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SHORT COMMUNICATION

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Report of a case with ferredoxin reductase (*FDXR*) gene variants in a Chinese boy exhibiting hearing loss, visual impairment, and motor retardation

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Abstract

Ferredoxin reductase (*FDXR*), located in 17q25.1, encodes for a mitochondrial NADPH: adrenodoxin oxidoreductase or ferredoxin reductase, the sole human ferredoxin reductase involved in the biosynthesis of iron-sulfur (Fe-S) clusters and heme formation. Iron-sulfur (Fe-S) clusters are involved in enzymatic catalysis, gene expression, and DNA replication and repair. Variants in *FDXR* lead to sensorial neuropathies, damage optic, and auditory neurons. Here, we report a Chinese boy with hearing loss, visual impairment, and motor retardation, with two novel compound heterozygous variants in *FDXR* (NM_004110), namely, c.250C > T (p.P84S) and c.634G > C (p.D212H), identified by whole-exome sequencing. Compared with the reported cases, except hearing loss and visual impairment, the clinical manifestations of this boy were more serious, who also had motor retardation and died in infancy after infection. The present study expands our knowledge of *FDXR* variants and related phenotypes, and provides new information on the genetic defects associated with this disease for clinical diagnosis.

KEYWORDS

ferredoxin reductase, hearing loss, iron-sulfur (Fe-S) clusters, motor retardation, visual impairment

Ferredoxin reductase (***FDXR***), which is on chromosome 17, encodes for a mitochondrial NADPH: adrenodoxin oxidoreductase or ferredoxin reductase, the sole human ferredoxin reductase involved in the biosynthesis of iron-sulfur (Fe-S) clusters and heme formation. Iron-sulfur (Fe-S) clusters are involved in enzymatic catalysis, gene expression, and DNA replication and repair. Here, we report a Chinese boy with hearing loss, visual impairment and motor retardation, with two novel compound heterozygous variants in the ***FDXR*** gene (NM_004110), namely, c.250C > T (p.P84S) and c.634G > C (p.D212H), identified by whole-exome sequencing.

1 | INTRODUCTION

Ferredoxin reductase (*FDXR*, OMIM 103270) are iron-sulfur proteins that promote the monooxygenase reactions catalyzed by P450 enzymes. The ferredoxin and ferredoxin reductase play important roles in iron–sulfur cluster assembly. The human genome includes two ferredoxins, ferredoxin 1 (*FDX1*) and ferredoxin 2 (*FDX2*). Rouault et al. found that interference with any of the three related genes, *FDX1, FDX2*, or *FDXR*, disrupts iron-sulfur cluster assembly and maintenance of normal cytosolic and mitochondrial iron homeostasis. In cell culture, including human erythroid leukemia (K562) cells and HeLa cells, by using RNAi techniques, knockdown of the sole human ferredoxin reductase, *FDXR*, diminished iron–sulfur cluster assembly and compromised iron-sulfur cluster formation, then caused a mitochondrial iron overload in conjunction with cytosolic depletion (Shi et al., 2012).

Paul et al. reported eight subjects from four independent families affected by auditory neuropathy and optic atrophy, and one of the children had a slight language delay. Whole-exome sequencing revealed biallelic variants in *FDXR* in affected subjects of each family. *FDXR* encodes the mitochondrial ferredoxin reductase, the sole human ferredoxin reductase implicated in the biosynthesis of iron-sulfur clusters (ISCs) and in heme formation. ISC proteins are involved in enzymatic catalysis, gene expression, and DNA replication and repair. Variants in several genes involved in ISC assembly have been reported in human diseases (Friedreich ataxia due to FXN variants is the most frequent). These variants result in a wide panel of variably severe clinical presentations, ranging from fatal infantile leukodystrophy to mitochondrial myopathy. Paul et al. showed that variants in *FDXR* result in sensorial neuropathies. These data highlight the wide clinical heterogeneity of mitochondrial disorders related to ISC synthesis (Paul et al., 2017).

Here, we report a Chinese boy suffering from hearing loss, visual impairment, and motor retardation. Whole-exome sequencing revealed compound heterozygous variants in *FDXR*, which is the second to find *FDXR* variants associated with auditory neuropathy and optic atrophy, preceded only by Paul et al. The boy eventually died of severe infection when he was 1 year old. According to the current reports and our report, the pathogenic variants in *FDXR* can lead to optic and auditory neuropathies, and also can lead to motor retardation.

2 | MATERIALS AND METHODS

A blood sample was collected from the proband and was sent to the AmCare Genomics Laboratory (GuangZhou, China) for whole-exome sequencing. The study was approved by the Medical Ethics Committee of Affiliated Hospital of Qingdao University. Genomic DNA was extracted using the Qiagen FlexiGene DNA kit (Qiagen) according to the manufacturer's instructions. The target DNA fragments from the amplified DNA library were captured using the SureSelect target enrichment system (Agilent). Paired-end sequencing was employed using the NextSeq500 (Illumina) instrument. Primary data came in fastq form after image analysis. Data were filtered to generate "clean reads" by removing adapters and low-quality reads (<Q20). Sequences were aligned to the hg19 reference genome by NextGENe software (SoftGenetics, State College, PA) using the recommended standard settings for single-nucleotide variant and insertion/deletion discovery. Sequence variants were annotated using population and literature databases, including 1,000 Genomes, dbSNP, GnomAD, Clinvar, HGMD, and OMIM, and computational analysis of variants was performed using PolyPhen-2, CADD, and MutationTaster. Sanger sequencing was utilized for further validation of variants. The obtained sequences were aligned with the previous results, and false-positive sites obtained by NGS (the next-generation sequencing) were ruled out. The frequency filter adopted the minor allele frequency (MAF) > 1% in the Asian population. Variants interpretation was manipulated according to the American College of Medical Genetics (ACMG) guidelines.

3 | CASE REPORT

A 9-month-old boy referred to the hospital for developmental delay. He was unable to lift his head stably, was unable to turnover, and his eyes did not track properly. He had hypotonia. He was born at 36 weeks of unexplained premature delivery. His birth weight was 2,800 g (50th centile) and his birth head circumference was 34 cm (75th centile). He was hospitalized because of unexplained jaundice when he was born. His parents were healthy and had no history of developmental delay in their families. He was diagnosed with developmental delay at seven months old. The brain MRI (Figure 1) showed that T1 signal of globus pallidus on both sides was slightly higher, T2W signal of paraventricular white matter on both sides was slightly higher, cerebral grooves in each lobe of the cerebral hemisphere were slightly deeper, ventricles on both sides were full, subarachnoid cavities on both sides were slightly wider, cerebellum and brain stem were normal, and midline structure was not displaced. The optic nerve was thin. Due to the lack of eye-tracking ability, a fundus examination was conducted: the fundus was unclear, the optic disc of both eyes was pale, the blood vessels were thin, white lines were observed, and the surrounding vitreous body of both eyes were point-like opacity. In addition, routine blood tests were performed: homocysteine was 8.7 μmol/L (1.6-16 μmol/L), lactate was 2.62 mmol/L

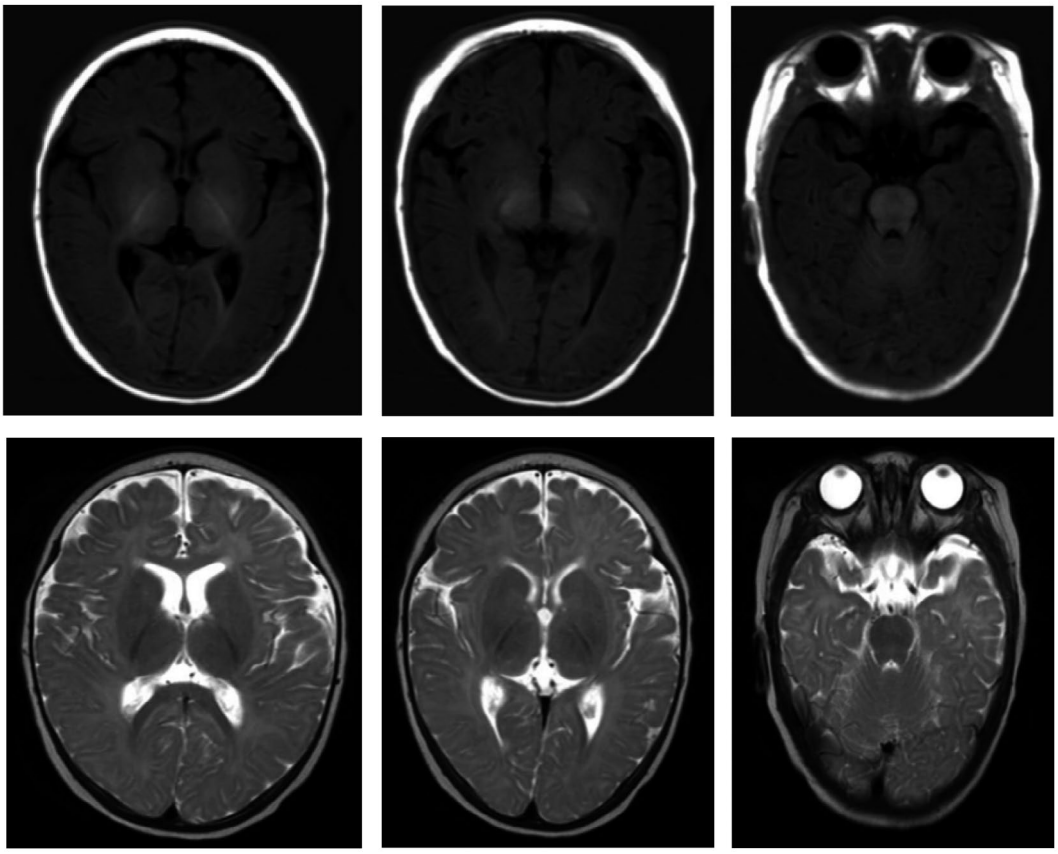

FIGURE 1 T1W signal of globus pallidus on both sides were slightly higher, T2W signal of paraventricular white matter on both sides were slightly higher, cerebral grooves in each lobe of cerebral hemisphere were slightly deeper, ventricles on both sides were full, subarachnoid cavity on both sides were slightly wider, cerebellum and brain stem were normal, and midline structure was not displaced. The optic nerve was thin

(0.5–2.2 mmol/L), AST was 91.1 U/L (5-40 U/L), ALT was 80.6 U/L (5-40 μ/L) and blood ammonia was 85 μmol/L (18-72 μmol/L). The cause of developmental delay in this boy remained unclear.

Due to movement and vision problems of the proband, it was hypothesized that it may have a genetic cause; thus, whole-exome sequencing was performed. A total of 270 variants remained after the filtering process. Combined with the clinical symptoms, family history, and genetic characteristics of the patients, the pathogenic mutation was screened. We identified two heterozygous ferredoxin reductase (*FDXR*) gene variants (NM_004110) (Figure 2): c.250C > T (p.P84S, maternally inherited), c.634G > C (p.D212H, paternally inherited) (Figure 3). These two changes have not been reported in the peer-reviewed literature. These two amino acid changes are highly conserved. In silico tools for the prediction of the pathogenesis indicated that the two missense variants were both damaging, with the results of CADD (25.8, Damaging), PolyPhen2 (0.997, Probably-damaging), and Mutation Taster (1, Disease-causing) in c.250C > T (p.P84S), and CADD (28.1, Damaging), PolyPhen2 (1, Probably-damaging), Mutation Taster (1, Disease-causing) in c.634G > C (p.D212H). According to the ACMG guidelines, both variants are classified with uncertain significance. The two variants are not reported in the reference population gene database. The regions where the two variants located are important components of this protein. The amino acid sequences of different species are highly conserved. Computer-aided analysis shows that the two variations will affect the structure and function of the protein, combined with the clinical manifestations of the patient, both variants are classified with uncertain significance. Upon the completion of a hearing test (Auditory brainstem response, ABR), profound bilateral hearing impairment was detected. At the same time, we conducted an otoacoustic emissions test on this patient. The test result was normal. So the diagnosis of auditory neuropathy was definite. This was consistent with *FDXR*-related phenotypic characteristics.

The proband was followed and he passed away of a serious infection at 1 year of age after having a fever for a full day. The boy was groaning in his mother's hands, he appeared ill, tired, and had tachycardia and tachypnea. Through physical examination and relevant laboratory examination, sepsis and septic shock were diagnosed in the local hospital. After active emergency rescue and treatment, the child still died, unfortunately.

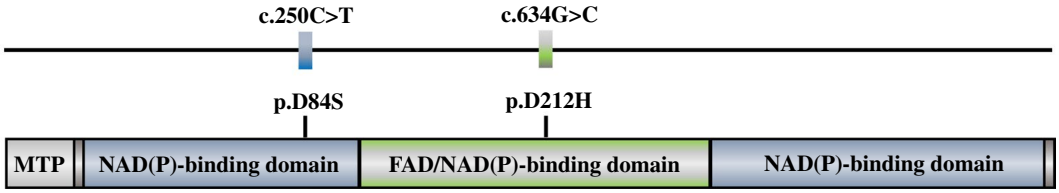

FIGURE 2 FDXR gene structure and locus position [Colour figure can be viewed at wileyonlinelibrary.com]

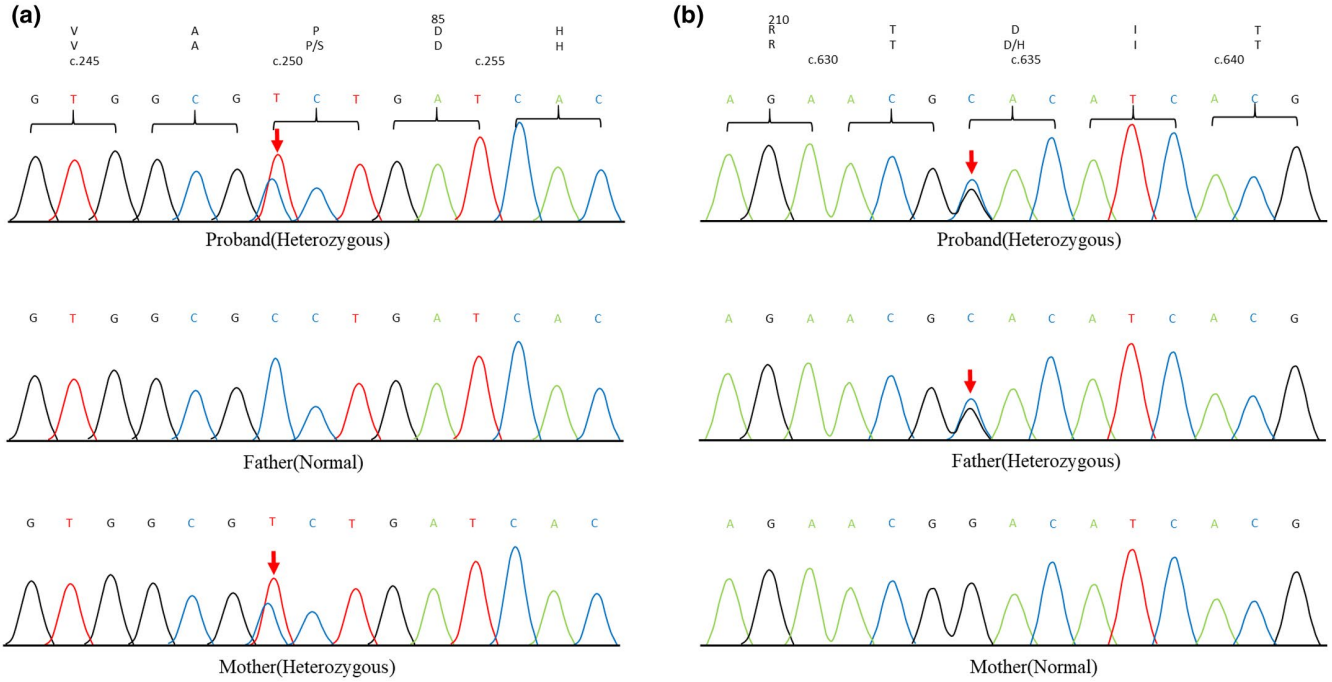

FIGURE 3 Compound heterozygous variants in the FDXR gene were confirmed by Sanger sequencing. A, Heterozygous c.250C > T (p.P84S) detected in the patient was maternally inherited. B, Heterozygous c.634G > C (p.D212H) detected in the patient was paternally inherited [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

4.1 | Pathogenesis studies

Iron-sulfur (Fe-S) clusters are widespread co-factors composed of iron and sulfur, which are important to various cellular processes, such as mitochondrial respiration, DNA repair, iron homeostasis, and metabolism (Beilschmidt & Puccio, 2014; Lill, 2009). Ferredoxin and ferredoxin reductase play important roles in iron–sulfur cluster assembly. *FDXR* encodes the mitochondrial ferredoxin reductase, the only human ferredoxin reductase implicated in the biosynthesis of ISCs and in heme formation (Ewen et al., 2011; Jung et al., 1999; Stehling et al., 2014). The human genome has two ferredoxins, ferredoxin 1 (FDX1) and ferredoxin 2(FDX2). Ferredoxin-1 and Ferredoxin-2, which are encoded by two homologous genes in mammals and expressed in mitochondria, serve as electron donors in Fe-S cluster biosynthesis (Cai et al., 2017; Sheftel et al., 2020). Mitochondrial membrane-associated ferredoxin reductase (*FDXR*) is a flavoprotein that initiates the mitochondrial electron transport chain reaction by transferring electrons from NADPH to the mitochondrial cytochrome P450 system via *FDX1* or *FDX2* (Hanukoglu & Jefcoate, 1980).

Fe–S clusters are parts of the mitochondrial complexes I and III. Generation of iron-sulfur (Fe–S) cluster scaffold proteins need a coordinated delivery of iron, sulfur, and electrons. The electrons required for Fe-S cluster assembly are delivered by ferredoxin, which is reduced by ferredoxin reductase using NADPH as the electron donor. Depletion of *FDXR* diminishes Fe-S cluster assembly and causes mitochondrial iron overload, indicating that *FDXR* has a crucial role in Fe-S cluster biogenesis in human cells. Variants of *FDXR* may impair Fe-S cluster biosynthesis and result in decreased complex I and complex III activity. Variants in *FDXR* have a broad effect on mitochondrial functions through the reactive oxygen species pathway (ROS pathway), contributing to more general mitochondrial dysfunction (Shi et al., 2012; Webert et al., 2014). Moreover, Yanbo Shi et al. detected a major iron overload and increased mitochondrial ROS

TABLE 1 Clinical characteristics of patients with *FDXR* gene deleterious variants

Patient	Present case	1 (Paul et al., 2017)	2 (Paul et al., 2017)	3 (Paul et al., 2017)	4 (Paul et al., 2017)	5 (Slone et al., 2018)	6 (Slone et al., 2018)
Gender	M	F	M	M	F	M	F
Hearing defect	BAN	BAN	BAN	BAN	BAN	No	No
Visual defect	BOA	BOA	Infraclinical BOA	BRP and BOA	BOA	BOA	BOA
Gross motor	Yes	No	No	No	No	Yes	Yes
FDXR variations	c.250C > T, c.634G > C	c.916, C > T Ho	c.916 C > T, c.1255 C > T	c.724C > T, c.979C > A	c.643C > G, c.1429G > A	c.1A > G, c.463C > T	c.947delG, c.578G > A
Protein change	p.P84S, p.D212H	p.Arg306Cys	p.Arg306Cys, p.Gln419*	p.Arg242Trp, p.Arg327Ser	p.Leu215Val, p.Glu477Lys	p.M1?, p.R155W	p.S316Tfs*19, p.R193H

Abbreviations: BAN, bilateral auditory neuropathy; BOA, bilateral optic atrophy; BRP, bilateral retinitis pigmentosa; F, female; Ho, homozygous; M, male.

production in fibroblasts of affected individuals, which is consistent with previous studies in human FDXR-knockdown cells (Shi et al., 2012).

In 2020, Slone et al. certificated that the *FDXR* variant leads to significant optic transport defects which may explain optic atrophy. At the same time, they indicated that *FDXR* variants can lead to mitochondrial iron overload and associated depolarization of the mitochondrial membrane. These results illustrated that *FDXR* variants cause neurodegeneration by affecting *FDXR*'s critical role in iron homeostasis (Slone et al., 2020).

Generally, variants in *FDXR* may impair protein functions through multiple mechanisms, including protein-protein interactions, protein stability, and binding affinity to the substrates. In this study, the patient carried two novel compound heterozygous variants in *FDXR* (NM_004110), namely, c.250C > T (p.P84S) and c.634G > C (p.D212H). These two variants are located in exon, which is predicted by software to affect protein function. Fe-S cluster biogenesis in human cells will be impaired and then neurodegeneration will happen. Sensorial nerves were destroyed, the patient suffered hearing loss and visual impairment. Motor nerves were destroyed, the patient suffered hypotonia and motor retardation. The detailed mechanism needs further study.

4.2 | Clinical manifestations and diagnosis

FDXR gene-related diseases have been observed to include auditory neuropathy and optic atrophy as clinical features and are inherited in an autosomal recessive manner. In 2017, Peng et al. demonstrated for the first time that germline hypomorphic variants in *FDXR* cause a novel mitochondriopathy and optic atrophy in humans. Mice carrying FDXR variants found progressive gait abnormalities and vision loss, suggesting a biallelic mode of inheritance for FDXR. In 17 individuals from 13 unrelated families, clinical manifestations consisted of microcephaly, optic atrophy, seizures, global development delay, hypotonia, spasticity, and ataxia. (Peng et al., 2018). Paul et al. have shown that biallelic variants in *FDXR* lead to sensorial neuropathies, confirming the critical role of the Fe-S biogenesis in the function of optic and auditory neurons (Paul et al., 2017) (Table 1).

In this study, the boy suffered hearing loss, visual impairment, hypotonia, and motor retardation. The boy's condition was serious because he has these clinical manifestations at the same time. The boy eventually died of a serious infection at 1 year of age. The blood test of the patient, including lactate, AST, ALT, and blood ammonia were all elevated, maybe it was related to the abnormal energy metabolism of mitochondria. Abnormal energy metabolism can lead to lactate accumulation and abnormal metabolism of many enzymes. The specific mechanism needs further study. Those points highlight the wide clinical heterogeneity of mitochondrial disorders even when the disease-causing variants arise in the same pathway. In this case, we further understand the refractory of mitochondrial-related diseases. At present, there is no radical cure for mitochondrial diseases. We can only provide some energy substances, such as idebenone and coenzyme Q10.

5 | CONCLUSION

In this sensorial neuropathy study, we reported one new human case with biallelic *FDXR* variants which expands our understanding of *FDXR*-related diseases. *FDXR* plays an important role in iron-sulfur (Fe-S) cluster assembly. Iron-sulfur (Fe-S) clusters are critical for various cellular processes. The boy suffered hearing loss, visual impairment, and motor retardation, and eventually died of serious infection. The prevalence of these disorders is unknown, but is suspected to be very rare. The world's awareness of the disease is also insufficient, resulting in a high rate of missed diagnosis. The molecular mechanism of how these variants affect the FDXR protein function and lead to the observed clinical manifestations need further to be investigated.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Chengqing Yang and Ying Zhang were responsible for writing the article. Zhi Yi collected clinical data and analyzed the pathogenicity of the variants in silico; Zhenfeng Song and Fei Li collected clinical data; Jiuwei Li edited figures; Chunli Wang and Jiao Xue searched the literature and provided language help; Ying Zhang and Victor Wei Zhang proofreading the article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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