A case of Bardet-Biedl syndrome caused by a recurrent variant in BBS12: A case report

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Abstract. Bardet-Biedl syndrome (BBS) is a clinically and genetically heterogeneous disorder that manifests as a result of primary cilia impairment. Cilia are present on most cell types, thus BBS is a multisystemic condition involving the majority of organ systems. The core features of the syndrome include retinal degeneration, obesity, polydactyly, cognitive impairment, renal anomalies and urogenital malformations. To date, pathogenic variants in 26 genes have been shown to be involved in the molecular basis of this rare ciliopathy. Of these causal loci, BBS12 accounts for ~8% of all cases. In this case report, an individual with BBS caused by a rare recurrent variant in BBS12 (NM_152618.3: c.1063C>T; p.Arg355*) is described and compared with others with the same DNA variant, placing this finding in the context of the current literature.

Introduction

Bardet-Biedl syndrome (BBS) [Mendelian Inheritance in Man (MIM), 209900] is a heterogenous disorder that is caused by the impairment of primary cilia. It belongs to a broad group of disorders known as ciliopathies, and represents a hallmark exemplar with a highly variable clinical presentation, likely due to second-site modification of primary causal loci (1-3). The predominant clinical features associated with BBS are retinal degeneration, obesity, polydactyly, cognitive impairment, renal disease and hypogonadism or urogenital malformations. Minor symptoms that may complicate a clinical diagnosis of BBS include developmental delay, behavioral and psychiatric abnormalities, metabolic and endocrine impairment, cardiovascular involvement, liver disease, Hirschsprung disease and olfactory deficits (4). Given the wide phenotypic variability that exists within and amongst BBS families, a clinical diagnosis of BBS may prove to be challenging. However, a diagnostic algorithm has been proposed by the presence of either four major features, or three major features and two minor symptoms (5). Moreover, it is difficult to make an accurate early diagnosis since the majority of the symptoms may only occur over time. Therefore, the median age of diagnosis is 9 years of age, and typically the diagnosis is associated with the occurrence of retinal degeneration (5,6). Although certain symptoms can be detected at an antenatal stage, such as polydactyly or genitourinary abnormalities, in the absence of a positive family history and established molecular underpinnings, such a diagnosis is rarely established in early childhood (7). Obesity, which is...
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noted in 72-92% of patients with BBS, becomes evident during the first 3 years of life. Typically, the birth weight is normal, and the weight gain commences during the first year (5,7). Obesity is associated with a higher risk of developing diabetes, metabolic syndrome or hypertension (8,9). Cognitive difficulties are common (>60% of individuals with BBS), although only 25% of those observed fulfill the intellectual disability consensus criteria (10). Some specific deficits, such as perceptual reasoning, attention capacity and functional independence, appear to be the most severely affected (10). Other neuropsychiatric abnormalities have been observed in BBS, including developmental delay, either motor or language impairment, and a broad spectrum of behavioral disturbances, such as emotional instability, disinhibition, aggressiveness, self-injury or obsessive-compulsive behavior (10). Kidney disease affects 53-82% of patients with BBS, and this represents the common cause of morbidity and mortality (11). The renal phenotype is highly variable, with renal dysfunction leading to end-stage renal failure in 42% of adult patients, as revealed by a large BBS cohort study (12). Individuals with BBS also display structural anomalies ranging from cysts, fetal lobulation, renal dysplasia, calyceal distortion and hydronephrosis to ectopic, atrophic, horseshoe kidney or renal agenesis (13,14). Hypogonadism and genital anomalies are observed in 59-98% of patients. Small penile length has also been identified in nearly all males with BBS, whereas hypoplastic labia minora is common in females. Less frequently, hydrometrocolpos may complicate many of the malformations, including vaginal atresia and septate or imperforate vagina, which may be identified antenatally or shortly after birth (5,11,15). In a minority of individuals, valvular stenosis, atrial/ventricular septal defects or cardiomyopathy are observed, which may be diagnosed at the prenatal or neonatal stage (5,16), whereas anosmia, hearing loss, liver disease, Hirschsprung disease and laterality defects have been reported at different ages of onset (4,17,18).

At the time of this report, 26 genes have been associated with the pathogenesis of BBS (Table I). The majority of the encoded BBS proteins localize to the base of the cilium, and all have been shown to be involved in ciliary biogenesis or function (Fig. 1) (11). The BBS1, BBS2, BBS4, BBS5, BBS7, TTC8/BBS8, BBS9 and BBIP1/BBS18 proteins are components of the BBSome, a macromolecular complex

Table I. Causal Bardet-Biedl syndrome genes.

| Gene no. | Gene name | Alias | MIM number | Chromosomal location | Subcellular location of related proteins |
|---------|-----------|-------|------------|----------------------|----------------------------------------|
| 1       | **BBS1** |       | 209901     | 11q13.2               | BBSome                                 |
| 2       | **BBS2** | RP74  | 606151     | 16q13                 | BBSome                                 |
| 3       | **ARL6** | BBS3, RP55 | 608845     | 3q11.2                 | BBSome associated                       |
| 4       | **BBS4** |       | 600374     | 15q24.1               | BBSome                                 |
| 5       | **BBS5** |       | 603650     | 2q31.1                 | BBSome                                 |
| 6       | **MKKS** | HMCS, KMS, MKS, BBS6 | 604896     | 20p12.2               | Chaperonin complex                      |
| 7       | **BBS7** |       | 607590     | 4q27                  | BBSome                                 |
| 8       | **TTC8** | BBS8, RP51 | 608132     | 14q31.3               | BBSome                                 |
| 9       | **PTHB1** | BBS9  | 607968     | 7p14.3                 | BBSome                                 |
| 10      | **BBS10** | C12orf58, FLJ23560 | 610148     | 12q21.2               | Chaperonin complex                      |
| 11      | **TRIM32** | HT2A, LGMDR8, BBS11 | 602290     | 9q33.1                 | Cilium base                            |
| 12      | **BBS12** | FLJ35630, C4orf24 | 610683     | 4q27                  | Chaperonin complex                      |
| 13      | **MKS1** | MKS, BBS13, JBT528 | 609883     | 17q22                 | Basal body                             |
| 14      | **CEP290** | KIAA03733H11AG, JBT55, SLSN6, LCA10, BBS14 | 610142     | 12q21.32              | Basal body                             |
| 15      | **WDPCP** | C2orf86, BBS15, CHDTHP | 613580     | 2p15                  | Basal body                             |
| 16      | **SDCCAG8** | CCCAP, SLSN7, BBS16 | 613524     | 1q43-q44              | Basal body                             |
| 17      | **LZTF1** | BBS17 | 606568     | 3p21.31               | BBSome associated                       |
| 18      | **BBP1** | NCRNA00081, BBP10, BBS18 | 613605     | 10q25.2               | BBSome                                 |
| 19      | **IFT27** | RABL4, BBS19 | 615870     | 22q12.3               | IFT                                    |
| 20      | **IFT74** | CCDC2, CMG1 | 608040     | 9p21.2                | IFT                                    |
| 21      | **CFAP418** | C8orf37, CORD16, RP64, BBS21 | 614477     | 8q22.1                | Cilium base                            |
| 22      | **NPHP1** |       | 607100     | 2q13                  | Transition zone                        |
| 23      | **IFT172** |       | 607386     | 2p23.3                | IFT                                    |
| 24      | **SCAPER** |       | 618195     | 15q24.3               | Cilium tip                             |
| 25      | **SCLT1** |       | 611399     | 4q28.2                | Distal appendage                       |
| 26      | **CEP164** |       | 614848     | 11q23.3               | Distal appendage                       |

BBS, Bardet-Biedl syndrome; IFT, intraflagellar transport; MIM, Mendelian Inheritance in Man.
that functions as an adaptor for intraflagellar transport (IFT) molecules (19,20). IFT molecules undergo bidirectional movement along the microtubule backbone (IFT-A and IFT-B protein complexes), acting as a carrier for proteins involved either in signaling pathways or in ciliary homeostasis (21). IFT27/BBS19, IFT74/BBS20 and IFT172 are components of the IFT-B complex, which confers anterograde IFT (22). IFT27/BBS19 has been suggested to interact with ADP ribosylation factor like GTPase 6 (ARL6)/BBS3, hence modulating the ciliary export of hedgehog signaling molecules. It has also been proposed that IFT27/BBS19 may interface with the BBSome complex through an interaction with leucine zipper transcription factor like 1 (LZTFL1)/BBS17 (23,24). IFT74/BBS20 has been shown to interact with IFT27/BBS19, whereas the remaining IFT-B molecules, including IFT172, play an important role in cilium stability (25,26). The position of the BBSome within the cilium is stabilized by ARL6/BBS3, a small GTPase that recruits the BBSome to ciliary membranes (19). The MKKS centrosomal shuttling protein (MKKS)/BBS6, along with the BBS10 and BBS12 proteins form part of the chaperonin-like complex that has an important role in BBSome assembly (27,28). Several proteins function at the basal body (MKS1/BBS13, CEP290/BBS14, WDPCP/BBS15 and SDCCAG8/BBS16) and are involved in ciliogenesis and the modulation of BBSome trafficking within the ciliary compartment (29-31). Tripartite motif containing 32/BBS11 is an E3 ubiquitin ligase that regulates components of the cytoskeleton, whereas LITTL1/BBS17 is hypothesized to regulate BBSome activity through transient interaction with BBS9 (32-34). Cilia and flagella associated protein (CFAP)418/BBS21 is located at the base of the cilium, and appears to have a role in facilitating protein transport, although its complete function and mode of interaction within the BBS protein network have yet to be fully elucidated (35).

Figure 1. Location and interaction of BBS proteins within cilium. (A) Overview of BBS protein complexes at the cilium. (B) Details of anterograde transport molecule assembly. The BBS proteins are shown in bold. BBS, Bardet-Biedl syndrome.
prevalence of ARL6/BBS3 pathogenic variants in consanguineous Saudi and Indian families (46,47).

BBS has been shown to be predominantly inherited in an autosomal recessive fashion, although it may also be inherited as an oligogenic trait (48,49). The underlying molecular mechanism is often complicated through the intervention of a third mutant locus, giving rise to ‘triallelic inheritance’, which may explain the extensive clinical variability of patients with BBS (50). Similarly, it has been hypothesized that the presence of second-site modifier or epistatic interactions are responsible both for intrafamilial or interfamilial clinical heterogeneity and for the severity of the phenotype (2,51,52). Copy number variants and retrotransposon insertions have been proposed to contribute to the pathogenesis of BBS (53,54). Furthermore, it has been suggested that even environmental events may be involved in defining the complexity of the BBS phenotype (55).

Here, a hitherto unreported case of BBS, clinically diagnosed in accordance with consensus criteria established by Beales et al (5), that was caused by a rare recurrent c.1063C>T; p.Arg355* variant in BBS12 is described. The molecular finding was identified by whole exome sequencing (WES) and confirmed by Sanger sequencing.

Case report

Written informed consent was obtained from the legal guardian of this patient and her family members, and they were all enrolled in the research study approved by Institutional Review Board of University of Medicine and Pharmacy ‘Carol Davila’ Bucharest (approval no. 29700, T.42; Oct 01, 2015). Additionally, the present study conformed to the guidelines of the Declaration of Helsinki (56). EDTA-treated peripheral blood samples from willing family members were collected (the patient, the patient’s sibling and their parents) subsequent to informed consent. Genomic DNA was extracted from the blood using the PureLink® Genomic DNA Extraction kit (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer’s instructions. WES was performed by the Advanced Center for Translational and Genetic Medicine, Ann & Robert H. Lurie Children’s Hospital of Chicago, IL, USA, according to a research study approved by the Lurie Children’s Hospital IRB (approval no. IRB 2019-3057; Aug 5, 2019). WES was performed on proband genomic DNA samples according to an established protocol (LC Sciences, LLC). Fragmented DNA samples generated via sonication were subjected to library construction. Exome capture was performed using an Agilent SureSelect Human All Exon V6 kit (Agilent Technologies, Inc.) according to the manufacturer’s instructions, and next generation sequencing was subsequently performed using an Illumina Novaseq6000 system at Lianchuan Bio for a 150 bp paired-end run, to a mean target depth of 147X, generating a total of 74,060,974 paired-end reads.

For bioinformatics analysis, and prior to alignment, low-quality reads (first, reads containing sequencing adapters, and secondly, nucleotides with a quality score <20) were removed to yield a total of 73,150,226 cleaned paired-end reads. The Burrows-Wheeler Aligner (57) was utilized to
perform reference genome alignment (hg19) with reads contained in paired FASTQ files. As the first post-alignment processing step, Picard (a collection of command-line tools for handling high-throughput sequencing data; broadinstitute.github.io/picard/) was utilized to identify and mark duplicate reads from BAM files. In the second post-alignment processing step, local read realignment was performed to correct for potential alignment errors around indels. Variant calls were generated using GATK HaplotypeCaller (gatk.broadinstitute.org/hc/en-us) (58) [which calls single-nucleotide polymorphisms (SNPs) and small insertions and deletions within local de novo assembly of haplotypes in an active region] or UnifiedGenotyper (59) (which calls SNPs and indels on a per-locus basis) (60). A Gaussian mixture model was used to assign accurate confidence scores to each putative variant call, and SnpEff (pcingola.github.io/SnpEff/) (an open-source tool that annotates variants and predicts their effects on genes by using an interval forest approach) was utilized to add biological information for the variants (61). Rare variants with gnomAD minor allele frequency <0.01 were retained, and functional DNA changes impacting amino acid sequence and intron-exon junctions in the 26 known BBS genes (Table I) were prioritized for further analysis using the Integrated Genomics Viewer (software.broadinstitute.org/software/igv/home) (62). BBS12 c.1063C>T; p.Arg355* was confirmed in the proband and available family members by PCR amplification with the following thermocycling conditions: Initial denaturation, 95˚C for 5 min; followed by 10 cycles of denaturation at 95˚C for 30 sec, annealing at 66˚C for 30 sec, and extension at 72˚C for 30 sec (-1˚C/cycle); 40 cycles of denaturation at 95˚C for 30 sec, annealing at 56˚C for 30 sec, and extension at 72˚C for 30 sec; and a final extension step at 72˚C for 10 min. The sequences of the primers used were: BBS12_PCR1 forward, 5'-TTGTGTTGCAACAAGGCAAC-3' and reverse, 5'-TTCACTGAGCCGATTACCAAC-3'. This was followed by capillary sequencing using BigDye terminator 3.1 chemistry using an ABI 3730xl DNA Analyzer according to the manufacturer's protocols (Applied Biosystems; Thermo Fisher Scientific, Inc.).

The proband was the first daughter of a young (mother 17 years-old, father 20 years-old) and apparently healthy Romani couple. The family self-reported as non-consanguineous. The second daughter was reported to be healthy. The family history included Down's syndrome in a paternal cousin, as well as several (>3 cases) familial cases of intellectual disability on the father's side of the family. The patient was born at 42 weeks by vaginal delivery after an uneventful pregnancy. The birth weight was 3,070 g (70th percentile); the occipitofrontal circumference (OFC) was not determined; the birth length was 50 cm (30th percentile); the bitemporal diameter, sparse eyebrow hypertelorism, long and smooth philtrum, large ears and full cheeks. Furthermore, oral/dental abnormalities were observed, namely a narrow forehead, a decreased bitemporal diameter, sparse eyebrow hypertelorism, long and smooth philtrum, large ears and full cheeks. Furthermore, oral/dental abnormalities were identified, including dysplastic teeth, a high-arched palate and digit anomalies, such as brachydactyly, conic fingers, partial cutaneous syndactyly of the second and third toes, and hypoplasia of the nails were also noted. An ophthalmological examination revealed retinal dystrophy; the patient's night vision was also very poor, and her daylight vision was weak (she frequently collides with objects while walking) as reported by her. However, specific measurements of visual acuity could not be obtained due to non-cooperation.
and severe intellectual disability. The neurological evaluation revealed language impairment (echolalia, bradylalia, a limited vocabulary and the use of expressions that the patient had heard on television) and no sphincter control. Psychiatric and psychological workup revealed severe intellectual disability (IQ score 36), behavioral disturbances, including emotional instability, self-aggressiveness, addictive behavior towards the phone and television (the patient liked to listen to music, sing and dance), severe hyperkinesia and abnormal food behavior (the patient asked repeatedly for food). The patient knew her name and age, and could count up to 10; however, she could not recognize colors or play with a puzzle. Abdominal ultrasound revealed the presence of hypoplastic genitalia, although her liver and kidneys appeared normal. Likewise, electroencephalography and brain MRI investigations were unremarkable. Over the course of the last year (at 7 years of age) slightly elevated levels of cholesterol [5.4 mmol/l (normal range <5.2 mmol/l)], creatinine [68 µmol/l (normal range 35-65 µmol/l)] and urea [8.4 mmol/l (normal range 1.4-8.3 mmol/l)] were recorded for the patient, and she displayed several episodes of high blood pressure that responded well to treatment.

Table II. Clinical findings of the patients with BBS harboring the BBS12:c.1063C>T homozygous variant.

| Patient characteristics | Present case | Case #1 and #2 | Case #3 | Case #4 |
|-------------------------|--------------|----------------|---------|---------|
| Reference               | -            | (57)           | (62)    | (63)    |
| Additional genomic variants | -            | -              | -       | BBS1:c.1016A>T |
| Age at time of report   | 7 years      | NP             | 5 months| NP      |
| Sex                     | Female       | NP             | Male    | Female  |
| Ethnic background       | Romani       | Romani         | NP      | NP      |
| Retinitis pigmentosa    | Yes          | NP             | No      | Yes     |
| Obesity                 | Yes (>6 standard deviations) | NP | Yes (>97th percentile) | No|
| Intellectual disability | Severe (IQ 36) (cognitive and language impairment) | NP | No | Yes (cognitive, language and motor impairment) |
| Polydactyly             | All limbs    | NP             | Feet    | Feet    |
| Genital anomalies/hypogonadism | Yes (hypoplastic genitalia) | NP | Yes (small penis, small testicles) | Yes (NS) |
| Kidney disfunction/ anomaly | Yes (elevated levels of creatinine and urea) | NP | No | Yes (NS) |
| Miscellaneous           | Severe behavioral abnormalities, hypercholesterolemia, hypertension, brachydactyly, syndactyly of 2-3 toes | NP | Heart anomaly, hypercholesterolemia of 5-6 left toes | Hypercholesterolemia |

Discussion

In this case report, a homozygous BBS12 NM_152618.3:c.1063C>T, p.Arg355* variant was identified using WES. This variant has been reported previously in dbSNP (rs121918327; ncbi.nlm.nih.gov/snp/), and in ClinVar (VCV000001147.9; ncbi.nlm.nih.gov/clinvar/) as being pathogenic according to the American College of Medial Genetics and Genomics guidelines (63) (PVS1, PP3, PM2). The variant is a nonsense mutation that is predicted to result in a premature stop codon within the apical domain of the protein (64). Experimental validation remains necessary to determine whether p.Arg355* produces an unstable protein that is targeted for degradation, or whether it generates a stable polypeptide with compromised function.

BBS12 (MIM 610683) is located on chromosome 4q27 and contains two exons, which code for a protein of 710 amino acids that belongs to a chaperonin-like complex, in addition to MKKS/BBS6 and BBS10 (41). The chaperonin-like complex, MKKS/BBS6-BBS10-BBS12, was initially considered to be vertebrate specific, and the proteins have similarity to the canonical type II chaperonins that are present in eukaryotic organisms (64-66). Subsequently, new evidence revealed that the proteins evolved earlier, due to the presence of several orthologs in ancient eukaryotes (67). Whereas canonical eukaryotic chaperonins utilize an ATP-specific hydrolytic site for protein folding, the rapidly evolved chaperonin-like proteins lost the ATPase hydrolytic site, but acquired novel functions, including the transduction of different morphogenetic signals from cilia (64,67).

The three chaperonin-like proteins have been shown to be localized at the base of the cilium, in the pericentriolar region of the basal body and centrosome. They are required for initial assembly of the BBSome, and operate through stabilizing
the BBS7 protein and subsequently recruiting BBS2 protein as an intermediary protein for the binding of a six prototypic chaperonin-containing tailless complex, which is responsible for completion of the folding process (27). Disruption of one of the chaperonin-like BBS genes leads to degradation of at least two subunits of the BBSome. The remaining BBSome proteins either stand as monomers or form aggregates with unspecified proteins (27).

As a consequence associated with this phenotype, it has been suggested that the deleterious variants in the MKKS/BBS6-BBS10-BBS12 complex may lead to a more severe phenotype and earlier onset of the disease compared with variants in the BBSome subunits (68,69). This may be accounted for by the existence of an intermediary complex that manages to retain some residual function in spite of a BBSome component being impaired, whereas alteration of the chaperonin-like complex components serve to restrain the aggregation of any functional complex (28). There is also some evidence to suggest that visual impairment is most severe in cases associated with alterations in the chaperonin-like BBS genes, with similar effects observed for all three genes (68). Furthermore, cognitive impairment is highly prevalent in individuals with BBS12 variants, whereas urogenital abnormalities are more common in those carrying BBS10 pathogenic variants (69).

Four cases with BBS12 c.1063C>T, p.Arg355*, have been reported previously (Table II) (64,70,71). In total, 3 of the 5 patients (including the presented case) reported are Romani; however, the ethnic backgrounds of the other 2 patients have not been provided, so it is not possible to conclude whether they share the same ethnicity. Interestingly, one of the patients reported previously is also Romanian, and although he is not located in the same geographic area as the current patient, the presence of a putative founder mutation cannot be excluded. Furthermore, the phenotypes of patients #1 and #2 were not reported. For the remaining 3 patients, some similarities have been observed: Genital anomalies were present in all cases. The other findings are variable, and may be explained either by the young age of patient #3 at the time of study, given that certain symptoms occur later in life, or by an additional genomic variant in patient #4 that may have influenced the phenotype. Even though they harbored the same variants, polydactyly was noted in all four limbs in the current patient, whereas in patient #3 polydactyly was observed only in the feet. The heart anomaly described in patient #3 was not present in the patient reported here. Therefore, further studies are required to elucidate the complex pathological mechanisms underpinning this highly heterogeneous ciliopathy.

In conclusion, the present case report has provided novel evidence in terms of defining the phenotype associated with this rare variant in BBS12.

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Availability of data and materials

Due to constraints of participant consent, whole exome sequencing data are not posted to public databases, but we will make portions of the dataset available to researchers upon reasonable request.

Authors’ contributions

JOF collected the data, wrote the manuscript, and prepared the figures and the tables. MBa and CB provided the clinical care of the patient. SK performed Sanger confirmation and segregation analysis. AS assisted with organization of clinical samples and data. LCB facilitated the initial preparation of samples. EED conducted the genetic testing and edited the manuscript. MBa designed the study, and revised the manuscript. All authors have read and approved the final manuscript. SK and EED confirm the authenticity of all the raw sequencing data.

Ethics approval and consent to participate

Willing family members were enrolled in the PhD research study approved by Institutional Board of University of Medicine and Pharmacy ‘Carol Davila’ Bucharest, (approval no. 29700, T.42; Oct 01, 2015), and all experiments conformed with the guidelines of the Declaration of Helsinki. The use of whole exome sequencing was approved by the Institutional Review Board of the Ann & Robert H. Lurie Children’s Hospital, Chicago (approval no. IRB 2019-3057, August 5, 2019).

Patient consent for publication

Written informed consent was obtained from legal guardians of the patient included in the study for genetic testing and publication of data and images, as well from all the other participants from whom samples were obtained.

Competing interests

The authors declare that they have no competing interests.

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