Prenatal diagnosis of Bardet-Biedl syndrome due to novel variants in the BBS10 gene in a fetus with multiple anomalies: A case report

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Abstract. Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder characterized by obesity, mental retardation, retinal dystrophy, hypogenitalism and renal and polydactyly malformations. The last two malformations may be observed antenatally and are highly variable, making the prenatal diagnosis of BBS challenging. The present study investigated the molecular etiology of BBS and validated a method for prenatal diagnosis. A Chinese couple who had conceived two fetuses with multiple malformations, including hyperechogenic kidneys, polydactyly, cardiac malformation and abdominal abnormalities, presented at the Prenatal Diagnosis Center of Boai Hospital of Zhongshan Affiliated to Southern Medical University (Zhongshan, China) in November 2018. BBS was suspected and whole-exome sequencing was performed for the second fetus. Two novel compound heterozygous variants were detected in the BBS10 gene, c.784_785delGA from the father and c.1812dupT from the mother, which are probably causative of the pathogenesis of BBS. This finding provided a basis for genetic counseling and prenatal diagnosis for the couple and enriched the variation spectrum of the BBS10 gene. The ultrasonic findings of the fetal abdomen are the first reported in fetuses with BBS, expanding the antenatal phenotypes of BBS.

Introduction

Bardet-Biedl syndrome (BBS; Online Mendelian Inheritance in Man #615987) is a rare autosomal recessive disorder that involves multiple systems, with clinical manifestations that may include obesity, mental retardation, retinal dystrophy, hypogenitalism, renal malformations and polydactyly (1). To date, 26 genes have been reported to be associated with BBS, and these BBS genes have crucial roles in both the composition and function of the cilia (2). The BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, BBS9 and BBS18 proteins are components of the BBSome that functions as an adaptor for intraflagellar transport molecules, and the BBS6, BBS10 and BBS12 proteins form the chaperonin-like complex that has an important role in BBSome assembly (3). The mutations of the genes coding for components of the BBSome are most frequently (57%) identified in the patients with BBS, and these are followed by the group of genes that encode chaperonin-like proteins (30%) (2). BSS10 was identified by Stoetzel et al (4) in 2006 and mutations in this gene account for 21% of BBS cases. A total of 115 variants distributed across the whole BBS10 gene have been reported in the Human Gene Mutation Database (HGMD). All types of mutations have been described; missense variants are the most frequent, followed by small deletions. Among them, the c.271dupT (p.Cys91LeufsX5) variant is the most common pathogenic variant in the BBS10 gene, accounting for up to 48% of cases with a BBS10 mutation (5). In the present study, a novel compound heterozygous variants in BBS10 was detected in a fetus with hyperechoic kidneys and severe cardiac malformation.

Case report

A 36-year-old G2P0A1 female was referred to the Prenatal Diagnosis Center of Boai Hospital of Zhongshan Affiliated to Southern Medical University (Zhongshan, China) in November 2018 for prenatal diagnosis at 25 weeks of gestation due to a fetus with multiple anomalies. The patient's previous pregnancy was terminated due to similar fetal anomalies, as indicated in the pedigree chart in Fig. 1. During the patient's

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first pregnancy, the 23-week sonography indicated bilateral enlarged hyperechogenic kidneys, polydactyly of both feet, bowel dilation and multiple abdominal calcifications (Fig. 2A-C). The pregnancy was terminated without any genetic analysis. During the second pregnancy, the first-trimester ultrasound was normal. The 25-week sonography indicated bilateral enlarged hyperechogenic kidneys, a ventricular septal defect combined with a single atrium, persistent left superior vena cava and seroperitoneum (Fig. 2D-F). Considering similar phenotypes of the kidneys in both fetuses, accompanied by polydactyly, cardiac malformations and abdominal abnormalities, BBS was suspected in this family. The parents were non-consanguineous and healthy, without any family history of congenital malformations.

Genetic testing and analysis were performed with umbilical cord blood in the second fetus. G-banding normal chromosomes (46, XX) was found in the second fetus. A genomic microarray of the umbilical cord blood using an Affymetrix CytoScan 750K array (Thermo Fisher Scientific, Inc.) revealed no abnormalities. Whole-exome sequencing was performed with umbilical cord blood on the MGIseq-2000 platform (MGI Tech Co., Ltd.), revealing that the fetus had two novel compound heterozygous BBS10 variants, a 2-bp deletion frameshift variant (NM_024685.3:c.784_785delGA, p.Glu262Asnfs* 41) and a nonsense variant (NM_024685.3:c.1812dupT, p.Asn605*). The two variants were also confirmed by Sanger sequencing with an ABI3730 DNA Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.), i.e. c.784_785delGA in the father and c.1812dupT in the mother (Fig. 1B and C). These two variants may be the reason for the disease in this family. Whole-exome sequencing and fetal ultrasound results were consistent with the diagnostic results of BBS. After detailed genetic counselling, the couple decided to terminate the pregnancy at 28 weeks. After induced labor, no unusual fetal face or polydactyly were observed.

The two variants detected in the present study have not been reported in any of the reference population gene databases, including the International Genome Sample Resource (http://www.internationalgenome.org; accessed March 11, 2022), Genome Aggregation Database (http://www. gnomad-sg.org/; accessed March 11, 2022), ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/; accessed March 11, 2022) and the Human Gene Mutation Database (http://www. hgmd.cf.ac.uk/ac/index.php; accessed March 11, 2022), which are used to screen pathogenic variants reported in published studies. BBS10 (NM_024685.3) c.784_785delGA causes a frameshift. This frameshift variant is located in the last exon of the BBS10 gene, which is not predicted to undergo nonsense-mediated decay (NMD) (6). However, the truncated protein loses the partial apical domain, the C-terminal equatorial domain and the intermediate domain including In3, which is important for the protein's function (7). According to the American College of Medical Genetics and Genomics (ACMG) (2015) guidelines (8), the evidence of the variant included PSV1-strong, PM2 and PP4. The c.784_785delGA variant is classified as likely pathogenic. The BBS10 mutation (NM_024685.3) c.1812dupT produces a termination codon. Similarly, this nonsense variant mutation is also located in the last exon of BBS10 and is not predicted to undergo NMD. As the role of the lost region on the protein function is unknown, PVS1 evidence cannot be used (6). MutationTaster (https://www.mutationtaster.org/) predicted the variant to be 'disease-causing; probability, 0.999'. According to the ACMG (2015) guidelines (8), variant evidence includes PM2, PP3, PM3 and PP4. The c.1812dupT variant is also classified as a likely pathogenic variant.

The amino acid sequences of the BBS10 protein (GenBank accession no. NP_078961.3) were obtained from the GenBank database. Modeling analysis was performed using the homology modeling program, SWISS-MODEL (http://swiss-model.expasy.org) to visualize the wild-type BBS10 structure with the 5GW4 template. Based on the structure of wild-type BBS10, the impact of the variant on the structure of the resulting truncated protein was analyzed according to SWISS-MODEL (Fig. 3). In addition, conformations of the wild-type BBS10 protein and the mutated BBS10 proteins were superimposed by PyMOL (9). The mutated BBS10 proteins had clearly different structures from the wild-type protein (Fig. 3D and F). Therefore, these compound heterozygous mutations were predicted to affect BBS10 function as well as interactions with other molecules.

Discussion

BBS is a rare autosomal recessive inherited ciliopathy (10). Ciliopathies are a group of diseases with variants in ciliary-associated genes (11). To date, >950 cilia-associated genes have been discovered (12). BBS10 is structurally similar to type II chaperonins and is involved in protein folding (7). Variants in BBS10 have been reported in patients with a wide range of clinical presentations, including obesity and rod-cone dystrophy, as well as renal and polydactyly malformations (13). Most symptoms of BBS appear postnatally, but kidney abnormalities, polydactyly and heart structural abnormalities may be detected antenatally. In 2019, Mary et al (14) reported 45 fetuses with BBS, with the most prevalent gene mutations in BBS1 (29%), BBS10 (20%) and BBS12 (18%). That study found no significant differences in phenotypes among fetal BBS cases with different mutations, and renal abnormalities (91%) and polydactyly (82%) were the most common ultrasound manifestations in BBS fetuses. In 2010, Emmanuelli et al (15) performed a retrospective analysis of 17 fetuses with hyperechogenic kidneys and found two cases that were diagnosed as BBS. Therefore, for fetuses identified to have prenatal renal abnormalities, detailed ultrasound scans of the fetal hands, feet and heart should be performed.

In the present case, enlarged and hyperechogenic kidneys, polydactyly of the feet and cardiac malformations on prenatal ultrasonography appeared in one family, suggesting a diagnosis of BBS. Combined with the clinical manifestations, family history and genetic testing results, the second fetus was diagnosed with BBS. However, no fetal specimen of the first pregnancy was obtained, and thus, it was not possible to clarify whether these *BBS10* variants also existed in the first fetus. BBS is a possible diagnosis for the first fetus according to the clinical symptoms. The clinical manifestations of both pregnancies were also not exactly the same: The first fetus had polydactyly and the second fetus had a ventricular septal defect combined with single atrium and persistent left superior vena cava. These differences are presumed to be due to the diversity



Figure 1. (A) Pedigree chart. The proband is indicated by an arrow. (B) By Sanger sequencing of exon 2 of the BBS10 gene, a heterozygous (NM_024685.3: c.784_785delGA, p.Glu262Asnfs* 41) variant was detected in the second fetus (II2) and the proband's father (II). The variant site is indicated by arrows. (C) By Sanger sequencing of exon 2 of the BBS10 gene, a heterozygous (NM_024685.3: c.1812dupT, p.Asn605*) variant was detected in the second fetus (II2) and the proband's mother (I2). The variant sites are indicated by arrows.



Figure 2. Ultrasonographic findings of the first fetus (II1) at 23 weeks were (A) hyperechogenic kidneys, (B) postaxial polydactyly of the right foot and (C) bowel dilation. Ultrasonographic findings of the second fetus (II2) at 25 weeks were (D) hyperechogenic kidneys, (E) a ventricular septal defect combined with a single atrium and (F) seroperitoneum. Right and left kidney are indicated by arrows in A and D. Bowel dilation is indicated by an arrow in C. Seroperitoneum is indicated by an arrow in F. RK, right kidney; LK, left kidney; RV, right ventricle; LV, left ventricle; SA, single atrium.



Figure 3. (A) Schematic view of protein structure of the BBS10 protein. (B) PyMOL view of the wild-type BBS10 structure: The equatorial domain (white), the intermediate domain (red), including In3 (purple) and the apical domain (yellow). (C) PyMOL view of the mutant (NM_024685.3: p.Glu262Asnfs* 41) BBS10 structure. (D) Superimposed conformations of wild-type (white) and mutated BBS10 structure (BBS10: p.Glu262Asnfs* 41, green) indicate that the mutant BBS10 protein had lost part of the apical domain, the C-terminal equatorial domain and the intermediate domain including In3. (E) PyMOL view of the mutant (NM_024685.3: p.Asn605*) BBS10 structure. (F) Superimposed conformations of the wild-type (white) and mutated BBS10 protein had lost protein (BBS10: protein had lost the C-terminal equatorial domain and was not able to form the normal 3D structure of In3 in the C-terminal equatorial domain and was not able to form the normal 3D structure of In3 in the C-terminal equatorial domain.

of clinical manifestations of BBS. The first fetus exhibited bowel dilation and multiple abdominal calcifications, indicating that fetal intestinal obstruction caused bowel dilation and peritonitis. The second fetus displayed seroperitoneum, indicating that the presence of intestinal obstruction resulted in intestinal perforation and exudation. The abovementioned ultrasonic findings of the abdomen in the present case were the first reported in a BBS fetuse, which enriched the antenatal phenotypes of BBS. Two BBS fetuses with malrotation of the midgut determined by autopsy have been reported, and one case with *BBS10* gene variants (14). In the case of the present study, the above-mentioned findings of the abdomen may be associated with malrotation of the midgut, but an autopsy was not performed. Therefore, the prenatal characteristics of the fetal ultrasound lacked specificity and were not sufficient to diagnose BBS. The application of whole-exome sequencing may clarify the diagnosis and help identify pathogenic genes and variants in the fetus.

In the present study, whole-exome sequencing was used to detect compound heterozygous variations in the BBS10 gene in the second fetus: Paternal c.784 785delGA (p.Glu262Asnfs* 41) and maternal c.1812dupT (p.Asn605*). BBS10 is located at 12q21.2, containing 2 exons and encoding a large protein of 723 amino acids. BBS10 encodes a vertebrate-specific chaperonin-like protein. The BBS10 protein regulates the formation of the BBS-chaperonin complex, which has three chaperonin functional domains: The equatorial domain, the intermediate domain and the apical domain (Fig. 3A). The intermediate domain contains three specific insertions (the flexible protrusion region specific to group II chaperonins): Two small insertions, In1 (amino acids at positions 128-139) and In2 (amino acids at positions 187-212), and one large insertion, In3 (amino acids at positions 426-596); these are responsible for ATP binding and hydrolysis. Both variants found in the BBS10 gene in the present study were novel, not reported in the literature or databases.

One of the two variants (c.784_785delGA) causes a frameshift that terminates in the protein in the apical domain, with partial deletion of the apical domain, the C-terminal equatorial domain and the intermediate domain including In3, which is consistent with the predicted structure of the protein. The other variant (c.1812dupT) causes termination in the C-terminal equatorial domain with an unknown function, but the region is conserved in BBS10 homologues by analyzing the conservation using MutationTaster (https://www.mutationtaster.org/). The predicted structure of the mutant BBS10 protein (c.1812dupT) indicated that the normal structure of In3 in the C-terminal equatorial domain was not able to form. This indicates that the C-terminal equatorial domain may have an important role in the formation of the normal structure of In3, but this requires further experimental confirmation. According to the ACMG (2015) guidelines, these two variants were classified as likely pathogenic.

In conclusion, whole-exome sequencing provided a definitive diagnosis for a fetus with multiple malformations. A total of two variants in BBS10 were determined as likely pathogenic, which provided a basis for genetic consultation, pre-implantation genetic diagnosis and prenatal diagnosis for the pedigree.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to concerns regarding participant/patient anonymity, but are available from the corresponding author on reasonable request.

Authors' contributions

XD, TM and YG performed genetic diagnoses and drafted and critically revised the manuscript. XD, ZL, DW, YX, HL, PY and LL clinically diagnosed patients, collected and analysed samples and performed imaging tests. All authors have read and approved the final manuscript. XD and YG confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the Ethics Committee of Zhongshan Boai Hospital Affiliated to Southern Medical University (Zhongshan, China) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The parents provided written informed consent for genetic testing.

Patient consent for publication

The parents provided written informed consent for the publication of their data in this study.

Competing interests

All authors declare that they have no competing interests.

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