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CLINICAL REPORT

Novel homozygous protein-truncating mutation of BBS9 identified in a Chinese consanguineous family with Bardet–Biedl syndrome

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Abstract

Background: Bardet–Biedl syndrome (BBS) is a rare and genetically heterogeneous disease with a broad spectrum of clinical features, including but not limited to rod-cone dystrophy, postaxial polydactyly, central obesity, intellectual disability, hypogonadism, and renal dysfunction. Twenty-one BBS (Bardet–Biedl syndrome) genes have been identified to date. There is minimal mutation information on BBS in Chinese populations and the exact pathogenic mechanism of the null mutation of BBS9 remains unknown.

Methods: A patient from a Chinese consanguineous family presented with polydactyly, truncal obesity, intellectual disability, genital anomaly, and retinitis pigmentosa was analyzed in this study. Blood DNA and RNA were extracted from the blood of the proband and the parents. The proband was screened for mutations by whole-exome sequencing. The likely pathogenic mutation detected in the proband was further confirmed by the Sanger sequence in the family. Real-time RT-PCR was used to measure the expression of BBS9 in the proband and the control.

Results: Targeted exome sequencing identified a novel homozygous null mutation (NM_198428.3: c.445C>T) in the 6th exon of the BBS9 gene in the proband and Sanger sequencing was used to validate the heterozygosity in the parents. The mutation was validated to induce the nonsense-mediated decay of BBS9 messenger RNAs by real-time RT-PCR.

Conclusions: The molecular findings helped to explain the clinical manifestations. The novel homozygous pathogenic variation expanded the mutational spectrum of the BBS9 gene in the Chinese population and will help to understand the pathogenic mechanism of BBS9 null mutation.

KEYWORDS
BBS, BBS9, NMD, null mutation

Hai-Yan Tang and Fen Xie are co-first author.

Ru-chun Dai and Xiao-liu Shi are co-corresponding author.

1 | BACKGROUND

Bardet–Biedl syndrome (BBS) is a multi-system non-motile ciliopathy primarily characterized by retinal cone-rod dystrophy, obesity, and related complications, postaxial polydactyly, cognitive impairment, hypogonadotropic hypogonadism and/or genitourinary malformations, and renal malformations and/or renal parenchymal disease (Forsyth et al., 1993). The signs and symptoms of this condition vary among affected individuals, even among members of the same family. The first signs and symptoms of BBS include rod-cone dystrophy, hypogonadism, intellectual disability, obesity, and polydactyly. Additional features can consist of strabismus, cataracts, astigmatism, neurodevelopmental delay, and dental anomalies (M'Hamdi et al., 2014). The prevalence of BBS was estimated to vary between 1 in 160 000 and 1 in 13 000 in worldwide, which depends on the level of consanguinity (Farag & Teebi, 1989; Klein & Ammann, 1969; M'Hamdi et al., 2011; Moore et al., 2005). To date, 21 genes have been implicated in the syndrome: BBS1, BBS2, ARL6/BBS3, BBS4, BBS5, MKKS/ BBS6, BBS7, TTC8/BBS8, BBS9, BBS10, TRIM32/ BBS11, BBS12, MKS1, CEP290, C2orf86, TMEM67, CCDC28B, BBIP1, IFT27, IFT74, and C8orf37 (M'Hamdi, Redin, et al., 2014). Within 21 BBS genes, the traditional Sanger sequence is not a good choice to look for the mutation in the patients. As Xing et al. (2014) showed, the exome sequence is an accurate and rapid method for the genetic diagnosis of BBS. Using next-generation sequencing, we reported a novel homozygous protein-truncating mutation (NP_001334965.1:p.[Arg149*; Arg149*]), in the exon 6 of the BBS9 gene (OMIM#607968) in a Chinese Han family. Pathogenic mutation in the BBS9 gene can cause the Bardet–Biedl syndrome 9 (BBS9, OMIM# 615986), which is an autosomal recessive and genetically heterogeneous ciliopathy that develops during childhood and affects many parts of the body, which is caused by the pathogenic mutation in the BBS9 gene (Abu-Safieh et al., 2012; Nishimura et al., 2005).

2 | METHODS

2.1 | Ethical compliance

The study is in accordance with the ethical principles of the Declaration of Helsinki. This study protocol was obtaining the approval of the Ethics Committee of the Second Xiangya Hospital, Central South University. The patient and his parents were enrolled with written informed consent.

2.2 | Whole exome sequencing analysis and Sanger sequence

We extracted DNA from whole blood using the QIAamp DNA Blood Mini Kit (QIAGEN, 51104). Exome capture was performed using a SureSelect Human All Exon V5 kit following the manufacturer's protocol. The captured libraries were sequenced using Illumina HiSeq 2000 Sequencer. The reads were mapped against UCSC hg19 (http://genome.ucsc.edu/) by BWA (http://bio-bwa.sourceforge.net/). The SNPs and Indels were detected by SAMTOOLs (http://samtools.sourceforge.net/). The candidacy of the resulting variants was based on their population frequency and predicted the effect on the protein. The likely pathogenetic mutations were validated using direct sequencing. Direct sequencing was performed on DNA from individuals of all available family members using the 3730xl DNA Analyzer (Applied Biosystems), and the samples were subjected to sequence analysis using Sequence Scanner v1.0 (Applied Biosystems).

2.3 | Real-time reverse-transcribed PCR

RNA from leukocytes was extracted with miRNeasy Mini Kit (QIAGEN, 217004), 1 μg of RNA was used to reverse-transcribe into cDNA using PrimeScript RT reagent Kit (RR047A). Real-time PCR was prepared with the QuantiTect SYBR Green PCR Kit (Qiagen, 204243), then run and analyzed on the Applied Biosystems 7500 Fast Real-Time PCR System. Data are based on two biological replicates of the proband, one normal control, normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the built-in Sequence Detection Software V.1.4.

3 | RESULTS

3.1 | Case presentation

The proband was a 17-year-old Chinese boy who was admitted to the hospital with a chief complaint of polydipsia, polyphagia, hyperglycemia, and truncal obesity for about 12 years. The patient's parents had consanguineous marriage and the patient was born full-term to a G2P2 mother (Figure 1). The first child of the family was a male with normal appearance and died from drowning at the age of 1 year. No fetal distress of the proband was reported. He spoke his first word at 12 months and started walking at 18 months. According to the Wechsler Intelligence Scale, the proband was diagnosed with intellectual disability. The patient displayed visual problems during both day and night since early childhood. The binocular papilla was

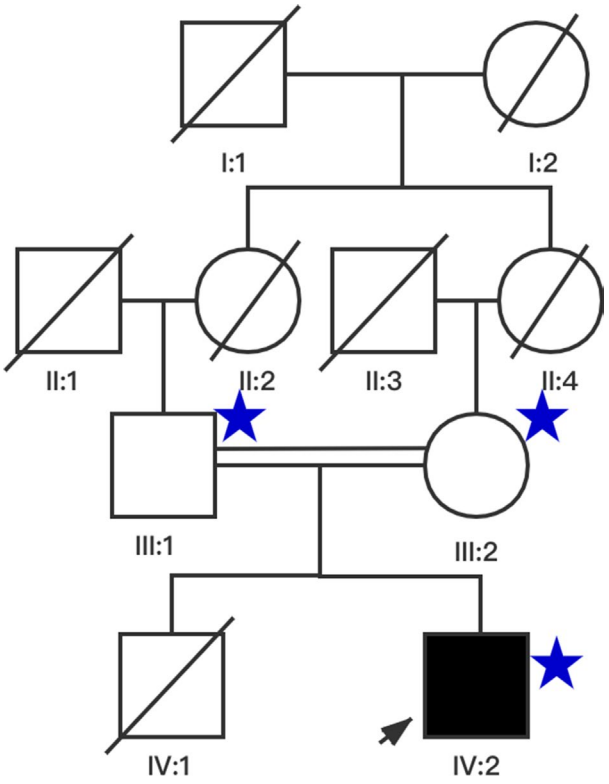

FIGURE 1 Family pedigree of patient. Symbols, tagged with asterisks, indicate individuals selected for genetic analysis

sallow-yellow, the retinal vessels in both eyes were thinner, and numerous bone-like cell deposits were visible in the axial portion of both eyes, which was suggestive of retinitis pigmentosa (RP) for both eyes (Figure 2a,b). The patient's waist circumference was 97cm, the hip circumference was 87cm, and the waist-to-hip ratio was 1.15. According to the WTO criteria, the patient was diagnosed with centripetal obesity (Figure 2e). The boy also had polydipsia, polyphagia, and hyperglycemia. Physical examination was remarkable for dysmorphism, with surgical scars visible on bilateral hands and feet (Figure 2c,d). Genitalia examination revealed the stretched phallic length was 12 cm and bilateral testicular volume was 20 ml bilaterally. There was no gynecomastia. The ultrasonography and the functional tests for the liver and kidneys showed abnormal structure, but no renal dysfunction was reported.

The proband was phenotyped as part of his clinical evaluation in a standard medical genetics clinic.

3.2 | Homozygous BBS9 variant identified by sequencing analysis and NMD identified by expression analysis

A homozygous nonsense mutation (chr7:33296850, NM_198428.3: c.445C>T) in the sixth exon of BBS9 gene

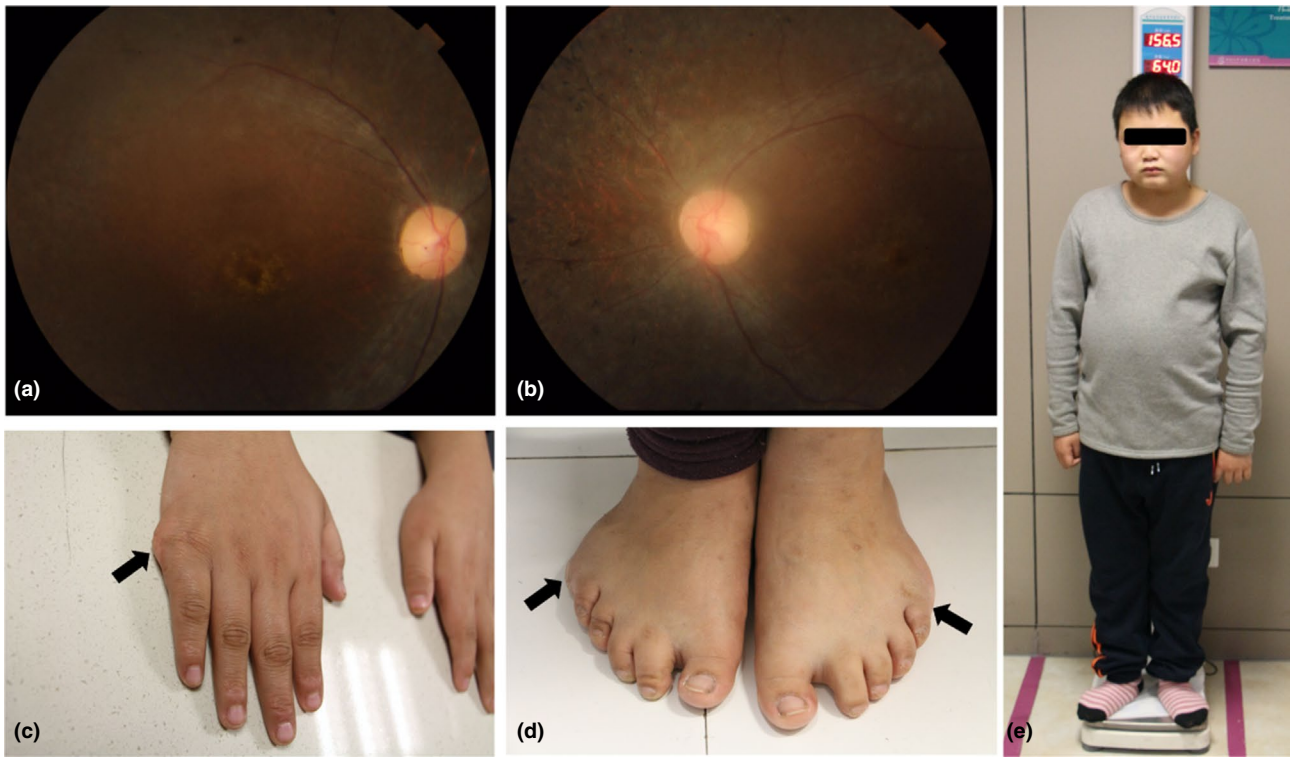

FIGURE 2 (a and b) Fundoscopy of the proband; (c and d) anomalies shown on the proband's hands and feet; (e) photography of the proband. The proband showed truncal obesity

was found in the proband, which generated a predicted premature termination codon (PTC) in the BBS9 protein (NP_001334965.1:p.[Arg149*; Arg149*]), with a prediction of "disease-causing" by MutationTaster (http://mutationtaster.org/). The proband's parents were heterozygotes for the nonsense mutant allele (Figure 3), which was consistent with the autosomal recessive inheritance mode of BBS. The PTC was predicted to result in the nonsense-mediated decay (NMD) of BBS9 mRNA. Real-time PCR revealed near-complete loss of the BBS9 transcript in the patient's lymphoblasts when compared with the control, thus confirming the nonsense-mediated decay (NMD) effect of this mutation and its likely null nature (Figure 4). The homozygous null mutation (NM_198428.3: c.445C>T) detected in the patient is a previously undescribed variant, which has not been curated in HGMD yet. Only three samples have been reported to date in Europeans from the Exome Aggregation Consortium database (http://exac.broadinstitute.org/) carrying the heterozygous variation. The population frequencies are 4.6×10^{-5} in Europeans and 0% in East Asians. The variant NM_198428.3: c.445C>T was interpreted as a pathogenic variant by ACMG standards and guidelines (Richards et al., 2015).

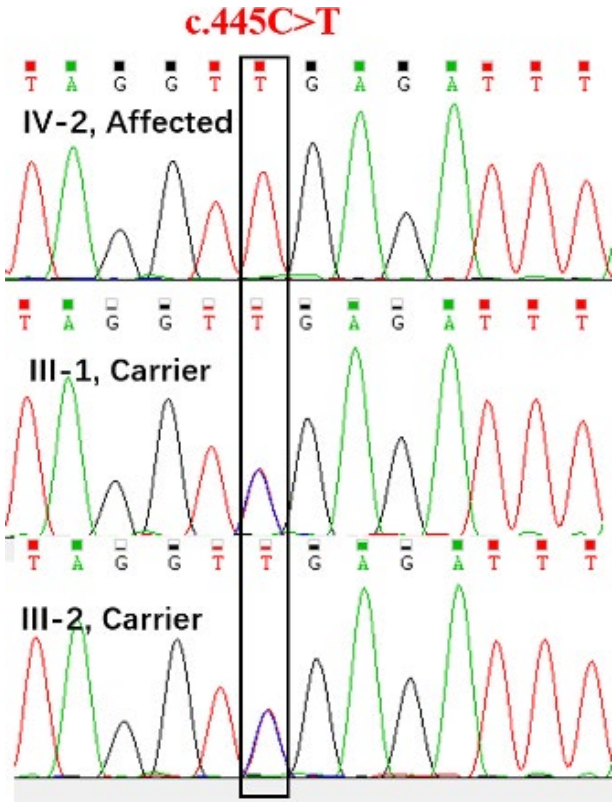

FIGURE 3 Sequence chromatogram of BBS9 exon six is showing a novel homozygous null mutation in an affected individual, a heterozygous mutation in two carriers

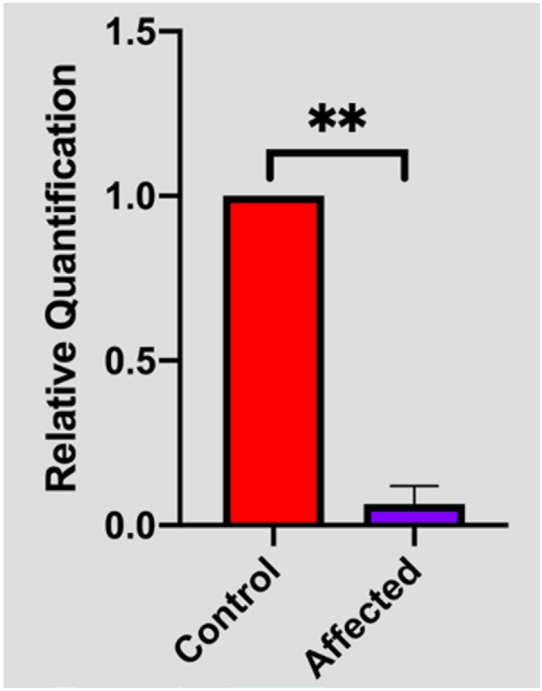

FIGURE 4 Real-time RT-PCR of patient and the control lymphoblast cells, revealing almost complete loss of the patient BBS9 transcript. Error bars indicate SE of the means; p-value < 0.05 (unpaired t test)

4 | DISCUSSION AND CONCLUSIONS

The present study described the clinical and genetic analyses of a Chinese consanguineous family with BBS9. The identified nonsense mutation (NM_198428.3: c.445C>T) in the BBS9 gene confirmed the clinical diagnosis of BBS and helped with the precise diagnosis of type 9. The protein-coding length of BBS9 is 889 amino acids. When the 445th nucleotide changed from C to T, a premature termination codon was generated in the 149 amino acid, which led to the complete absence of the gene product by NMD. To date, a total of 69 pathogenic or likely pathogenic single nucleotide variants of BBS9 have been reported to be associated with HGMD. However, the null variations have not been validated with real-time PCR experiment to understand that the exact pathogenic mechanism is NMD. The present study showed the near-complete loss of the BBS9 transcript in the patient's lymphoblasts when compared with the control.

Khanna et al. (2009) and Putoux et al. (2011) found that some alleles may contribute to or exacerbate the phenotype of other ciliopathies, particularly BBS. These genes include CCDC28B, MKS1, MKS3, C2ORF86, KIF7, and RPGRIP1L. In the present study, no rare variation of these genes was found in the proband.

The mutation information on BBS is minimal in Chinese populations, and only three studies involving seven Chinese

cases have been reported to date (Li et al., 2014; Xing et al., 2014; Yang et al., 2008). The present study reported the phenotypes of the first Chinese family with p. Arg149* of BBS9, which will help the doctors to distinguish the phenotype of BBS from other similar syndromes and expand the understanding of BBS. Also, the current study validated the presence of NMD in the nonsense mutation of BBS9, which will help to interpret the null variation in BBS9.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

HYT was involved in DNA isolation, genetic analysis of data, and manuscript drafting. F.X. was involved in recruiting the BBS family, clinical analysis of data. RCD carried out family recruitment, blood sampling, and clinical analysis. XLS supervised the whole study, helped in data analysis, edited, and refined the manuscript. All authors have read and approved the refined manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study is in accordance with the ethical principles of the Declaration of Helsinki. This study protocol was obtaining the approval of the Ethics Committee of the Second Xiangya Hospital, Central South University. The patient and his parents were enrolled with written informed consent.

CONSENT FOR PUBLICATION

Written informed consent was obtained from the patient's parents for publication. A copy of the written consent is available for review by the Editor of this journal. The authors know and consent for publication.

DATA AVAILABILITY STATEMENT

The data generated or analyzed during the current study are available from the corresponding author on reasonable request.

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