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Clinical features of a novel compound heterozygous genotype of the *BBS2* gene: a case report

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Abstract

Bardet–Biedl syndrome is a rare autosomal recessive genetic disorder with heterogenous clinical manifestations. The present study reports the clinical features of a novel compound heterozygous genotype of the *BBS2* gene in a 14-year-old girl and her 6-year-old sister who had complaints of early-onset low vision. Fundus images revealed retinitis pigmentosa-like changes, and full-field electroretinograms showed no amplitude for the rod or cone response in both patients. Interestingly, nystagmus was observed in the older sister. On physical examination, the sisters had moderate obesity without polydactyly, hypogonadism, or intellectual disability. Exome sequencing revealed a novel compound heterozygous genotype of *BBS2* in the sisters, namely the paternally inherited NM_031885.5:c.534 + 1G > T variant and the maternally inherited NM_031885.5:c.700C > T (p.Arg234Ter) variant. Both variants were classified as pathogenic according to the American College of Medical Genetics and Genomics guidelines. This study provides useful information on the genotype-phenotype relationships of the *BBS2* gene for genetic counseling and diagnosis.

Keywords

Bardet–Biedl syndrome, *BBS2*, retinitis pigmentosa, low vision, case report, exome sequencing

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Introduction

Bardet–Biedl syndrome is a rare and heterogeneous group of autosomal recessive ciliopathies primarily characterized by retinal dystrophy, obesity, polydactyly, cognitive impairment, hypogonadism, and kidney disease.[1] The secondary features of Bardet–Biedl syndrome may include neurologic, olfactory, oral/dental, cardiovascular, gastrointestinal, endocrine/metabolic, and/or other abnormalities.[2] The clinical diagnosis is usually made based on four primary features or three primary features plus two secondary features. According to reports, the prevalence of Bardet–Biedl syndrome varies among different ethnic groups, from 1:160,000 in northern European populations to as high as 1:13,500 in Kuwait—Bedouin populations.[3]

At least 24 genes (*BBS1-21*, *NPHP1*, *IFT74*, and *SCAPER*) have been reported to be associated with Bardet–Biedl syndrome.[4] These genes are closely related to cilium structure and function. BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, and BBS9 form the BBSome complex, which is localized at the basal body of cilia and regulates protein sorting to and from the ciliary membrane.[5,6] The chaperonin complex, comprising BBS6, BBS10, and BBS12, mediates assembly of the BBSome.[7] Mutations in members of the BBS gene family can lead to immotile cilia dysfunction, involving multiple organs and showing variable phenotypic expressivity.

BBS2 is located on chromosome 16q13 and encodes a full-length product consisting of 721 amino acid residues.[8] The relationship of the *BBS2* gene with Bardet–Biedl syndrome 2 and non-syndromic retinitis pigmentosa 74 has been established.[9] According to a recent review of the genotypes and phenotypes of 90 patients with Bardet–Biedl syndrome, *BBS2* ranks second among the causative genes and is associated with almost 100% penetrance of ocular symptoms.[10] Although the early onset of visual problems may bring individuals to medical attention, a diagnosis of Bardet–Biedl syndrome could be missed because of an atypical presence or absence of other systemic symptoms.

The present study reports the clinical characteristics of a novel compound heterozygous genotype of *BBS2* in a Chinese family. The clinical and genetic findings of this study provide useful information regarding the disease for genetic counseling and diagnosis.

Patients and methods

Medical ethics

The study was approved by the Ethics Committee of Hunan Provincial Maternal and Child Health Care Hospital (No. K202031). Written informed consent was obtained from the patients or the patients' legal representative for treatment and publication of the case.

Study participants

The study involved a 14-year-old girl (proband) and her younger sister and non-consanguineous Chinese parents. Optometric, ophthalmic, and physical examinations were conducted for the sibling pair. The previous clinical information of the sisters was collected from medical records. Peripheral venous blood samples were collected from the family members for exome sequencing.

Exome sequencing

Genomic DNA was extracted from peripheral blood using a Qiagen DNA Blood Mini kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol. Initially, 50 ng of purified genomic DNA was fragmented into approximately 200 base pair (bp) segments using enzymes. The DNA fragments were then subjected to

end repair, and an adenine base was added to the 3′ end. Subsequently, the DNA fragments were ligated with barcoded sequencing adaptors, and fragments of approximately 320 bp were collected using AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). After polymerase chain reaction (PCR) amplification, the DNA fragments were hybridized and captured using NanoWES V2.1 (Berry Genomics, Beijing, China) according to the manufacturer's instructions. The hybrid products were eluted and collected for PCR amplification, followed by purification. The libraries were then quantified using quantitative PCR, and the size distribution was determined using an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The Novaseq6000 platform (Illumina, San Diego, USA), with a 150-bp pair-end sequencing mode, was used for sequencing. Raw image files were processed using CASAVA v1.82 (Illumina Inc. San Diego, CA, USA) for base calling and generation of raw data. Sequencing reads were aligned with the human reference genome (hg38/GRCh38) using the Burrows–Wheeler Aligner tool (https://bio-bwa.sourceforge.net) and PCR duplicates were removed using Picard v1.57 (http://picard.sourceforge.net/). GATK software (https://software.broadinstitute.org/gatk/) was employed for variant calling. Variant annotation and interpretation were conducted using ANNOVAR (http://www.openbioinformatics.org/annovar). Public databases including the Genome Aggregation Database (gnomAD) (http://gnomad.broadinstitute.org/), the 1000 Genomes Project (http://browser.1000genomes.org), the Single Nucleotide Polymorphism Database (dbSNP) (http://www.ncbi.nlm.nih.gov/snp), Online Mendelian Inheritance in Man (OMIM) (http://www.omim.org), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar), and the Human Gene Mutation Database (HGMD) (http://www.hgmd.org) were used for annotation.

Sanger sequencing

Primers targeting the mutation of interest were designed using the Primer3 program (http://bioinfo.ebc.ee/mprimer3) based on the human genome reference sequence hg38/GRCh38 and were used for PCR amplification. The purified PCR product was subjected to capillary electrophoresis on an ABI 3730XL DNA analyzer (ABI PRISM, Foster City, CA, USA). Mutation Surveyor software (SoftGenetics, PA, USA) was used for sequence analysis.

Results

Case presentation

The reporting of this study conforms to the CARE guidelines.[11] A 14-year-old girl and her 6-year-old sister presented with complaints of poor vision. For the teenage girl, the best corrected visual acuity (BCVA) was 0.2 in either eye with the following refraction prescription: −2.0 sphere, −3.7 cylinder, axis 180° in the right eye and −1.25 sphere, −4.5 cylinder, axis 5° in the left eye. Intermittent exotropia of both eyes and nystagmus were noted. On physical examination, the girl had moderate central obesity and a round wide face without polydactyly/syndactyly/brachydactyly or hypogonadism. The patient had no signs of intellectual disability, and her academic performance in middle school was average. Medical records showed that she had low vision since the age of 5 years and that her BCVA degraded from 0.4 to 0.2 over time. Fundus images revealed the appearance of bone spicules along with attenuated retinal vessels indicative of retinitis pigmentosa (Figure 1a). A scotopic full-field electroretinogram (ffERG) at the age of 8 years showed the absence of either the rod or cone response (see Figure S1, Supplemental Digital Content 1). At the age of 5 years, chest radiography showed

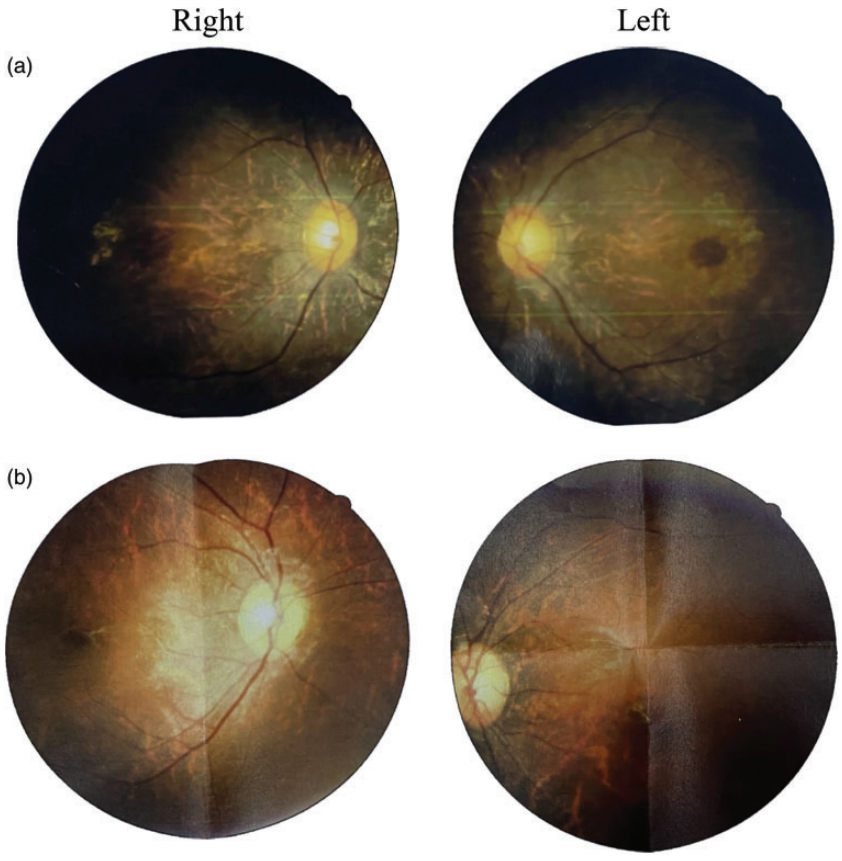

Figure 1. Fundus images showing retinal dystrophy in the (a) 14-year-old proband and (b) her 6-year-old sister.

no abnormal findings. Ultrasound examination at the age of 11 years revealed increased parenchymal echogenicity in both kidneys. At the latest follow-up visit, the girl reported increased urination at night.

In the younger sister, the BCVA was 0.3 in the right eye and 0.08 in the left eye with the following prescription: −0.5 sphere, −3.5 cylinder, axis 180° in the right eye and −0.5 sphere, −3.5 cylinder, axis 180° in the left eye. Concomitant exotropia was also observed. A physical examination revealed moderate central obesity and a round-shaped wide face with no polydactyly/syndactyly/brachydactyly, hypogonadism, or intellectual disability. Fundus examination at the age of 3 years showed the appearance of bone spicules (Figure 1b). An ffERG at the age of 5 years revealed that no waves were generated by the rod or cone system (Figure S2, Supplemental Digital Content 1).

Genetic analysis

Exome sequencing revealed that the proband and her younger sister were compound heterozygous for the *BBS2* NM_031885.5:c.534 + 1G > T variant of paternal origin and the NM_031885.5: c.700C > T (p.Arg234Ter) variant of maternal origin (Figure 2).

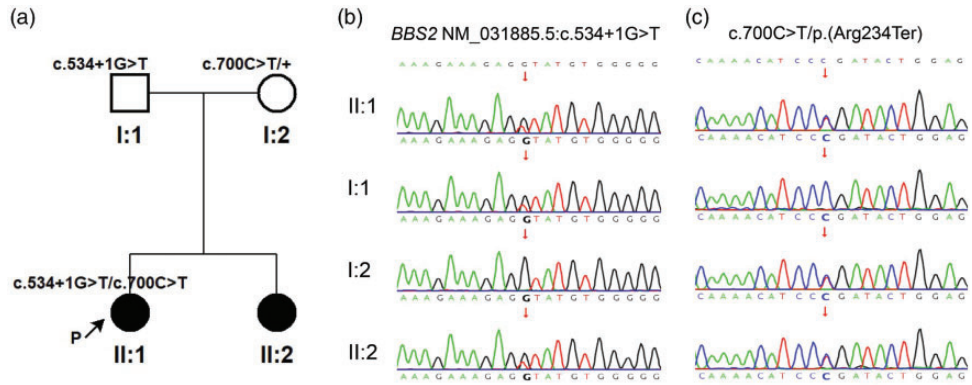

Figure 2. (a) Genotypes of the members of the affected family as validated by Sanger sequencing. (b) Pedigree diagram of the family and (c) detection of the c.534 + 1G > T variant and the c.700C > T variant, respectively.

The c.534 + 1G > T variant affects a conserved splicing donor site and leads to alterations in function of the protein (PVS1). Because the c.534 + 1G > T variant was in the trans position with the c.700C > T variant causing it to be compound heterozygous, rule PM3_strong could be applied according to the American College of Medical Genetics and Genomics (ACMG) guidelines. A literature search identified one patient previously diagnosed with Bardet–Biedl syndrome who was homozygous for the c.534 + 1G > T variant[12] and three patients from two affected families with Bardet–Biedl syndrome carrying variants of uncertain significance in trans with the c.534 + 1G > T variant.[13] Co-segregation of the c.534 + 1G > T variant with the disease has been observed in previous reports (PP1_strong).[12,13] This variant was not found in the 1000 Genomes database but had documented allele frequencies of 1.153e−04 and 1.359e−03 in the Exome Aggregation Consortium (ExAC) database and gnomAD, respectively (PM2). The variant was classified as 'pathogenic (P)' in Clinvar and as 'disease-causing (DM)' in the HGMD. In the present case, the c.534 + 1G > T variant was graded as 'pathogenic' according to the ACMG guidelines (evidence categories PVS1 + PM3_strong + PP1_strong + PM2).[14]

The c.700C > T variant produces a truncated loss-of-function protein because of the premature termination of translation at codon 234 (PVS1). The variant was in compound heterozygosity with the c.534 +1G > T variant in the present case, and a literature search identified one patient with Bardet–Biedl syndrome carrying a *BBS2* likely pathogenic variant in trans with the c.700C > T variant[15] (PM3_strong). The variant was not found in the 1000 Genomes database but possessed allele frequencies of 1.654e−05 and 5.783e−05 in the ExAC database and gnomAD, respectively (PM2). In our study, the c.700C > T variant co-segregated with the disease (PP1). The c.700C > T variant has been documented in Clinvar and the HGMD with classifications of 'P' and 'DM', respectively. Therefore, the c.700C > T variant was graded as 'pathogenic' according to the ACMG guidelines (evidence categories PVS1 + PM3_strong + PM2 + PP1).

Discussion

It has been estimated that *BBS2,* as a causative gene of Bardet–Biedl syndrome,

accounts for approximately 8% of all cases.[3] According to a recent survey involving 34 patients with Bardet–Biedl syndrome from 31 unrelated Chinese families, *BBS2* and *BBS4* were mutated in half of the probands and were the most prevalent causative genes in the cohort. High frequencies of retinal dystrophy, polydactyly, and obesity, ranging from 78.6% to 100%, were observed in the Chinese patients.[13] However, in our study, the major features of the proband and her younger sister during childhood included retinal dystrophy (low vision) and mild to moderate obesity without polydactyly, hypogonadism, or intellectual disability, resembling a phenotype of non-syndromic retinitis pigmentosa. The possibility of a syndromic disease might have been neglected, and genetic testing was not performed. Because the proband and her affected younger sister simultaneously presented to us, we realized that a genetic factor might be associated with the condition in the family. This study reminds us that obtaining a detailed medical and family history of a patient is recommended in ophthalmology outpatients when the observed visual problems can be related to certain syndromes.

Both homozygosity and compound heterozygosity of the c.534 + 1G > T variant have been recurrently observed in Chinese patients who were diagnosed with Bardet–Biedl syndrome.[12,13] The phenotypic profile of the c.534 + 1G > T variant in these patients is summarized in Table 1. Individuals who were homozygous for the c.534 + 1G > T variant seemed to manifest a more severe phenotype than individuals who were compound heterozygous for the c.534 + 1G > T variant and a nonsense or splice-site variant. Homozygosity of the c.534 + 1G > T variant is more likely to be associated with the presence of polydactyly and cognitive impairment in patients. We infer that the c.534 + 1G > T variant may have a more profound impact on the BBS signaling pathways than that of a complete loss-of-function variant.

Early diagnosis of Bardet–Biedl syndrome is difficult because of the progressive onset of symptoms.[15] Furthermore, there are phenotypic overlaps between ciliopathies.[16] For instance, obesity and retinitis pigmentosa are common clinical features of Bardet–Biedl syndrome and Alstrom syndrome. In addition, the symptoms of the patients might be atypical. These factors further impede the diagnosis of Bardet–Biedl syndrome. On the basis of the increasing availability of next-generation sequencing technologies, genetic testing is becoming more necessary to confirm the diagnosis of Bardet–Biedl syndrome, help identify carriers, and provide genetic counseling and prenatal diagnosis.[17] In the present study, exome sequencing was meaningful to the couple because they were identified as recessive pathogenic *BBS2* variant carriers. In vitro fertilization and preimplantation genetic diagnosis might be adopted for the next pregnancy in the family. Importantly, because of the involvement of multiple systems and variable onset of symptoms, multidisciplinary monitoring and management of patients with Bardet–Biedl syndrome is necessary. The multidisciplinary approach may involve nephrologists, ophthalmologists, neurologists, endocrinologists, and many other specialists for supportive treatment and preventive medical care.[17,18]

This study has characterized the clinical features of a novel compound heterozygous genotype of the *BBS2* gene in a Chinese family with Bardet–Biedl syndrome. Exome sequencing is an effective tool to improve the diagnosis of the syndrome. The study also emphasizes the awareness of clinicians toward patients with vision problems associated with genetic and syndromic conditions.

Table 1. Comparison of the clinical features of patients with Bardet-Biedl syndrome with the *BBS2* c.534 + 1G > T variant.

Patient No.	Sex	Diagnostic age	Genotype of *BBS2*	Retinal dystrophy	Obesity	Polydactyly	Cognitive impairment	Hypogonadism	Kidney disease	Reference
1	F	14 y	Het c.534 + 1G > T; Het c.700C > T (p.Arg234Ter)	Low vision, fundus with retinitis pigmentosa-like changes	Mild	No	No signs of intellectual disability	No	Night urination	This study
2	F	6 y	Het c.534 + 1G > T; Het c.700C > T (p.Arg234Ter)	Low vision, fundus with retinitis pigmentosa-like changes	Mild	No	No signs of intellectual disability	No	No symptoms yet	This study
3	M	39 y	Hom c.534 + 1G > T	Degraded vision at age 17, blindness at age 28	33.3 kg/m^2	Postaxial hexadactyly and brachydactyly of the four limbs	Mental retardation	Micropenis	End-stage renal disease	Ref[12]
4	M	32 y	Hom c.534 + 1G > T	Progressive visual loss with blindness at age 27	44 kg/m^2	Postaxial hexadactyly of the four limbs	Developmental delay	Imperceptible penis	Mild proteinuria	Ref[12]
5	M	30 y	Het c.534 + 1G > T Het c.1278A > G (p.Glu426Glu)	Yes	Yes	No	No	Yes	No	Ref[13]
6	M	29 y	Het c.534 + 1G > T Het c.1278A > G (p.Glu426Glu)	Yes	Yes	No	No	Yes	No	Ref[13]
7	M	26 y	Het c.534 + 1C > G Het c.2059 + 1G > T	Yes	Yes	Yes	No	_	_	Ref[13]

y, years; F, female; M, male; Het, heterozygous; Hom, homozygous; '–', not available

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Author contributions

ML, YL, and TW recruited the study participants and obtained the clinical data. HZ performed the sequencing analysis. WX designed the study, wrote the manuscript, and takes responsibility for the integrity of the work. All authors have read and approved the final version of the manuscript.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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Supplementary material

Supplemental material for this article is available online.

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