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ORIGINAL ARTICLE

Identification of a homozygous *BBS7* frameshift mutation in two (related) Chinese Miao families with Bardet-Biedl Syndrome

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Abstract

Background: Bardet-Biedl Syndrome (BBS) is a genetically heterogeneous autosomal recessive disorder with a wide spectrum of clinical features. To date, mutations in 21 different genes (*BBS1-21*) have been identified as causing isolated or complex BBS phenotypes. In this report, we present three Chinese Miao ethnic patients who were diagnosed with BBS on the basis of characteristic clinical features and investigated the exsome of these patients.

Methods: To evaluate disease genes, the Agilent SureSelect system and Illumina HiSeq 2000 platform for whole exome enrichment and sequencing (WES) were used on the proband and her mother. Variants that fit a recessive model of inheritance only were compared and filtered using public databases. Variants detected by exome sequencing were validated by Sanger sequencing. A total of 981 phenotypically normal subjects were enrolled as control data set.

Results: A frameshift homozygous germline mutation in *BBS7* was detected by WES and identified by Sanger sequencing in affected individuals. This mutation was predicted to result in premature termination of exon5 (c.389_390delAC, p.Asn130ThrfsX3; RefSeq NM_176824.2) and lead to a 133 amino acid truncated protein. The inheritance patterns in the families are consistent with autosomal recessive inheritance, and no such homozygous mutation was found in the other 981 controls.

Conclusion: This mutation has not yet been described in any reported literature, and this is the first report on *BBS7* mutation in Chinese Miao families with BBS phenotypes.

Keywords: Bardet-Biedl syndrome; BBS7 gene; Frameshift; Whole exome sequencing

1. INTRODUCTION

Bardet-Biedl syndrome (BBS1; OMIM 209900) was first reported by Bardet and Biedl in the 1920s. BBS is a genetically heterogeneous autosomal recessive disorder. Its phenotypes are extremely variable, including four of six major symptoms (obesity, rod-cone dystrophy, renal abnormalities, polydactyly, male hypogonadism, and learning disabilities), or three major symptoms and at least two minor symptoms (hepatic fibrosis, diabetes mellitus, neurological, speech and language deficits, behavioral traits, facial dysmorphism, dental anomalies, and developmental delay).[1,2] The prevalence rate of BBS varies between different populations, ranging from 1:160 000 in North Europe and to 1:13 500 and 1:175 000 in isolated communities in Kuwait and Newfoundland, respectively.[3,4] In China, no such data was available, and we could only identify <80 reported cases of BBS from literature review.[5-9]

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To date, a minimum of 21 disease-causing *BBS* genes (*BBS1-21*) have been identified in 80% of BBS patients, with the remaining 20% lacking a molecular diagnosis.[10] Some *BBS* genes appear to have a greater ethnicity-specific frequency than others do.[11,12] This includes *BBS1* M390R and *BBS10* C91LfsX5, which are the most common alleles in Northern European individuals, but not found in patients of Middle Eastern or North African descent. However, few *BBS* mutations have been reported in Chinese populations.[6,8,9]

In this report, we describe three BBS patients from two related Miao families from a mountain village of Miao nationality in the Yunnan Province of China. The affected individuals' parents all married through *Huanqin*, a type of traditional arranged marriage in some parts of rural China. In this tradition, a daughter from one family marries a son from another family and in "exchange," a daughter from that family marries a son from the first family. Our observations suggest a consanguineous relationship in generation I, despite no confirmation from the family (Figure 1). In the sixth nation-wide census in 2010, the Miao population accounted for approximately 0.70% of total population (http://www.stats.gov.cn/z). To keep the whole genome information, Miao BBS patients' B cells were collected and immortalized, and B lymphoblastoid cell lines were successfully established by Epstein-Barr virus transformation as described in our previous study.[7,13] Whole exome enrichment and sequencing (WES) in combination with direct Sanger sequencing of candidate genes identified an AC deletion mutation in *BBS7* (c.389_390delAC, p.Asn130ThrfsX3; RefSeq NM_176824.2). This frameshift mutation was predicted to lead to the truncation

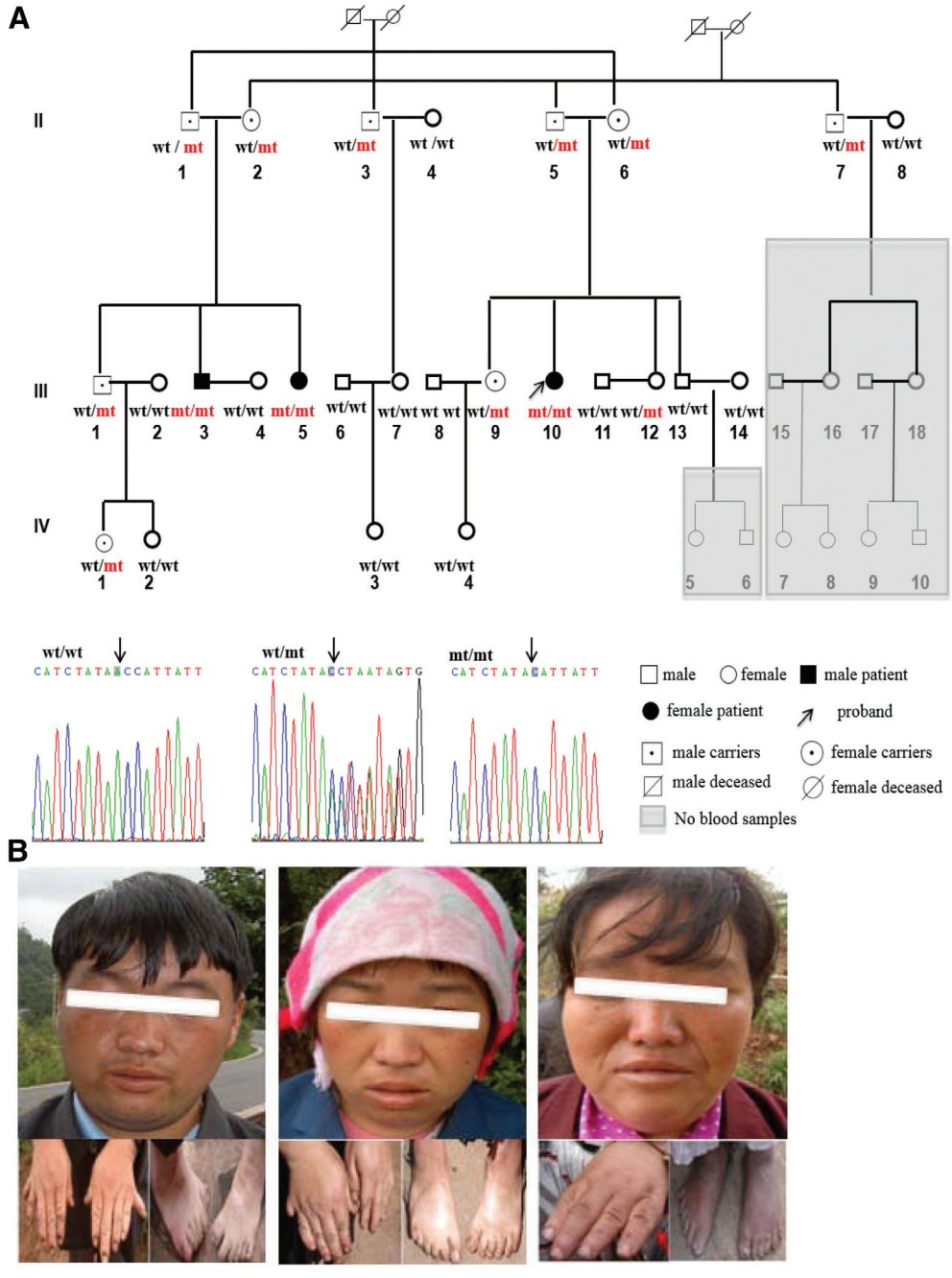

Fig. 1 Two Chinese Miao families with BBS and photographs of patients III-3, III-5, and III-10. A, Results of Sanger Sequencing on exon5 of *BBS7*. III-3, III-5, and III-10 showed a homozygous c.389_ 390delAC (RefSeq NM_176824.2) germline mutation in BBS7. II-6 and III-10 were evaluated by whole exome sequencing. wt/mt, heterozygous carrier of the BBS7 c.389_390delAC mutation; mt/mt, homozygous carrier of this mutation. B, Photographs of patients III-3, III-5, and III-10. Top, typical BBS facial features of affected individuals; bottom, typical polydactyly of hands and feet of affected individuals.

of 133 amino acids from the protein. This is the first report of *BBS7* mutation in Chinese Miao families with BBS phenotypes.

2. METHODS

2.1. Subjects

This study was approved by the Ethics Review Board of the First People's Hospital of Yunnan Province, China (2013YL061). Informed consent was obtained from the patients' parents and from all other participants. The two related families for the presented molecular investigation were identified in a Miao village in the Yunnan Province of China. A total of 51 Miao individuals from the same Miao village (unrelated to the two BBS families), and an additional 930 individuals outside this village (including 300 Miao people, 300 Dai people, 300 Hani people, and 30 Han people), were enrolled as phenotypically normal controls. Blood samples were collected for DNA extraction and laboratory examination. Physical examination was performed. Total body photographs were taken and included the hands, feet, and any specific dysmorphic features. Ophthalmic examination, abdomen ultrasound, and urogenital system examination were also conducted.

2.2. Whole-exome enrichment, sequencing, and bioinformatic analysis

WES was performed on proband (III-10) and her mother (II-6) (The Beijing Genomics Institute, China). Qualified genomic DNA was randomly sheared by Covaris (KBioscience, Herts, UK), and

the mean fragment size was 150 to 200 bp; this was then followed by library preparation using Agilent SureSelect Biotinylated RNA Library "baits." Sequencing was performed on an Illumina HiSeq 2000 (Illumina, San Diego, CA) to generate 90-bp paired-end reads following the manufacturer's protocol. SOAPaligner/SOAP2 was used to map reads onto the reference genome (http://soap.genomics.org.cn/). Only mapped reads were used for subsequent analysis. Variants were compared and filtered using public databases, including dbSNP (v129), 1000 Genome Project (20100208 release), and eight HapMap exomes. Only recessive models of inheritance (autosomal recessive model and X-linked recessive model) were considered because of the normal phenotypes of the parents.

2.3. Sequencing

Primers for candidate genes were designed using the online version of Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Polymerase chain reaction amplification of *BBS7* exon 5 was performed with primers *BBS7*-E5f: 5′-GGCCTTAACATCCTCATTTTCAGCT-3′ and *BBS7*-E5r: 5′-CCTCCCTCCAACCCAATTTCTTC-3′. The sequencing reactions were performed using BigDye Terminator v3.1 and a Genetic Analyzer 3130 (Applied Biosystems). The sequence data were then aligned with the *BBS7* reference sequence via the NCBI online blastn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

3. RESULTS

3.1. Clinical findings

Three individuals (2 females and 1 male) from two families were diagnosed with BBS on the basis of criteria established elsewhere. Symptoms included retinal dystrophy and progressive night blindness, truncal obesity, bilateral postaxial polydactyly of hands and feet (III-3: six digits for each site), bilateral postaxial polydactyly of feet and unilateral brachydactyly of the hands (III-5, III-10: six digits for each foot; III-5: six digits on right hand; III-10: six digits on left hand), learning difficulties, renal abnormality, and other clinical features (Table 1; Figure 1).

3.2. WES results and sequencing analysis

Initial filtering of WES data through the public databases and recessive models revealed a homozygous c.389_390delAC (RefSeq NM_176824.2) germline mutation in *BBS7* of the proband. In the proband, 35 reads (100%) across the mutation site showed the c.389_390delAC mutation, while in the mother, 7 out of 19 reads (36.8%) across the mutation site showed the two base deletion. This mutation was identified by Sanger sequencing, and the results revealed that the affected cousins of the proband carried this homozygous *BBS7* defect as well, while their parents and some siblings were heterozygous carriers of the c.389_ 390delAC allele (Figure 1). We further analyzed a collection of 981 DNA samples obtained from phenotypically normal controls and show that the homozygous mutation was absent, with the exception of seven (0.7%) additional individuals (all of whom were from the same Miao village and were later confirmed to be relatives of the two families under study), with the heterozygous deletion in *BBS7*. Data from all known BBS genes were analyzed; however, sequences were filtered out of our analysis if they did not fit the recessive model of inheritance or if they were not considered a functional mutation. These data are available from the authors upon request.

4. DISCUSSION

In this report, we studied three affected subjects from two Miao families, who were referred to the hospital by their local town health center. Their phenotype assessments are summarized in Table 1. All patients were presented with five established major symptoms of BBS, including obesity, rod-cone dystrophy, renal abnormalities, polydactyly, and learning disabilities. Other

Table 1

Clinical description of BBS features presented by all three patients

	Case 1 (III-3)	Case 2 (III-5)	Case 3 (III-10)
Sex/Age	Male/37	Female/35	Female/39
Weight, kg/Height, m	72.5/1.49	60/1.36	70/1.36
Pressure, mmHg	140/100	130/90	140/104
Major BBS phenotypes			
Retinitis pigmentosa	Yes	Yes	Yes
Obesity (BMI, kg/m^2)	Yes (33)	Yes (32)	Yes (38)
Renal anomalies	Right renal cyst	Right renal cyst	Right renal cyst
Polydactyly	Yes	Yes	Yes
Learning/comprehension	Delay	Delay	Delay
Hypogonadism	Yes	No	No
Minor features			
Speech development	Delay	Delay	Delay
Motor skill	Normal	Normal	Normal
Strabismus	Yes	Yes	Yes
Dental architechure	Normal	Tooth crowded	Tooth crowded
Behavior	Normal	Normal	Normal
Development delay	Mild	Mild	Mild
Brachydactyly	Yes	Yes	Yes
Short neck, low nose bridge	Yes	Yes	Yes
Diabetes mellitus	No	No	No
Heart problems	No	No	No
Hearing loss	No	No	No
Menstruation in female	NA	Irregular	Irregular
Nystagmus	No	No	No
Cataract	No	No	No
Micropenis	Yes	NA	NA

BBS = Bardet-Biedl Syndrome; BMI = body mass index; NA = not available.

minor clinical features were also observed in the three affected individuals. Our patients did not show nystagmus and/or cataracts compared with that in some patients with *BBS7* frameshift mutations described in previous reports.[14-15]

BBS is a ciliopathy involving multiple systems. Eight highly conserved BBS proteins (BBS1, 2, 4, 5, 7, 8, 9, and BBIP10) form a complex known as the BBSome,[16] which functions in ciliary membrane biogenesis. BBS7 is an integral part of the BBSome and physically interacts with the BBS chaperonin complex (BBS6, BBS10, BBS12, and CCT/TRiC family chaperonins).[17] Dysfunction or abnormality of the BBS7 protein can cause structural and functional defects in cilia. Both missense mutations or absent BBS7 can affect the formation of the BBSome, which can adversely affect various organs in the body.[18-20] *BBS7* is located on chromosome 4q27 and consists of 19 exons encoding a 715 amino-acid protein. To date, mutations within *BBS7* were reported in 4.2% of BBS families.[14] Homozygous and compound heterozygous mutations, including nonsense mutations, copy-number variants, and frameshift mutations in *BBS7* were identified in affected individuals.[9,21-24] BBS7 was identified as a novel BBS protein in 2003, and since then frameshift mutations including K237fsX296,[25] M284LfsX7,[11] R238EfsX59,[19] K237fsX60,[26] Q448RfsX13,[27] R238EfsX59,[15] and H29QfsX12[20] have been reported in the literature. In this study, we identified a homozygous c.389_390delAC (RefSeq NM_176824.2) germline mutation in *BBS7* in all BBS patients. This mutation, which resulted in a frameshift, is predicted to lead to premature termination of exon5 (p.Asn130ThrfsX3), thereby abolishing approximately 81.4% of the wild-type BBS7 protein (133aa versus 715aa) (Ref NP_789794.1). To our knowledge, this mutation has not been previously described in any reported literature. Interestingly, the heterozygous mutation of c.389_390delAC was found not only in unaffected individuals of these two families, but also in their nonlineal relatives who live in the same village. The two families denied the consanguineous relationship of generation I, though it is difficult to trace when and how this BBS7 frameshift variation began to distribute within this village. The fact that none of the controls outside this village carried this specific mutation could support a hypothesis that *Huanqin* marriage of patients' parents increases the risk of carrying this *BBS7* homozygous mutation.

InterPro-based analyses[28] on wild-type *BBS7* showed a hypothetical WD40/YVTN repeat-like-containing domain lying in the area between residues 26 and 378. Both the WD40 and the YVTN repeated motifs consist of approximately 40 residues and share a similar seven-bladed, β-propellers structure. Mutations of these residues might be involved in the disassembly activity of filaments, transcriptional corepression, and phosphorylation.[29-31] In this report, InterPro-based analyses on BBS7 mutant (c.389_390delAC, p.Asn130ThrfsX3) showed no domains, and repeats could be predicted between residues 1 and 130. This indicated that the deletion position may be essential to the formation of bladed, β-propeller structure, and as a result, BBS7 protein may undergo a loss of function.

Additionally, local alignment of BBS1, BBS2, and BBS7 indicates that BBS7 exhibits similarity with BBS2 (between residues 147 to 398) and BBS1 (between residues 171 to 315), which may indicate that these genes belong to a distinct subfamily of proteins. This raises the possibility that variation in the area between residues 26 and 378 of BBS7 could yield a common mechanism to the phenotype caused by mutations at each locus.[23] Interestingly, BBS7(–/–) knock-out mice show similar phenotypes to other BBS gene mutant mice including retinal degeneration, obesity, ventriculomegaly, and male infertility characterized by abnormal spermatozoa flagellar axonemes.[16]

In addition to the *BBS7* deletion mutation in the three BBS patients discussed, abnormal expression of some proteins (including ANXA1, CISH, and KIF2A) was also observed, which may correlate with the function or structure of cilia.[32] Mutations in noncoding sequences were also found by WES (data not shown). These data indicated that genotype-phenotype correlation could be affected not only by BBS genes,[33] but also by other etiologies, such as protein defects associated with noncoding RNA regulatory mechanisms or epigenetic modification.

In conclusion, we have found a mutation c.389_390delAC within *BBS7* that is predicted to result in the premature termination of exon5 (p.Asn130ThrfsTer3) and may be essential to the correct formation of BBS7 protein structure. However, there is no single disease that is "monogenic" in the strict sense of the word.[34] Therefore, further studies are needed to better characterize the genotype-phenotype correlation of the mutation in this report, which is also a limitation of this study.

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