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Clinical findings and molecular diagnosis in children with Bardet-Biedl Syndrome in Turkey: Identification of novel variants

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ABSTRACT

Aims: Bardet-Biedl syndrome (BBS) is a rare autosomal recessive ciliopathy with multisystemic involvement and variable phenotypic features. A diagnosis is usually made through the clinical diagnostic criteria. However, the clinical diagnosis can be difficult due to the absence of clear phenotype-genotype correlation and overlapping findings with other ciliopathies. Next-generation sequencing (NGS) is a rapid and cost-effective diagnostic method for this group of diseases. Besides correct diagnosis, detection of novel variants also contributes to establishing new phenotype-genotype correlations and delineating the pathophysiology of the syndrome. Here, we aimed to present clinical and molecular findings of patients with BBS using NGS panel analysis to contribute to the genotype-phenotype correlation in this rare syndrome.

Methods: We retrospectively reviewed the medical records of patients with a suspicion of BBS admitted to the Pediatric Genetics Department in Umraniye Training and Research Hospital. Patients who met the BBS clinical diagnostic criteria were included in the study. Targeted NGS analysis, including 20 genes associated with BBS, was performed on an Illumina Next-Seq-500 platform.

Results: The final analyses included 6 patients (age, mean±standard deviation: 14.3±6.6 years, female: 50%). Rod cone dystrophy (100%), polydactyly (100%), and intellectual disability (100%) were the most common findings followed respectively by obesity (83%), renal anomalies (83%), liver anomalies (67%), dental problems (67%), metabolic problems (50%), genital anomalies (33%), psychiatric disorders (33%), and sleep apnea (33%). The 5th metatarsal shortness and camptodactyly were anomalies reported for the first time in BBS. Seven variants were detected in the BBS1, BBS7, BBS5, BBS9, and MKKS, two of which were novel. BBS7 was the most common gene.

Conclusions: Our study expanded the genotypic spectrum of the disease with two novel variants reported. Besides, by defining novel/rare clinical features, including camptodactyly, the fifth metatarsal, the 4th-5th metacarpal shortness, and nephrocalcinosis, it formed a source for the phenotype-genotype correlation trying to be established in the literature.

Introduction

Bardet-Biedl syndrome (BBS) is a rare autosomal recessive ciliopathy with multisystemic involvement. Retinal rod-cone dystrophy, obesity, polydactyly, intellectual disability, hypogonadism, and renal disease are the characteristic findings of the syndrome. In addition to these findings, variable other neurological, gastrointestinal, endocrinological, and cardiovascular system involvements have been reported (1-5). The prevalence of the syndrome differs according to geographical regions, reported as 1/160.000 in Europe and rises to 1/13.500 in regions such as the Middle East, where the consanguinity rate is high (1,3,6).

In BBS, the key element in the pathophysiology is cilia dysfunction. Cilias are organelles that have functions primarily in the determination of cell polarity, regulation of the cell cycle, and mechano-sensation. Also, they take part in the

signaling pathways involved in vertebral development and organ differentiation in the embryological period (7). These widespread functions of the cilia are reflected in the phenotype as multi-systemic involvement, as in BBS. Bi-allelic/tri allelic loss of function mutations in 23 genes (BBS1-21) associated with cilia structure, biogenesis, and function, are reported in approximately 80% of patients with BBS (8).

The clinical diagnosis of the syndrome is made through the clinical diagnostic criteria presented in Table 1 (9,10). According to those criteria, four major features or the coexistence of three major and two minor features are adequate to establish the diagnosis (3,4,6,11,12). However, the diagnostic criteria may be insufficient when evaluating patients in early childhood, where some clinical findings have not yet emerged. Additionally, phenotypic variability is expected in BBS, and some patients may not meet the diagnostic criteria despite a confirmed molecular diagnosis. There is also no clear phenotype-genotype correlation in the syndrome, and an accurate clinical diagnosis can be difficult due to overlapping findings with other ciliopathies (6,13). Considering all these, it is undeniable that a molecular diagnosis is an essential tool for timely and accurate diagnosis. Next-generation sequencing (NGS) technologies, which have become widespread in clinical use recently, are a rapid and cost-effective diagnostic method in this group of diseases with genetic heterogeneity. Novel variants to be detected by this method will also contribute to establishing new phenotype-genotype correlations and delineating the pathophysiology in this rare group of diseases, apart from achieving accurate diagnosis in patients.

Here, we aimed to present the clinical and molecular findings of patients with BBS using NGS panel analysis to contribute to the genotype-phenotype correlation in this rare syndrome.

Methods

We retrospectively reviewed the medical records of patients with a suspicion of BBS admitted to the Pediatric Genetics Department in Umraniye Training and Research Hospital. Patients who met at least four major criteria or three major and two minor criteria according to the BBS clinical diagnostic criteria were included in the study (9,10). Beyond the demographic data, family history, clinical presentation; renal function test, urinalysis, liver function test, thyroid function test, abdominal-renal ultrasonography, echocardiography, and the examination results performed by the eye, eye-nose-throat, and cardiology departments were collected from the medical records of the patients. The local ethics committee approved the protocol of the study with an accession number of BS.GP.0.01/177. The study was conducted following the Declaration of Helsinki. Informed consent was obtained from each patient and their legal guardians for molecular analysis.

Targeted NGS analysis and variant interpretation

After obtaining written informed consent, peripheral blood samples were collected from all individuals. Following the standard protocols of the QIAAmp DNA Mini (Qiagen) kit, automatic DNA isolation was performed in EDTA-anticoagulated blood samples. Targeted NGS analysis was performed on the Illumina Next-Seq 500 platform using SOPHIA Clinical Exome Solution using Illumina V2 chemicals as outlined previously (14,15). The gene panel consisted of 20 genes associated with BBS; *ARL6* (NM_001278293), *BBS1* (NM_024649), *BBS10* (NM_024685), *BBS12* (NM_001178007), *BBS2* (NM_031885), *BBS4* (NM_001252678), *BBS5* (NM_152384), *BBS7* (NM_176824), *BBS9* (NM_001033604), *C8ORF37* (NM_177965.4), *CCDC28B* (NM_001301011), *CEP290* (NM_025114), *LZTFL1* (NM_001276378), *MKKS* (NM_018848), *MKS1* (NM_001165927), *SDCCAG8* (NM_006642), *TMEM67* (NM_001142301), *TRIM32* (NM_001099679), *TTC8* (NM_001288781), *WDPCP* (NM_001042692). Sequence analysis covers the coding regions of each gene, including all coding exons, +/− 10 base pairs of adjacent intronic sequences, and each nucleotide is read at a depth of at least 50X. Variants that fall outside these regions and exonic variants with a minor allele frequency of less than 10% were considered false positives and unanalyzed. Copy number variations were not examined.

Table 1. Clinical diagnostic criteria for Bardet-Biedl syndrome (9,17)

Major features	Minor features
Retinal cone-rod dystrophy	Neurologic abnormalities (ataxia, poor coordination, mild spasticity, speech delay)
Central obesity*	Olfactory dysfunction (anosmia, hyposmia)
Postaxial polydactyly	Oral/dental abnormalities (dental crowding, hypodontia, small roots, high arched palate)
Cognitive impairment	Gastrointestinal abnormalities (liver disease**, inflammatory bowel disease, celiac disease, Hirschsprung disease)
Hypogonadism	Cardiovascular and other thoraco-abdominal abnormalities (congenital heart diseases, situs ambiguous)
Renal disease	Endocrine/metabolic abnormalities (T2DM, DI, hypothyroidism, metabolic syndrome)

*Features associated with obesity (including endocrine/metabolic abnormalities and non-alcoholic fatty liver disease) are defined as minor features of BBS.
**Liver disease is considered as abnormalities in liver imaging and/or abnormal transaminase levels.
T2DM: Type 2 diabetes mellitus, DI: Diabetes inspidus, BBS: Bardet-Biedl syndrome

The DNA sequences were aligned to the NCBI Build37 (hg18) version of the human genome. Alignments were confirmed using the Integrative Genomics Viewer v.2.313. The Sophia-DDM-V5.2 bioinformatics analysis program performed variant calling and data analysis. The interpretation of the variants was performed according to the 2015 American College of Medical Genetics (ACMG) standards and guidelines (16). Iranome and GnomAD data were used as the control population. The variants' effects on protein function were investigated using *in silico* prediction programs such as SIFT, PolyPhen2, M-CAP, Mutation Taster, and MVP. The Human Gene Mutation Database and ClinVar and PUBMED databases were used to investigate mutations previously associated with BBS. Only variants of unknown significance (VUS), pathogenic (P), and likely-P variants were reported in the results section. Segregation analysis was performed by Sanger sequencing. Primer sequences and reaction conditions are available on request.

Results

Demographic and clinical characteristics

We identified 10 patients who were followed up with a suspicion of BBS. Four patients who did not fulfill the BBS clinical criteria were excluded and the final analyses included 6 patients (age, mean±standard deviation: 14.3±6.6 years, female: 50%). In the study group, while all major criteria were present in four of the patients, two had only four major criteria. Rod cone dystrophy (100%), polydactyly (100%), and intellectual disability (100%) were the most common findings in all patients. These findings were followed respectively by obesity (83%), renal anomalies (83%), gastrointestinal abnormalities anomalies (67%), dental problems (67%), endocrine/metabolic problems (50%), hypogonadism (33%), psychiatric disorders (33%) and sleep apnea (33%). Among the renal abnormalities, renal agenesis/hypogenesis (40%) and nephrocalcinosis (40%) were the most common abnormalities. All male patients had hypospadias regarding hypogonadism. Hepatosplenomegaly and elevated transaminase levels were the findings detected in all patients with liver abnormalities. In the dental anomalies group, malocclusion was the most common dental anomaly (75%); hypodontia was present in only one patient (Figure 1). Anxiety was the most common problem detected in 75% of patients with neuropsychiatric disorders. In the metabolic/endocrine finding group, diabetes mellitus (DM) was present in only one patient; however, hyperlipidemia (75%) stood out as the major metabolic problem. Cardiac abnormalities were present in only one patient with the atrial septal defect. Hearing loss and olfactory problems were not observed in the study group. Clinical features and the details of the anomalies are presented in Table 2.

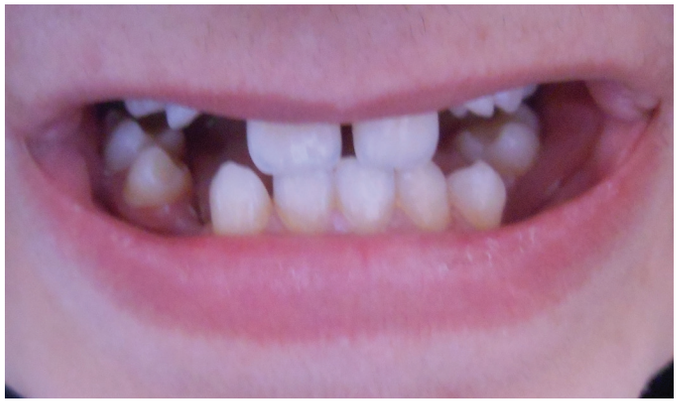

Figure 1. Image of the oral region showing the hypodontia in Patient 2

Molecular analysis

A molecular diagnosis was achieved in all the patients. Seven different variants were detected in the *BBS1, BBS7, BBS5, BBS9*, and *MKKS* genes. There were three frameshift (fs), two splice-site, and two missense variants, two of which were novel variants that were not previously reported in patients with BBS (Figure 2). The details of the variants are presented in Table 2.

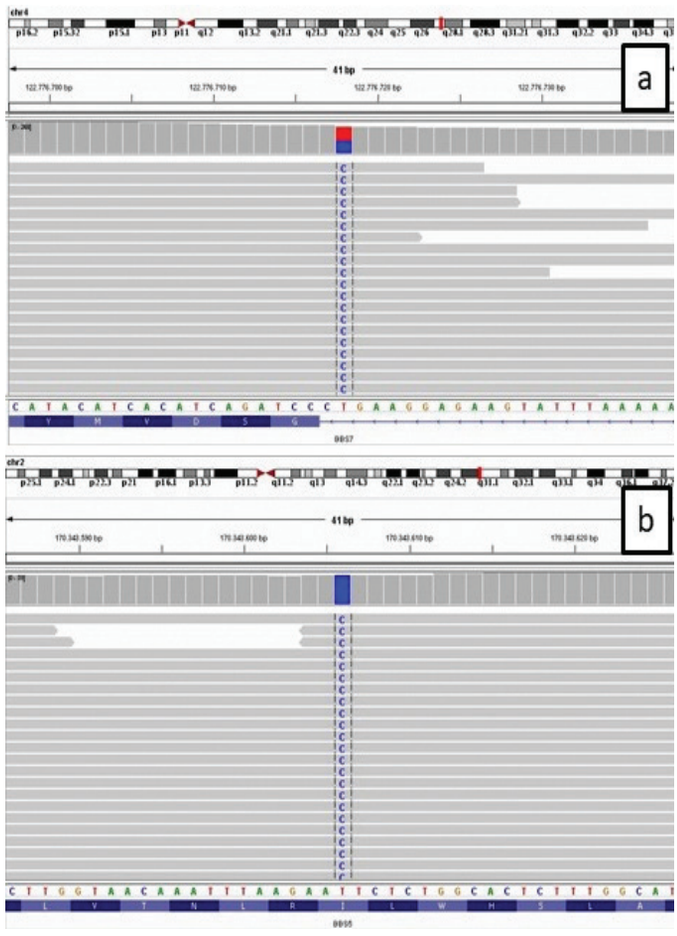

Figure 2. Integrative genomics viewer images of the novel variants reported in Patient 1 (BBS7 c.529-2A>G) (a) and Patient 2 (BBS5 c.170T>C) (b)

Table 2. Clinical and molecular findings of the patients

Patient ID	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Percentage
Gene	*BBS7/BBS7*	*BBS5*	*BBS7*	*MKKS*	*BBS9*	*BBS1*	
NM number	NM_176824	NM_152384	NM_176824	NM_018848	NM_001033604	NM_024649	
Depth	215/213	47	125	726	47	110	
50x	99.11%	98.87%	99.92%	99.98%	86.77%	84.83%	
Mutation type	Frameshift/Splice	Missense	Splice	Missense	Frameshift	Frameshift	
cDNA	c.712_715delAGAG/ c.529-2A>G	c.170T>C	c.849+1G>T	c.541G>A	c.310del	c.1232_1235del	
Protein	p.Arg238Glufs*59/p.(?)	p.Ile57Thr	p.(?)	p.Ala181Pro	p.(Cys104Valfs*20)	p.(Gly411Glufs*12)	
Reference	Reported/Novel	Novel	Reported	Reported	Reported	Reported	
Zygosity	Het/Het	Hom	Hom	Hom	Hom	Hom	
ACMG class	P/P	VUS	P	LP	P	P	
ACMG evidence	PVS1, PP5, PM2/PVS1, PM2	PM2, PP2, PP3	PVS1, PM2, PP3	PM2, PP2, PP3	PVS1, PM2, PP3, PP5	PVS1, PM2, PP3, PP5	
Age (years)	18	17	2	14	14	21	
Gender	M	F	M	F	F	M	
BMI (kg/m²)*	29.4	30.5	28.1	42.3	34.5	43	
CDC BMI-for-age percentiles	(95^{th} -97^{th})	(95^{th} -97^{th})	(>97^{th})	(>97^{th})	(>97^{th})	(>97^{th})	
Rod cone dystrophy	Yes	Yes	Yes	Yes	Yes	Yes	100
Limb abnormalities	Yes	Yes	Yes	Yes	Yes	Yes	100
Polydactyly	Yes	Yes	Yes	Yes	Yes	Yes	
Syndactyly	Yes	No	No	No	No	No	
Others	No	5^{th} metatarsal, 4^{th}-5^{th} metacarpal shortness	No	No	No	Camptodactyly Brachydactyly	
Intellectual disability	Moderate	Mild	NA	Moderate	Mild	Mild	83
Developmental delay	Yes	Yes	Yes	Yes	Yes	Yes	100
Renal abnormalities	No	Left renal agenesis Right renal cyst	Grade 2 pelvic ectasia Bilateral medullar nephrocalcinosis	Bilateral renal cyst	Nephrocalcinosis Proteinuria	Bilateral renal hypogenesis Renal insufficiency	83
Genital abnormalities	Hypospadias	No	Hypospadias	No	No	Hypospadias	50

Table 2. Continued

Patient ID	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Percentage
Liver abnormalities	No	No	No	Hepatosplenomegaly Hepatosteatosis High transaminase levels	No	Hepatosplenomegaly High transaminase levels	33
Diabetes mellitus	No	No	No	Yes	No	No	17
Dyslipidemia	Yes	No	Yes	Yes	No	Yes	67
Dental abnormalities	Malocclusion	Multiple caries Erosion Hypodontia	No	Malocclusion	Malocclusion	No	67
Neuropsychiatric problems	Anxiety	Anxiety	No	OCD/seizures	Anxiety	No	67
Sleep apnea	No	No	No	Yes	No	Yes	33
Hearing loss	No	No	No	No	No	No	0
Anosmia	Yes	No	No	No	No	No	17

*CDC BMI-for age charts (2-20 years) were used to determine the weight status (underweight: <5th percentile, healthy weight: 5th-85th percentile, overweight: 85th-95th percentile, obesity: >95th percentile).
Hom: Homozygous, Het: Heterozygous, P: Pathogenic, LP: Likely pathogenic, VUS: Variant of insignificance, M: Male, F: Female, BMI: Body mass index, CDC: Centers for disease control and prevention, OCD: Obsessive-compulsive disorder

One of the novel variants was the *BBS7* c.529-2A>G, a null variant detected in Patient 1. We predicted that this variant would cause exon skipping by affecting the acceptor site of the *BBS7*. This was not previously reported in community databases such as GnomAD and Iranome. The variant was classified as P with the evidence of PVS1 and PM2.

The other novel variant was the homozygous missense c.170T>C variation in *BBS5*. It was not reported in the GnomAD and Iranome databases. Most *in silico* prediction tools (M-CAP, MutationTaster, and SIFT) showed that the variant had a damaging effect on the protein. The variant was classified as VUS with the ACMG evidence of PM2 and PP3.

Discussion

BBS is characterized by both phenotypic and genotypic heterogeneity (2). Thus, identifying new variants and deep phenotyping in patients is vital in elucidating this rare syndrome's pathophysiology and advancing therapeutic research. Our study is the third study conducted for this purpose in patients with BBS in Turkey (1,11). We managed to detect the underlying molecular pathology in all of our patients using the targeted NGS panel. Although the targeted panel analysis yielded a diagnostic rate up to 86% in previous studies, we achieved the molecular diagnosis in all of our patients (1,2). We suggest this finding may be related to the inclusion of patients who only fulfilled the BBS clinical diagnostic criteria in our study.

BBS1 and *BBS10* stand out as the genes with the most common disease-associated variants in the patient cohorts reported so far (5,17). Consistent with these findings, *BBS10* and *BBS1* were reported as the most common genes in the largest series conducted in our country (1). However, in our study, while no variant was detected in *BBS10*, a *BBS1*-related variant was detected in only one patient. Also, with three variants detected, *BBS7* stood out as the most common gene. Variations in *BBS7* are defined as a rare cause of the syndrome, with a frequency of 1.5-3% in multiethnic groups (18-21). This rate was up to 15% in studies conducted in our country (1,11). When evaluated with the data from our research, one can suggest that *BBS7*-associated BBS is more frequent in the Turkish population than in other ethnic groups.

Missense variations were reported as the most common type of variation in BBS-related genes (2,22). Unlike this finding, null variants (fs, splice site) were detected more frequently in our study, as in the other two studies conducted in Turkey (1,11). We can suggest that this finding may be related to Turkish ethnic origin and geographical region; however, studies involving more patients from Turkey are needed to make an accurate assessment.

We contributed to the BBS-related genotypic spectrum with the two novel variants detected. The first of these was the heterozygous *BBS7* c.529-2A>G variant. The fact that it was a

null variant unreported in population databases was important evidence to be considered P associated with BBS. Furthermore, we thought that this variant would explain the clinical findings related to BBS if it was found in a trans with the previously reported *BBS7* c.712_715delAGAG, fs variant detected in the same patient. However, although we assumed that these two variants were found to be compound heterozygous based on clinical findings, we could not conduct a segregation analysis in terms of compound heterozygosity because the parents were deceased, and we could not confirm this situation. The other novel variant was the *BBS5* c.170T>C variant. It was absent in population databases and determined as damaging according to most of the *in silico* prediction tools. These findings provide important evidence for associating the variant with the disease phenotype. Additionally, the interspecies conservation score of the genomic position (PhyloP100way score: 6.026, GERP score: 5.5) was also high. The patient's clinical findings had met five of the major and two minor diagnostic criteria of BBS. Therefore, although it was classified as VUS according to ACMG, we thought the variant explained the patient's phenotype. However, it was also kept in mind that functional studies should be conducted, or the same variant should be reported in another patient to prove the pathogenicity of the variant.

In terms of phenotypic findings, rod-cone dystrophy, polydactyly, developmental delay, and intellectual disability, which are the major features of the syndrome, were present in all of our patients. Rod-cone dystrophy has been reported in approximately 80-90% of patients in previous studies and is usually detected after three years of age, at a mean age of 8.5 years (1,2,4). In this study, all but one patient was diagnosed with rod-cone dystrophy in adolescence, in line with these data. The youngest patient with rod-cone dystrophy in our study was a two-year-old girl diagnosed at the age of 1.5 years when examined for nystagmus (Patient 3). She had a homozygous null variant in the *BBS7* gene, so we conclude that the retinal pathology observed at such an early age may be related to the null variant. Nystagmus has only been reported in two patients with BBS (1,23). Since they both had variants of *BBS7*, authors have suggested that there is a relationship between *BBS7*-related variants and nystagmus (1,23). Although the number of reported cases is scant, our patient appears to be a case that supports this correlation.

Polydactyly has been reported in 60-80% of patients with BBS (1,2,6). In addition to polydactyly; syndactyly, brachydactyly, and clinodactyly are among the other extremity anomalies reported in BBS. Among these anomalies, syndactyly and brachydactyly were also detected in two patients in our study group. Moreover, isolated 5th metatarsal, 4th, and 5th metacarpal shortness in the patient with the homozygous missense variant in *BBS5* and camptodactyly in the patient with homozygous fs variant in *BBS1* were the extremity anomalies that we reported for the first time in association with BBS (Patient 2 and Patient 6) (Figure 3).

Developmental delay is reported in approximately 90% of patients with BBS while varying degrees of intellectual disabilities are described in 60% of patients (1,2,24). While there is no clear genotype-phenotype correlation in terms of intellectual disability, milder intellectual disability is reported with *BBS1* and *BBS12* variants (2). Mild intellectual disability was observed in most patients, and moderate intellectual disability was found in only two of the patients with *BBS7* and *MKKS* variants in our study (Patient 1 and Patient 4). However, there is no data in the literature to support this phenotype-genotype correlation considering moderate intellectual disability.

Another major feature, obesity, was found in 67% of the patients in our study group, similar to the rate of 70-90% reported in the literature (1,3,4). DM and liver abnormalities that are reported to be associated with obesity were also present mainly, in obese individuals in the study group (2,8). However, contrary to DM and liver abnormalities, it has been suggested that dyslipidemia can also be detected in patients with BBS without obesity (2). Dyslipidemia observed in 18-year-old and 2-year-old individuals who were not obese but overweight may support this view (Patient 1 and Patient 3). Therefore, it is crucial to follow the patients from an early age in terms of these metabolic problems to minimize the related complications.

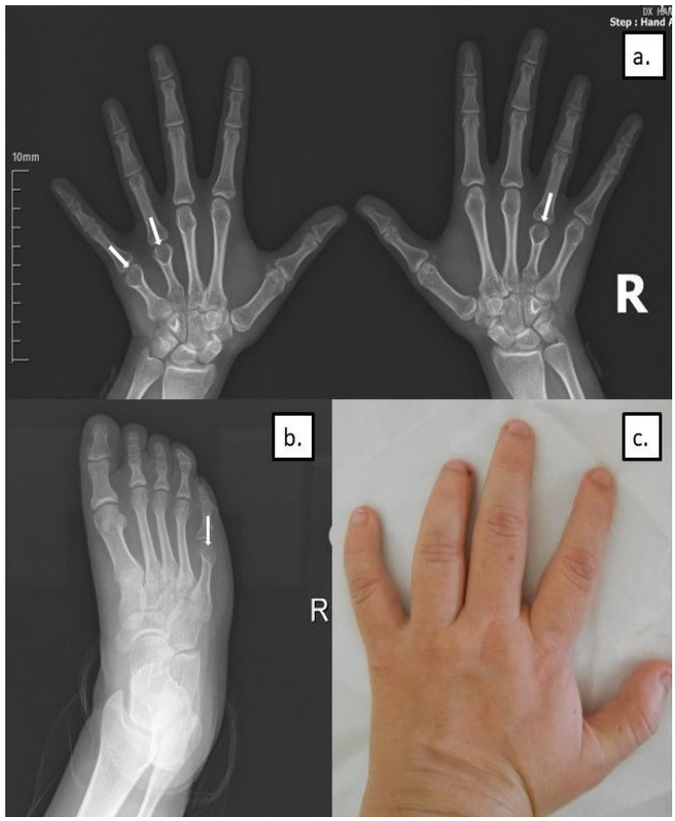

Figure 3. Foot and hand X-rays of Patient 2 and hand image of Patient 6. Note the 4th and 5th metacarpal shortness in left, 4th metacarpal shortness in right hand (a), metatarsal shortness in right foot (b), camptodactyly in third and fourth fingers in the left hand (c)

Renal anomalies are reported in 50-80% of individuals with BBS and cause severe morbidity and mortality with advancing age, particularly if not properly managed (1,2,25,26). Renal involvement was present in 67% of patients, and it stood out as the most common organ involvement causing morbidity in this study. The patient with the most severe renal involvement was a 21-year-old male followed up for end-stage renal disease (Patient 6). It is thought that the presence of the homozygous null variant in *BBS1* in this patient may have led to a severe clinical course. Nephrocalcinosis, an infrequent renal finding in individuals with BBS (27), had been observed in two patients with variants of *BBS7* and *BBS9*, respectively in our cohort (Patient 3 and Patient 5). However, more cases are needed to establish a genotype-phenotype correlation for nephrocalcinosis.

Among the non-classical findings, psychiatric problems observed in 66% of the study group were remarkable. These psychiatric disorders, primarily including autism, obsessive-compulsive disorder, and anxiety, have been defined in 30-50% of individuals with BBS and can affect the functionality of a patient's daily lives (1,2,11,24). Especially in patients with severe multisystemic involvement, psychiatric problems may remain in the back and have been ignored by the patient and the family. So it is of great importance that health professionals do not overlook these problems but make appropriate interventions in the early period (2,24).

This study has some limitations. First, the number of patients and the heterogeneity of the detected genes prevents us from establishing any widespread phenotype-genotype correlations in the study. However, in this rare syndrome, the phenotype and genotype information reported in the literature are of great value. Second, since a wide phenotypic heterogeneity is defined in BBS, the use of strict clinical criteria for inclusion in the study group may have resulted in some patients being missed. The enrollment of patients in the study with looser criteria would have allowed broadening of the disease's genotypic/phenotypic spectrum.

Conclusion

Our study expanded the genotypic spectrum of the disease with two novel variants reported. Besides, by defining novel/rare clinical features, it formed a source for the phenotype-genotype correlation trying to be established in the literature.

Ethics

Ethics Committee Approval: Ethical approval was obtained at the Health Sciences University, Istanbul Umraniye Training and Research Hospital Local Clinical Research Ethics Committee (approval number: B.10.1.TKH.4.34.H.GP.0.01/177, date: 27.05.2021).

Informed Consent: Retrospective study.

Peer-reviewed: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Ö.A.D., Design: Ö.A.D., N.B.A, Data Collection, or Processing: Ö.A.D., N.B.A., Analysis, or Interpretation: Ö.A.D., N.B.A., Literature Search: Ö.A.D., N.B.A., Writing: Ö.A.D.

Conflict of interest: No conflict of interest was declared by the authors.

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