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 exome 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 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). 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. In China, no such data was available, and we could only identify <80 reported cases of BBS from literature review.

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. Some BBS genes appear to have a greater ethnicity-specific frequency than others do. 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.

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/e). 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. 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

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Conflicts of Interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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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’-GGCCTTAACATCCTCATTTGCAGCT-3’ and BBS7-E5r: 5’-CCTCCCTCAGCAAACCATTTGCTTC-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/m²) | 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 architecture | 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 | NA | Irregular | Irregular |
| Mensesation in female | NA | No | No |
| 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.14-16

BBS is a ciliopathy involving multiple systems. Eight highly conserved BBS proteins (BBS1, 2, 4, 5, 7, 8, 9, and BBS10) form a complex known as the BBSome,14 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). Inactivation or absence 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 and H29QfsX1220 have been reported in the literature. In this study, we found a frameshift variation in BBS7 within all BBS patients. This mutation, which resulted in a frameshift, is predicted to lead to premature termination of exon 5 (p.Asn130ThrfsTer3), 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 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 consanguineous marriage of patients’ parents increases the risk of carrying this BBS7 homozygous mutation.

InterPro-based analyses24 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 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 consanguineous marriage of patients’ parents increases the risk of carrying this BBS7 homozygous mutation.

In conclusion, we have found a mutation c.389_390delAC within BBS7 that is predicted to result in the premature termination of exon 5 (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.19 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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REFERENCES


