Exome Sequencing of a Family with Bardet-Biedl Syndrome Identifies the Common Russian Mutation c.1967_1968delTAinsC in *BBS7*

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Key Words

Bardet-Biedl syndrome · *BBS7* gene · Ciliopathy · Exome sequencing · Founder alleles

Abstract

Bardet-Biedl syndrome (BBS) is a rare autosomal recessive ciliopathy characterized by obesity, postaxial polydactyly, retinitis pigmentosa, mental retardation, and kidney abnormalities. At least 19 genes have been shown to be associated with BBS, and therefore, genetic testing is highly complicated. We used an Illumina MiSeq platform for whole exome sequencing analysis of a family with strong clinical features of BBS. A homozygous c.1967_1968delTAinsC (p.Leu656fsX673; RefSeq NM_176824.2) mutation in *BBS7* was identified in both affected children, while their healthy sibling and the non-consanguineous parents were heterozygous for this allele. Genotyping of 2,832 DNA samples obtained from Russian blood donors revealed 2 additional heterozygous subjects (0.07%) with the c.1967_1968delTAinsC mutation. These findings may facilitate the genetic diagnosis for Slavic BBS patients.

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Bardet-Biedl syndrome (BBS; OMIM 209900) is a rare genetic ciliopathy, manifesting with blindness-causing retinal dystrophy, postaxial polydactyly, obesity, renal dysfunction, hypogonadism, cognitive abnormalities, and some other severe defects. Molecular diagnosis of this disorder is highly complicated, because mutations in at least 19 genes (*BBS1–BBS19*) may cause the BBS phenotype [Marion et al., 2012; Forsythe and Beales, 2013; Aldahmesh et al., 2014; Scheidecker et al., 2014]. Sequencing of candidate genes is associated with high setup expenses for appropriate assays, requires significant costs and labor input to analyze the multitude of relevant loci, and has a risk of missing causative mutations in yet unknown BBS genes [Marion et al., 2012; Redin et al., 2012; Scheidecker et al., 2014]. The recent invention of whole exome sequencing (WES) has provided a viable alternative for BBS genetic diagnosis [Ajmal et al., 2013]. To our knowledge, BBS patients have not yet been systematically described in Russia or other Slavic countries. Here, we present a Russian family with BBS which was identified via WES.

Fig. 1. Russian family with BBS. wt/mut = Heterozygous carrier of the *BBS7* c.1967_1968delTAinsC mutation; mut/mut = homozygous carrier of this mutation. The pedigree was drawn using Pedigree Chart Designer (CeGaT, Germany).

Case Report

A family with 2 cases of presumable BBS requested genetic counseling at the St. Petersburg City Medical Genetic Center, Russia (fig. 1). The parents, being clearly non-consanguineous, were born in distinct regions of Russia (Bryansk and Volgograd). The 35-year-old mother (I.1) reported chronic anemia and mild gastrointestinal dysfunction, while the 41-year-old father (I.2) was healthy. Both parents did not have prior marriages. By the time of the referral, 6 pregnancies had occurred within this family. The first (II.1), the third (II.3), and the fifth (II.5) pregnancy was terminated spontaneously at 6–8 weeks for unknown reasons. The second pregnancy (II.2) resulted in the birth of an apparently healthy boy who is now 16 years old. Unfortunately, children from the fourth (II.4, 7-year-old boy) and the sixth (II.6, 1.5-year-old girl) pregnancy demonstrated characteristic clinical features of BBS.

BBS signs in the affected boy included macrosomy (height: 145 cm; weight: 52 kg; head circumference: 54 cm; chest circumference: 85 cm), abdominal obesity, retinitis pigmentosa, history of surgically corrected postaxial polydactyly, prominent forehead, flat nasal bridge, micropenis, mild mental retardation, and a decreased emotional responsiveness. The affected girl had a similar phenotype, including macrosomy (height: 93 cm; weight: 20 kg; head circumference: 50 cm; chest circumference: 66 cm), abdominal obesity, history of surgically corrected postaxial polydactyly, prominent forehead, flat nasal bridge, speech delay, and an obstinate attitude. An ophthalmological examination was not carried out due to resistance of the child.

Materials and Methods

DNA samples from the affected girl and her healthy father were subjected to WES. Exome enrichment was performed using the Nextera Exome Enrichment Kit (Illumina, USA) which is supposed to cover 37 Mb of coding sequences (214,405 exons; 98.3% of sequences annotated in RefSeq database) and includes all 19 known *BBS* genes. Massive parallel sequencing was carried out using Illumina MiSeq and included multiple 150-bp reads with approximately 50× coverage. Sequencing depth across coding re-

gions of *BBS1–BBS19* did not significantly differ from the average (online suppl. table 1; www.karger.com/doi/10.1159/000371408). The conversion of nucleotide-specific fluorescent signals was done using MiSeq Reporter software. Reads were aligned to the Human Reference Genome (version hg19) by Burrows-Wheeler Aligner. The obtained files were analyzed using GATK (Genome Analysis Tool Kit) software. The identified differences from the reference sequence were annotated using Annovar resource (www.openbioinformatics.org/annovar/).

Results and Discussion

WES led to the identification of a homozygous c.1967_1968delTAinsC (p. Leu656fsX673; RefSeq NM_176824.2) germline mutation in the *BBS7* gene in the analyzed girl. Her affected brother carried this homozygous *BBS7* defect as well, while the healthy brother and both parents were heterozygous carriers of the c.1967_1968 delTAinsC allele. This mutation has already been described by Muller et al. [2010] who detected compound *BBS7* heterozygosity (c.1967_1968delTAinsC and c.528+1G>A) in a patient with BBS. *BBS7* encodes a subunit of the BBSome complex and is essential for proper cilia functioning. Mutations in *BBS7* have been revealed in only 2% of analyzed BBS patients [Forsythe and Beales, 2013].

The presence of an identical mutation in unrelated parents of patients with BBS is not unexpected. Surprisingly, the Slavic population of Russia and neighboring countries is characterized by pronounced founder effects, so a number of genetic diseases in this part of the world are attributed to recurrent alleles [Dörk et al., 2000; Sokolenko et al., 2010; Jurecka et al., 2012]. By allele-specific PCR, we further analyzed a collection of 2,832 DNA samples obtained from Russian healthy blood donors and revealed 2 additional subjects (0.07%) heterozygous for the c.1967_1968delTAinsC allele in *BBS7*.

This study exemplifies the power of WES for the diagnosis of genetically heterogeneous diseases. Importantly, the DNA analysis was performed using a relatively accessible, laboratory-scale next generation sequencing device. The recurrent nature of the identified mutation, c.1967_1968delTAinsC in *BBS7*, has now to be taken into account while considering a BBS diagnosis in patients of Slavic origin.

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