually accompanied by the involvement of extra-skeletal organs such as retinopathy and fibrocystic changes in liver and kidney. However, the SRPS phenotype is usually more severe than JATD, and often leads to embryonic developmental disorders and perinatal death, the survival period is short. while the relative survival rate of JATD is high, about 40%, could survive to adulthood[13, 15].

In the present report, the proband was an adult patient and displayed modest phenotype of skeletal abnormalities, such as a relatively small thoracic cage, which is less conspicuous compared to neonate cases,

possibly attributing to the improvement of respiratory function with age. Whereas, short fingers (toes) of the extremities are very conspicuous in the patient and no other typical radiological features such as cone-shaped epiphyses can be distinguished in the patient's radiographs, further illustrating the high variability of JATD manifestations. It is worth concerned that, for this patient, the extra-skeletal phenotype - progressive renal failure and retinal degeneration were considerably more noticeable than the skeletal changes, which were not been concerned until visiting our hospital. So, this case implied the crypticity and confusability of JATD phenotype in adult patient. Notably, the proband's only sibling died of respiratory failure at infancy stage without definite diagnosis. It is estimated that 60% of JATD cases accompanied with lethal respiratory distress after birth[14]. Once overcome the respiratory dysfunction through a careful nursing at early stage after birth and the survival rate of infants will be improved. Whereas, 30% of the survival JATD patients developed end stage renal disease and 50% of the JATD cases presented retinal alteration just as the proband in this report, however, the age of onset of extraosseous manifestations are still unevaluated to date[14, 25].

To make a definite diagnosis and find the pathogenic gene, JATD/SRPS panel including fifteen genes were screened by high-throughput sequencing, a novel homozygous variant c.2789C>T (p.S930L) in exon 24 of WDR60 gene was found, and multiple sequences alignment indicated the evolutionary conservation of the site p.S930L among different species (figure 4). In silico analysis by four software highly suggested the variant was a pathogenic form. Current evidence has proved that WDR60 mutations could cause varying degree phenotypes of JATD or SRPS[13, 17, 20]. Moreover, one report also confirmed the destructive effect of WDR60 mutation on cilia structure and assemble by immunofluorescence in fibroblast derived from affected patient[17]. In our case, the patient was in a very serious condition and denied biopsy, so we could not acquire the in vivo evidence of variant disrupting ciliogenesis from this patient. Even so, we have made a point mutation mouse model and we will provide more intensive investigation on the pathogenic mechanism of c.2789C>T variant in future study.

In summary, in this report, we identified a novel homozygous variant c.2789C>T (p.S930L) in a delayed diagnosis of JATD patient. This report will help to expand our understanding for this disease in China and enrich the mutational spectrum of WDR60 gene.

Authors' Contributions

L.S. conceived and designed the experiments. X.Z., L.C., and A.S. performed the experiments. Z.L., R.Z. and Y.H. performed the data analyses. X.Z. wrote the manuscript and L.S. revised the manuscript. All authors have reviewed the final manuscript and approved submitting for publication.

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Conflict of Interest Statement

The authors declare no competing interests regarding the contents of this case report.

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Reference

[1] *Elliott KH, Brugmann SA.* Sending mixed signals: Cilia-dependent signaling during development and disease.Developmental biology. 2019; *447:*28-41.*http://www.ncbi.nlm.nih.gov/pubmed/29548942*

[2] *Goetz SC, Anderson KV.* The primary cilium: a signalling centre during vertebrate development. Nature reviews. Genetics. 2010; *11:*331-344.*http://www.ncbi.nlm.nih.gov/pubmed/20395968*

[3] *Christensen ST, Morthorst SK, Mogensen JB, Pedersen LB.*Primary Cilia and Coordination of Receptor Tyrosine Kinase (RTK) and Transforming Growth Factor beta (TGF-beta) Signaling. Cold Spring Harbor perspectives in biology. 2017;*9 .http://www.ncbi.nlm.nih.gov/pubmed/27638178*

[4] *Goggolidou P.* Wnt and planar cell polarity signaling in cystic renal disease. Organogenesis. 2014; *10:*86-95.*http://www.ncbi.nlm.nih.gov/pubmed/24162855*

[5] *Gleeson JELaJG.* A systems-biology approach to understanding the ciliopathy disorders. Genome Medicine. 2011;*3:* 1-9.

[6] *McInerney-Leo AM, Wheeler L, Marshall MS, Anderson LK, Zankl A, Brown MA, Leo PJ, Wicking C, Duncan EL.* Homozygous variant in C21orf2 in a case of Jeune syndrome with severe thoracic involvement: Extending the phenotypic spectrum. American journal of medical genetics. Part A. 2017; *173:*1698-1704.*http://www.ncbi.nlm.nih.gov/pubmed/28422394*

[7] *Chen CP, Ko TM, Chang TY, Chern SR, Chen SW, Lai ST, Chuang TY, Wang W.* Prenatal diagnosis of short-rib polydactyly syndrome type III or short-rib thoracic dysplasia 3 with or without polydactyly (SRTD3) associated with compound heterozygous mutations in DYNC2H1 in a fetus. Taiwanese journal of obstetrics & gynecology. 2018;*57:* 123-127.*http://www.ncbi.nlm.nih.gov/pubmed/29458881*

[8] *Wang L, Dynlacht BD.* The regulation of cilium assembly and disassembly in development and disease. Development. 2018;*145 .http://www.ncbi.nlm.nih.gov/pubmed/30224385*

[9] *Jeune M BC, Carron R.* Asphyxiating thoracic dystrophy with familial characteristics. Arch Fr Pediatr. 1955; *12:886–91* .

[10] *Handa A, Voss U, Hammarsjo A, Grigelioniene G, Nishimura G.* Skeletal ciliopathies: a pattern recognition approach.Japanese journal of radiology. 2020; *38:*193-206.*http://www.ncbi.nlm.nih.gov/pubmed/31965514*

[11] *Oud MM, Latour BL, Bakey Z, Letteboer SJ, Lugtenberg D, Wu KM, Cornelissen EAM, Yntema HG, Schmidts M, Roepman R, Bongers E.*Cellular ciliary phenotyping indicates pathogenicity of novel variants in IFT140 and confirms a Mainzer-Saldino syndrome diagnosis.Cilia. 2018; *7:*1.*http://www.ncbi.nlm.nih.gov/pubmed/30479745*

[12] *Lin AE, Traum AZ, Sahai I, Keppler-Noreuil K, Kukolich MK, Adam MP, Westra SJ, Arts HH.* Sensenbrenner syndrome (Cranioectodermal dysplasia): clinical and molecular analyses of 39 patients including two new patients. American journal of medical genetics. Part A. 2013; *161A:*2762-2776.*http://www.ncbi.nlm.nih.gov/pubmed/24123776*

[13] *Cossu C, Incani F, Serra ML, Coiana A, Crisponi G, Boccone L, Rosatelli MC.* New mutations in DYNC2H1 and WDR60 genes revealed by whole-exome sequencing in two unrelated Sardinian families with Jeune asphyxiating thoracic dystrophy. Clinica chimica acta; international journal of clinical chemistry. 2016; *455:*172-180.*http://www.ncbi.nlm.nih.gov/pubmed/26874042*

[14] *Baujat G, Huber C, El Hokayem J, Caumes R, Do Ngoc Thanh C, David A, Delezoide AL, Dieux-Coeslier A, Estournet B, Francannet C, Kayirangwa H, Lacaille F, Le Bourgeois M, Martinovic J, Salomon R, Sigaudy S, Malan V, Munnich A, Le Merrer M, Le Quan Sang KH, Cormier-Daire V.* Asphyxiating thoracic dysplasia: clinical and molecular review of 39 families. Journal of medical genetics. 2013; *50:*91-98.*http://www.ncbi.nlm.nih.gov/pubmed/23339108*

[15] *Schmidts M, Arts HH, Bongers EM, Yap Z, Oud MM, Antony D, Duijkers L, Emes RD, Stalker J, Yntema JB, Plagnol V, Hoischen A, Gilissen C, Forsythe E, Lausch E, Veltman JA, Roeleveld N, Superti-Furga A, Kutkowska-Kazmierczak A, Kamsteeg EJ, Elcioglu N, van Maarle MC, Graul-Neumann LM, Devriendt K, Smithson SF, Wellesley D, Verbeek NE, Hennekam RC, Kayserili H, Scambler PJ, Beales PL, Uk10K, Knoers NV, Roepman R, Mitchison HM.* Exome sequencing identifies DYNC2H1 mutations as a common cause of asphyxiating thoracic dystrophy (Jeune syndrome) without major polydactyly, renal or retinal involvement. Journal of medical genetics. 2013; *50:*309-323.*http://www.ncbi.nlm.nih.gov/pubmed/23456818*

[16] *McInerney-Leo AM, Harris JE, Leo PJ, Marshall MS, Gardiner B, Kinning E, Leong HY, McKenzie F, Ong WP, Vodopiutz J, Wicking C, Brown MA, Zankl A, Duncan EL.* Whole exome sequencing is an

efficient, sensitive and specific method for determining the genetic cause of short-rib thoracic dystrophies. Clinical genetics. 2015;*88:* 550-557.*http://www.ncbi.nlm.nih.gov/pubmed/25492405*

[17] *McInerney-Leo AM, Schmidts M, Cortes CR, Leo PJ, Gener B, Courtney AD, Gardiner B, Harris JA, Lu Y, Marshall M, Consortium UK, Scambler PJ, Beales PL, Brown MA, Zankl A, Mitchison HM, Duncan EL, Wicking C.* Short-rib polydactyly and Jeune syndromes are caused by mutations in WDR60. American journal of human genetics. 2013;*93:* 515-523.*http://www.ncbi.nlm.nih.gov/pubmed/23910462*

[18] *Patel-King RS, Gilberti RM, Hom EF, King SM.* WD60/FAP163 is a dynein intermediate chain required for retrograde intraflagellar transport in cilia. Molecular biology of the cell. 2013;*24:* 2668-2677.*http://www.ncbi.nlm.nih.gov/pubmed/23864713*

[19] *Toropova K, Zalyte R, Mukhopadhyay AG, Mladenov M, Carter AP, Roberts AJ.* Structure of the dynein-2 complex and its assembly with intraflagellar transport trains. Nature structural & molecular biology. 2019; *26:*823-829.*http://www.ncbi.nlm.nih.gov/pubmed/31451806*

[20] *Kakar N, Horn D, Decker E, Sowada N, Kubisch C, Ahmad J, Borck G, Bergmann C.* Expanding the phenotype associated with biallelic WDR60 mutations: Siblings with retinal degeneration and polydactyly lacking other features of short rib thoracic dystrophies.American journal of medical genetics. Part A. 2018; *176:*438-442.*http://www.ncbi.nlm.nih.gov/pubmed/29271569*

[21] *Shaheen R, Schmidts M, Faqeih E, Hashem A, Lausch E, Holder I, Superti-Furga A, Consortium UK, Mitchison HM, Almoisheer A, Alamro R, Alshiddi T, Alzahrani F, Beales PL, Alkuraya FS.* A founder CEP120 mutation in Jeune asphyxiating thoracic dystrophy expands the role of centriolar proteins in skeletal ciliopathies. Human molecular genetics. 2015; *24:*1410-1419.*http://www.ncbi.nlm.nih.gov/pubmed/25361962*

[22] *Kessler K, Wunderlich I, Uebe S, Falk NS, Giessl A, Brandstatter JH, Popp B, Klinger P, Ekici AB, Sticht H, Dorr HG, Reis A, Roepman R, Seemanova E, Thiel CT.* DYNC2LI1 mutations broaden the clinical spectrum of dynein-2 defects. Scientific reports. 2015;*5:* 11649.*http://www.ncbi.nlm.nih.gov/pubmed/26130459*

[23] *Rix S, Calmont A, Scambler PJ, Beales PL.* An Ift80 mouse model of short rib polydactyly syndromes shows defects in hedgehog signalling without loss or malformation of cilia. Human molecular genetics. 2011; *20:*1306-1314.*http://www.ncbi.nlm.nih.gov/pubmed/21227999*

[24] *D'Asdia MC, Torrente I, Consoli F, Ferese R, Magliozzi M, Bernardini L, Guida V, Digilio MC, Marino B, Dallapiccola B, De Luca A.*Novel and recurrent EVC and EVC2 mutations in Ellis-van Creveld syndrome and Weyers acrofacial dyostosis. European journal of medical genetics. 2013; *56:*80-87.*http://www.ncbi.nlm.nih.gov/pubmed/23220543*

[25] *Tuysuz B, Baris S, Aksoy F, Madazli R, Ungur S, Sever L.*Clinical variability of asphyxiating thoracic dystrophy (Jeune) syndrome: Evaluation and classification of 13 patients. American journal of medical genetics. Part A. 2009; *149A:*1727-1733.*http://www.ncbi.nlm.nih.gov/pubmed/19610081*

Figure legends

Fig. 1 Pedigree of the Chinese family with JATD. , male *, female; , male patient; *, female patient; /, proband.

Fig.2 Clinical and radiological features of the JATD proband. A. the proband manifested with small chest, short extremities with short fingers and toes, and deformity teeth. B. X-rays of the proband show shortening of the ribs, fingers and toes.

Fig.3 The funduscopy revealed the presence of bilateral retinitis pigmentosa in the proband.

Fig.4 The verification of c.2789C>T variant in the porband and normal individual by Sanger sequence (A) and Evolutionary conservation of the site of 930 Serine by multiple sequence alignment of WDR60 protein

across different species (B).

Table1Clinical features and biochemical data of the patienton admission in our hospital for the first time.

Clinical characteristics	Proband	Normal range
age (yrs)	45	-
Gender	Male	-
Height (cm)	170	167.1(average)
Weight (kg)	60	-
BP^{+} (mmHg)	163/66	100-120/60-80
$eGFR^{++}$ (ml/min)	7.52	90-120
$BUN^{\S}$ (mmol/l)	12.58	3.1-8
Serum $Cr^{\P}$ (μmol/l)	921.11	57-97
Uric Acid (μmol/l)	259.15	208-428
Urine gravity	(anuria)	1.005-1.025
Urine pH	(anuria)	4.6-8.0
Proteinuria	(anuria)	-

BP^{+}=blood pressure, GFR^{++}=glomerular filtration rate, eGFR was estimated by MDRD formula. $BUN^{\S}$= blood urea nitrogen, $Cr^{\P}$=creatinine