Original Article

Two novel mutations of *PEX6* in one Chinese Zellweger spectrum disorder and their clinical characteristics

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Background: Zellweger spectrum disorder (ZSD) is an autosomal recessive peroxisome biogenesis disorder (PBD) caused by bi-allelic mutations in any of the 13 *PEX* family genes.

Methods: We reported a Chinese PBD-ZSD patient with compound heterozygous mutations of *PEX6* detected by target sequencing and Sanger sequencing. The clinical materials were collected. In silico analysis were used to evaluate the pathogenicity of the two mutations. An updated review summarized the genotype-phenotype correlation of PBD patients with *PEX6* mutations.

Results: The patient was diagnosed as PBD-ZSD and displayed retinitis pigmentosa, bilateral sensorineural hearing loss, hypotonia, developmental delay, ovarian and enamel dysplasia. Elevated very long chain fatty acids were shown and a pattern of leukodystrophy was displayed through MRI. The two mutations were novel with p.Cys358* and p.Leu83Pro, both classified as pathogenic according to American College of Medical Genetics and Genomics guideline. Phenotype-genotype correlations were shown in the reported patients with PBD-ZSD continuum.

Conclusions: we reported the first Chinese PBD-ZSD patient with 2 novel mutations in PEX6. Target sequencing and VLFAC were helpful in diagnosis.

Keywords: Zellweger spectrum disorder (ZSD); *PEX6*; gene

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Introduction

Zellweger spectrum disorder (ZSD) is a subtype of peroxisome biogenesis disorder (PBD) caused by defects in peroxisomal biogenesis proteins. ZSD was used to be classified into Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD), among which ZS was the most severe form and IRD was the least (1). Recently, this classification has been updated by regarding ZSD as an overall spectrum disease, with clinical features from severe, intermediate to mild phenotype (2). In addition, Heimler syndrome, another recessive PBD with mild clinical manifestations, is included in the ZSD spectrum as well. Clinical manifestations of ZSD range from dysmorphic features, sensory deficits to hypotonia, spastic ataxia, and life-threatening problems in multiple organs.

Bi-allelic mutations in any of the 13 *PEX* family genes can lead to ZSD, among which mutations in *PEX1* and *PEX6* are the most frequent, accounting for 60–70% and 10–15% respectively (3). Both *PEX1* and *PEX6* encode members of AAA (ATPases associated with diverse cellular

activities) family of ATPases and form complexes anchored to the peroxisomal membrane by interaction with *PEX26*, a peroxisomal membrane protein. Pathogenic mutations in either *PEX1* or *PEX6* will cause export deficits of *PEX5*, a peroxisomal matrix protein receptor important for peroxisomal matrix protein import (4). This results in the damage of peroxisome-dependent metabolism pathway leading to accumulation or shortage of metabolites such as very long chain fatty acids (VLCFAs), C27-bile acid intermediates and erythrocyte plasmalogens (1).

Here we reported a Chinese female patient with PBD-ZSD due to two novel compound heterozygous mutations of p.Cys358* and p.Leu83Pro in *PEX6*. The phenotype-genotype association of *PEX6* in PBD-ZSD is also reviewed.

Methods

Clinical material

Clinical materials of the proband were collected. Biochemistry analysis and cranial magnetic resonance imaging (MRI) were carried out. The diagnosis of PBD-ZSD was made by the combination of clinical evaluations and genetic testing.

Genetic test

Genomic DNA was isolated from peripheral blood sample of the patient through a standard method (Qiagen, German). Leukoencephalopathy gene panel sequencing was performed by target sequencing of the exons. In brief, all exons and their corresponding flanking regions of these genes were selected as target regions. Paired-end sequencing was performed on Illumina HiSeq X-ten platform. All variants different from the reference sequence were further screened by allele frequency <1% according to 1000 Genomes Project (http://www.internationalgenome.org/data), Inhouse database, ESP6500 (*evs*.gs.washington.edu/EVS/) and ExAC (exac.broadinstitute.org). The synonymous variants were excluded. The phenotypes of the screened genes were compared with the clinical manifestations of the proband and the inherited modes were considered to further exclude irrelevant genes. The mutations left were confirmed by Sanger sequencing. SIFT (5), Provean (6), Polyphen2 (7), Mutation Taster (8) and MUpro (9) were used to predict the pathogenicity of the variants.

Interpretation of the variants was based on the American College of Medical Genetics and Genomics (ACMG) recommended standards (10).

Updated review of the literature

An updated review was performed to identify primary articles reporting individuals of PBDs due to mutations in PEX6. As the diseases associated with *PEX6* include both ZSD and Heimler syndrome (11), a rare kind of PBDs, a PubMed search with search terms of ("*PEX6*" OR "*PAF-2*") AND (("ZSD" OR "Zellweger Spectrum Disorder" OR "ZSS" OR "Zellweger Spectrum Syndrome") OR ("PBD" OR "peroxisome biogenesis disorder") OR ("Heimler Syndrome") OR ("ZS" OR "Zellweger Syndrome") OR ("NALD" OR "Neonatal Adrenoleukodystrophy") OR ("IRD" OR "Infantile Refsum Disease")) was performed. All studies included had to meet the following criteria: (I) containing patients who were diagnosed as PBDs with at least the mutation information and population were provided; (II) the pathogenicity is derived from *PEX6* mutations rather than any other *PEX* genes. Secondary articles such as reviews were screened for additional information.

Results

Clinical evaluation

The patient was a 29-year-old female with flattened face and broad nasal bridge. She referred to Huashan Hospital after a five-year history of hand and head tremor as well as torticollis under no predisposing cause. The patient was born with retinitis pigmentosa and bilateral sensorineural hearing loss. Not only intellectual disability but ovarian and enamel dysplasia were shown during her growth and development. Family history was denied (*Figure 1A*). During her last physical examination, the patient displayed less cooperation, poor vision, weak light reflex of both eyes and a wide base gait. The muscle strength and muscle tone were normal. Biochemical analysis showed elevated VLCFAs level (C26:0 1.79 nmol/mL, normal range of ≤1.3 nmol/mL; C26:0/C22:0 =0.052, normal range of ≤0.023). Cranial MRI performed at age 24 indicated obvious brain atrophy and symmetrical subcortical and periventricular high signals (*Figure 1B*).

Mutation analysis

The mean depth of target sequencing of the patient was 84.780X. The percentage of the target regions with mean depth over 20X was 99.2%. Two heterozygous variants of c.1074T > A and c.248T > C in *PEX6* (NM_000287.3)

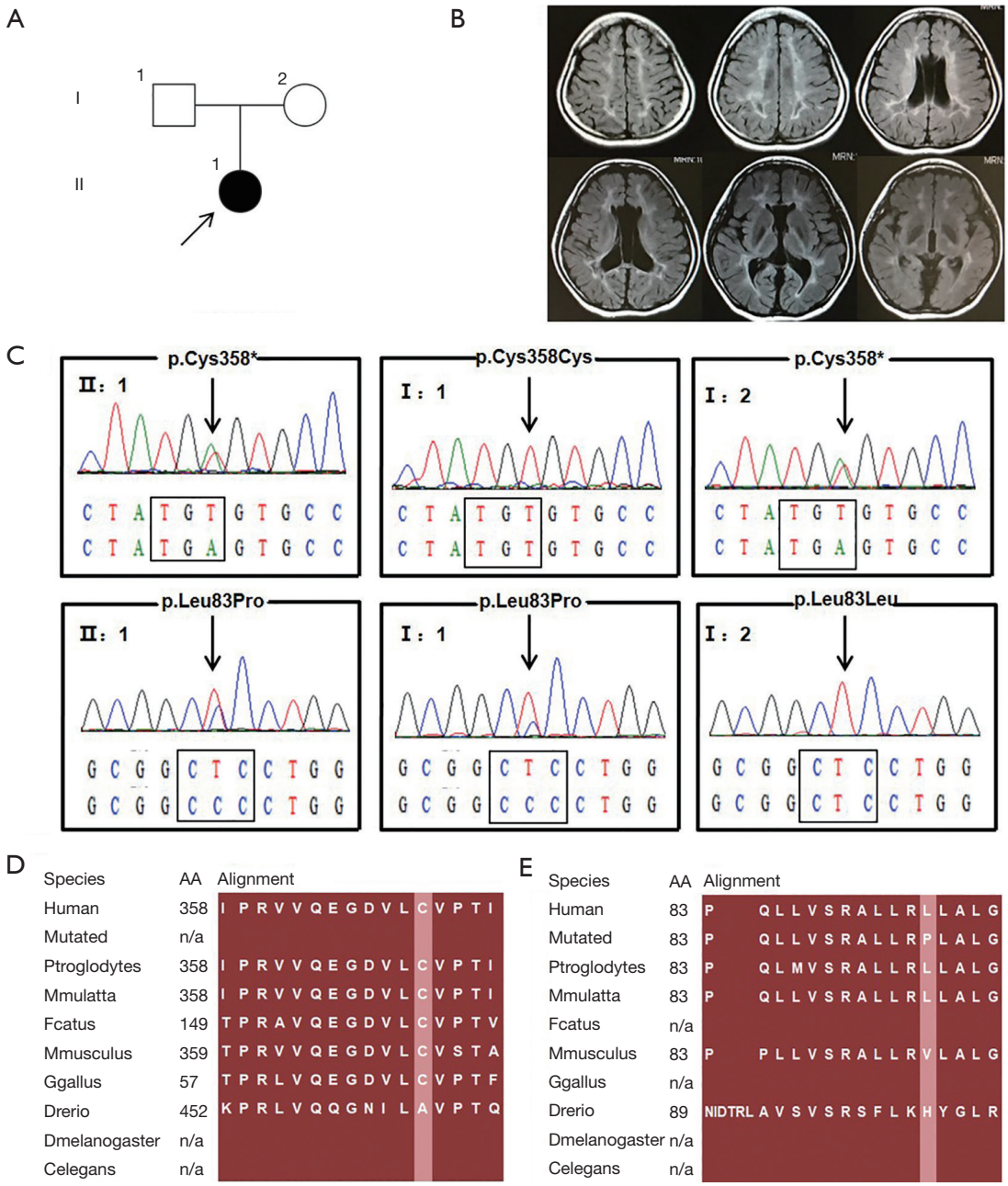

Figure 1 MRI pattern and genetic analysis of the patient. (A) Pedigree chart of the patient with p.Cys358* and p.Leu83Pro mutation on *PEX6*. Arrow: proband; square: male; circle: female. (B) Cranial MRI showed obvious brain atrophy and symmetrical subcortical and periventricular high signals. (C) Sanger sequencing of the pedigree. Compound heterozygous mutations of PEX6 were found in the proband (II:1). Heterozygous mutation p.Cys358* of *PEX6* in I:2 and p.Leu83Pro in I:1. (D) Conservation among multiple species at position 358. (E) Conservation among multiple species at position 83. MRI, magnetic resonance imaging.

were found, leading to p.Cys358* and p.Leu83Pro change in exon3 and exon1 respectively (*Figure 1C*). No possible variants in another 12 *PEX* family genes were found. The variant was not detected in 200 Chinese seniors without medical history of neurological diseases from the community by Sanger sequencing. The parents

Table 1 In silico analysis of the *PEX6* p.Cys358* and p.Leu83Pro pathogenicity

Mutation	cDNA	SIFT (5)	Provean (6)	Polyphen-2 (7)	Mutation Taster (8)	MuPro (9)
p.Cys358*	c.1074T > A	NA	Deleterious (–6.13)	NA	Disease causing (6.0)	Decrease stability (–1.32)
p.Leu83Pro	c.248T > C	Damaging (0.02)	Neutral (–0.53)	Benign (0.424)	Disease causing (98.0)	Decrease stability (–2.29)

NA, not assessed.

of the patient were identified as heterozygous carriers of the two mutations separately by Sanger Sequencing (*Figure 1C*). Online bioinformatics prediction tools were used to estimate the pathogenicity of two variants (*Table 1*). Both variants have not been reported and were absent from ExAC database. In addition, according to the online protein analysis (http://www.cmbi.ru.nl/hope/), the mutant residue of p.Leu83Pro was considered to be smaller than the wide-type residue and disrupted a α-helix structure, which may consequently result in loss of interaction with other proteins. The wild-type residue was partially conserved at two positions (*Figure 1D,E*).

According to ACMG standards, both p.Cys358* (PVS1+PS3+PM2+PM4+PP3+PP4) and p.Leu83Pro (PS3 + PM2 + PM3 + PP3 + PP4) were classified as pathogenic variants (10). We diagnosed the patient as ZSD in combination of the clinical evaluations and genetic analysis.

Phenotype-genotype analysis of PBD patients with PEX6 mutation

Here, an updated review was performed to identify primary articles reporting individuals of PBDs due to mutations in *PEX6*. In all, we identified 16 articles containing 53 patients diagnosed as PBDs with *PEX6* mutations (11-25) (http://fp.amegroups.cn/cms/atm.2019.06.42-1.pdf). Although ZSD was as an autosomal recessive inheritance disease, there were 11 ZSD patients showed the mode of autosomal dominant inheritance, which could be explained by allelic expression imbalance (13). In the remaining 42 patients, 47.6% showed homozygous mutations and 52.4% carried compound heterozygous mutations.

Among the fifty-three patients with ZSD, thirty-six were reported with clinical manifestations, of whom 61.1% displayed metabolism deficits including liver dysfunction and adrenal insufficiency. Other clinical characteristics include psychomotor development delay (50%), hypotonia (47.2%), vision disability (77.8%), sensorineural hear loss (66.7%), seizures (16.7%) and enamel dysplasia (36.1%).

In these patients who survived into adulthood (11,13,18,23), onset age was less than 3 years old, and the disease duration is usually over 18 years. Most of them have sensorineural hearing loss, pigmentary retinopathy and liver dysfunction. Other clinical manifestations include development delay, white matter abnormality, enamel dysplasia and facial dysmorphism. The patients with partial deficiency of peroxisomal functions may have normal reproductive capability (23). However, the patient in our case had displayed ovarian dysplasia. Since hypothalamic and pituitary lesions, Turner's syndrome as well as toxic effect have been excluded according to the patient's past medical history, we suspected ovarian dysplasia might be related to the *PEX6* mutations, which needs to be further confirmed by more cases.

Discussion

We described a case of ZSD. The diagnosis was based on clinical characteristics of hypotonia, developmental delay, leukodystrophy and disease course, together with biochemical analysis and the detection of compound-heterozygous mutations of p.Cys358* and p.Leu83Pro in *PEX6*.

Disease caused by mutations of *PEX6* include ZS, NALD, IRD and Heimler syndrome, all of which are now considered as the clinical continuums of PBD-ZSD, with the severity ranging from fatality to mildness (11). In our case, the patients displayed hand and head tremor as well as torticollis, which is consistence with the previous report by Kumar *et al.* in a patient with *PEX16* mutations (26). Autosomal recessive cerebellar ataxia has been reported in patients with *PEX16*, *PEX2* and *PEX10* mutations (27-29) which was also noted in our patient. Besides, other neurological phenotypes such as peripheral neuropathy and progressive spastic paraparesis were not observed in our patient.

VLCFA is biosynthesized in the endoplasmic reticulum. The fatty chain of VLCFA is so long that it can only be metabolized in peroxisomes rather than mitochondria. Defects in multiple peroxisome enzyme pathways caused

by mutations in *PEX* family lead to the accumulation of downstream fatty chains. According to our up-dated review, metabolism deficits including liver dysfunction and adrenal insufficiency is one of the most common phenotypes in PBD-ZSD patients with *PEX6* mutations. Thus, screening of plasma metabolites such as VLCFAs can be a strong evidence for PBD-ZSD.

There are some limitations in this study. First, the functional assays to identify the pathogenicity of *PEX6* mutations failed to be carried out. Second, since there is some missing information in the previous literature included for up-dated review, the genotype-phenotype correlation is not precise enough. A more comprehensive cohort study of PBD-ZSD is needed to further clarify the associations between genetics and clinical features.

Conclusions

In this article, we reported a Chinese PBD-ZSD patient with 2 novel compound heterozygous mutations of *PEX6*. Clinical evaluation and in silico analysis were performed. Since the variable combinations of the clinical manifestations in ZSD, Biochemical test such as VLFAC and Target sequencing was quite important in the diagnosis. We also found ovarian dysplasia in the patient but whether it is related to the *PEX6* mutation should be further explored. As this is the first case of ZSD with *PEX6* mutations in Chinese patient, further studies will focus on the contribution of PEX6 mutations to Chinese PBD patients and the pathogenicity of two novel mutations.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was conducted after receiving written informed consent from the patient. This research was approved by the Institutional Ethics Committee of Huashan Hospital Fudan University. The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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