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Case Report

A Novel Homozygous PEX1 Pathogenic Variation in a Chinese Newborn with Zellweger Syndrome

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Abstract Zellweger syndrome (ZS) is the most severe phenotype in peroxisomal biogenesis disorders (PBDs). 14 Peroxin (*PEX*) genes have been identified to attribute PBDs, and about 70% of the ZS patients harbor gene mutations in *PEX1*. Recently gene mutation screening combined with clinical manifestations such as distinct facial features and congenital malformations is considered a suitable test for ZS patients. Here, a Chinese newborn patient with clinical features of ZS confirmed by molecular findings was reported. A novel pathogenic variation of the *PEX1* gene was identified by exome sequencing. The patient is a homozygote of c.1671_1672delAG variation in the *PEX1* gene and was inherited from her heterogenous parents, respectively. This variation leads to early termination of translation and produces a non-functional truncated protein. We report a novel pathogenic variation in the *PEX1* gene, providing valuable information for genetic counseling and reproductive options.

Key words *Exome sequencing; PEX1 gene; Zellweger syndrome*

Introduction

Peroxisomes are dynamic organelles that primarily involve in fatty acid metabolic pathways and contain hundreds of enzymes. Peroxisome disorders including single enzyme deficiency and peroxisome function and assembly defects, which lead to the rare autosomal recessive disorder, peroxisomal biogenesis disorder (PBDs), have highlighted the functional importance of peroxisomes in humans.[1,2] About 80% of the patients with PBDs are Zellweger spectrum disorder (ZSD). According to the severity of clinical features, ZSD is classified into Zellweger syndrome (ZS), having the most severe phenotype, neonatal adrenoleukodystrophy (NALD), infantile Refsum disease (IRD) and Heimler syndrome.[3,4] The manifestations are clinically heterogeneous, and the affected newborns and infants always have distinctive faces, congenital malformations, severe liver diseases, and die in the first year of life. But cases with milder phenotype only have progressive peroxisome dysfunction and can grow into teenagers or adults.[5] Inherited gene mutations in fourteen *PEX* genes encoding Peroxin have been identified as the causes of PBDs. Nearly 70% of the ZSD patients harbour mutations in the *PEX1* gene.[1] *PEX1* is located on chromosome 7q21-q22 and encodes a 143-kDa cytosolic protein that belongs to the AAA (ATPases associated with diverse cellular activities) protein family. Pex1p functions to recycle the PEX5 receptor and import proteins to the peroxisome matrix.[6]

In this study, we report a Chinese newborn with clinical features of ZS and identified a novel pathogenic variant of *PEX1* (c.1671_1672delAG, p.G558fsX34) that were inherited from the patient's parents.

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Patient Description

This report involves a female newborn who was delivered vaginally with a birth weight of 3,100 g at 39+3 weeks of gestation to a 27-year-old native Chinese female (gravida 1, para 1). Pregnancy was complicated with polyhydramnios from 38 weeks. The baby was born with respiratory distress and needed oxygen administration. The Apgar scores were 6 at 1 minute, 9 at 5 minutes, and 9 at 10 minutes. The infant was hypotonic, neonatal seizures and dysmorphic features were noted, including widely spaced eyes and depressed nasal bridge. The parents were healthy, non-consanguineous, and no family history of any particular disease or mental retardation. The patient was their first baby.

Further workup revealed the patient had cloudy cornea with sluggish light reflex, large anterior fontanelle, about 5 cm x 5 cm in size. Her temperature was 32.5°C, pulse: 88 times/min, blood pressure: 62/35 mmHg. After the oxygen-absorption by nasal trachea, breathe 40 times/min, oxygen saturation (SPO_2) 90%. She had no spontaneous breathing and activity. The physiologic reflexes such as embracing, sucking, grasping, or foraging reflexes were not elicited. Breath sounds were symmetrical but rough with moist rales. The heart sounds were low and recorded with cardiac murmurs. The hepatomegaly was palpable 3 cm below the costal margin.

Laboratory studies showed hyperbilirubinemia with a marked elevation in liver transaminases, which revealed liver dysfunction. Various biochemical indicators suggested the patient had severe neonatal pneumonia, impaired myocardium, neonatal asphyxiation, and respiratory failure shock. The chest radiograph showed the double lung texture got thickening and fuzzy, thymus participating in the enlargement of the heart. Head B-sonography revealed transparent septum was not closed, bilateral lateral ventricles cyst (Left 20.2 mm, Right 26.4 mm), and high signal of the white matter in the lateral ventricle. Head CT suspected intracranial hemorrhage. Echocardiography showed patent ductus arteriosus, aorta shunted from right to left, patent foramen ovale, room interval shunted from left to right, severe pulmonary arterial hypertension.

Considering the patient's symptoms (Table 1), doctors treated her with symptomatic therapy, but her vital signs still couldn't maintain stable. Until the 4th day of age, she died due to her patients' choice of withdrawing treatment.

Exome Sequencing and Analysis

Genomic DNA extraction from the patients and their parents was extracted from the whole blood by column method. Exome sequencing was performed with the genomic DNA of the patient by using the SureSelect V5 capture kit (Agilent) and HiSeq 2000 sequencer (Illumina). After mapping to the reference human genome (UCSC hg19), the sequence data were sorted, merged, and analysed. We achieved an average percentage of >95% of

Table 1 Patient clinical features

	Patient
Age	0 year
Gender	Female
Health care in pregnancy	Polyhydramnios at 38 weeks
Hyporeflexia	Yes
Dysmorphic features	Ocular abnormalities with widely spaced eyes, turbid cornea and sluggish light reflex; depressed nasal bridge; large anterior fontanelle and cranial joint dehiscence.
Head B-sonography	Transparent septum was not closed, bilateral lateral ventricles cyst and high signal of the white matter in the lateral ventricle.
Liver dysfunction	Yes
Mutation in the *PEX1* gene	NM_000466:exon10:c.1671_1672delAG;p.Gly558fs
Treatment	Keep warm, intravenous nutrition, ventilator support, prevent bleeding, reduce intracranial pressure, protect liver, anti-infection, anti-shock and improve pulmonary hypertension.
Prognosis	Died on day 4

covered consensus coding DNA sequence at 20x, providing sufficient depth to analyse variants. Candidate pathogenic variants were defined as missense, nonsense, frameshift, and splice-site mutations with a minor allele frequency <0.01, using the 1000 Genomes Project database, Human Gene mutation database (HGMD), ClinVar, NHLBI Exome Sequencing Project (ESP6500), UniProt and dbSNP. We used programs Polyphen2, and other prediction programs like SIFT, and MutationTaster to predict the effect of amino acid substitution on protein function and structure.

Referring to the data filtering approach, we detected a novel small deletions variant in the homozygous state in the *PEX1* gene. The mutation corresponds to two bases change c.1671_1672delAG and leads to an early termination after a frameshift by deletion of AG at the 1671st and 1672nd bases of the 10th exon cause disruption of the reading frame from position 558 of the protein with the generation of a premature stop codon (p.G558fsX34). Sanger sequencing results confirmed segregation and revealed that the two parents are heterozygous, while the dead patient carried the same homozygous variant (Figure 1a and b). The mutation c.1671_1672delAG of the PEX1 gene had neither been reported before nor in the common genetic variation database. The p.G558fsX34 is predicted to be probably deleterious and disease-causing.

Discussion

Here, we described a Zellweger syndrome case with homozygous variation (c.1671_1672delAG) of the *PEX1* gene, which was from heterozygous parents, respectively. The individual was hypotonic and had distinctive facial features of ZS patients, including a high forehead, widely spaced eyes, depressed nasal bridge, and sizeable anterior fontanelle. She is also characterised by developmental malformations of the brain, liver, and kidney.

Although the function of Pex1p is not fully elucidated, previous reports highlighted its essential role in peroxisome assembly and protein import. Pex1p contains PEX-2N, PEX-1N, and AAA domains (Figure 1c). PEX-2N and PEX-1N domains can interact with ubiquitin and proteins with a ubiquitin-like domain. The N terminal region is presumed to contain adapter binding sites allowing for peroxisome biogenesis. The AAA cassette is for ATP binding and ATP hydrolysis.[7,8] The two base-pair deletions (c.1671_1672delAG) in the patient results in a frameshift at the 558 aa (p.G558fsX34) and predicted to cause premature termination of translation and a truncated protein. Patients with this homozygous mutation generally suffer from severe peroxisome disorders such as Zellweger syndrome.

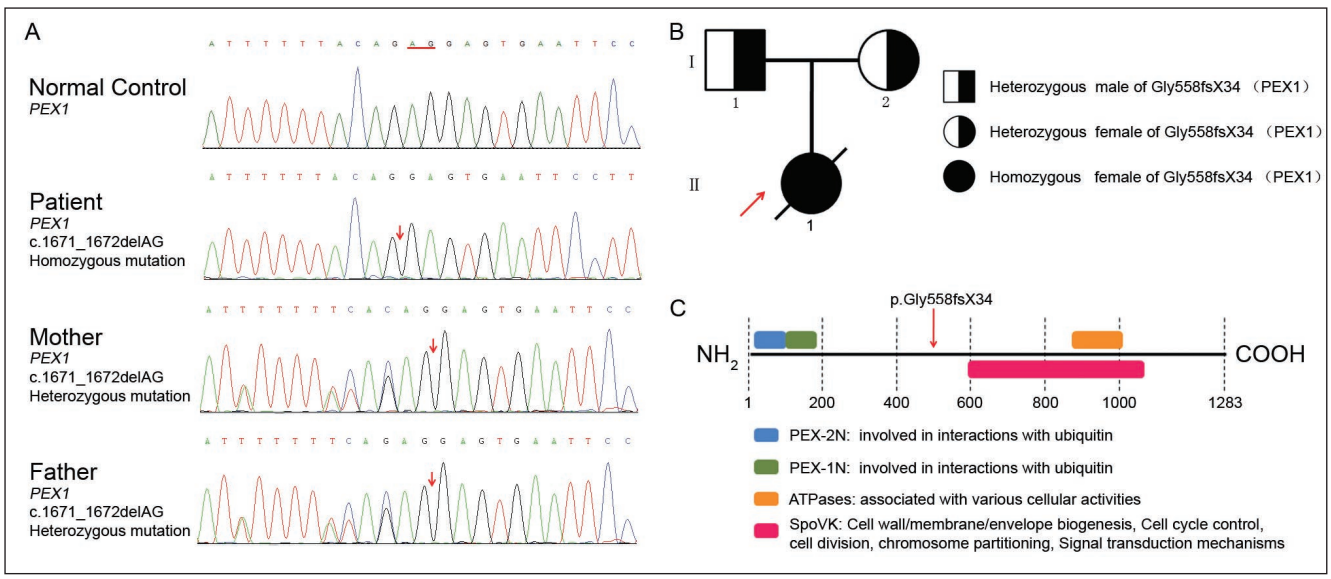

Figure 1 The pedigrees and sequencing results of the family with *PEX1* variation. (A) Sequencing results, the site of the variant was marked by arrows. (B) Family pedigree, the proband was indicated by arrow. (C) Schematic representation of the *PEX1* structure showing the localisation of a novel homozygous *PEX1* pathogenic variation, the p.Gly558fsX34.

More than 114 different mutations have been identified in the *PEX1* genes, including missense/nonsense mutation, splicing, small deletions/insertions/indels, and gross deletions/insertions/duplications (data from HGMD). Patients with milder phenotypes commonly harbour missense mutations, while nonsense, frame-shifts, and deletions mutations are more likely to be found in severe cases.[5]

One of the weaknesses of our study is the lack of biochemical analysis. Impaired peroxisomes lead very-long-chain fatty acids (VLCFAs) and branched-chain fatty acids accumulation in tissues and decreased levels of docosahexaenoic acid. These metabolites are predicted to impair the development of the brain, liver, kidneys, and endocrine glands. However, the detection of these metabolites is not inevitable in making a diagnosis of ZS patients. Moreover, the metabolite assay is a technique that requires doctor's experience and can't make an accurate diagnosis. It was reported that VLCFA analysis is hard to dissimilate ZS with other phenotypes.[9,10]

Exome sequencing has considerable potential in making a more accurate and comprehensive diagnosis for Zellweger syndrome. Our patient only received symptomatic treatment during her illness because there are no uniform guidelines for ZS treatment in China. As a result, our patient was in a dangerous condition and died early. This case is the first report of a Chinese newborn patient with the severe classic form of Zellweger syndrome caused by a novel mutation c.1671_1672delAG in the *PEX1* gene using High-throughput exome sequencing. Our findings provide information for genetic counseling and prenatal diagnosis for the family members.

Conflict of Interest

The authors declare no conflicts of interest.

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