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Targeted multi-gene panel testing for the diagnosis of Bardet Biedl syndrome: Identification of nine novel mutations across BBS1, BBS2, BBS4, BBS7, BBS9, BBS10 genes

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ABSTRACT

Bardet-Biedl Syndrome (BBS) is a rare, autosomal-recessive ciliopathy characterized by obesity, rod-cone dystrophy, postaxial polydactyly, renal abnormalities, genital abnormalities and learning difficulties. To date, mutations in 21 different genes have been described as being responsible for BBS. Recently sequential gene sequencing has been replaced by next generation sequencing (NGS) applications. In this study, 15 patients with clinically diagnosed BBS were investigated using a next generation sequencing panel which included 17 known BBS causing genes (BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12, MKS1, NPHP6, WDPCP, SDCCAG8, NPHP1).

A genetic diagnosis was achieved in 13 patients (86.6%) and involved 9 novel and 3 previously described pathogenic variants in 6 of 17 BBS causing genes. BBS10 and BBS1 were the most commonly involved genes with frequencies of 31% and 23% respectively. Three of the 13 patients had an affected sibling. All affected siblings were found to be homozygous for the mutation detected in the proband. No evidence of triallelic inheritance was detected.

Although limited association between certain genes and phenotypic features has been observed in this study, it is considered that additional studies are needed to better characterize the genotype–phenotype correlation of BBS. Our results demonstrate that NGS panels are feasible and effective method for providing high diagnostic yields in the diseases caused by multiple genes such as BBS.

1. Introduction

Bardet-Biedl syndrome (BBS, OMIM 209900) is a rare autosomal recessive pleiotropic disorder caused by defects of primary cilia (Zaghloul and Katsanis 2009). The main features of the syndrome are truncal obesity, rod-cone dystrophy, postaxial polydactyly, genital abnormalities, renal anomalies and learning disabilities (Baker and Beales 2009, Bardet 1995, Biedl 1995). Moreover, a wide spectrum of additional features has been described to date (Deveault et al., 2011, Forsythe and Beales 2013). The prevalence of BBS ranges from 1:100,000 in Europe to 1:160,000 in North America (Forsythe and Beales 2013). Higher prevalence has been reported in isolated populations and/or populations having a high rate of consanguineous marriages such as Kuwait Bedouins and Newfoundland with frequencies of 1/13,500 in and 1/17,500 respectively (Farag and Teebi 1989, Green et al., 1989).

To date, causative mutations have been identified in 21 different genes (BBS1–BBS19, NPHP1, IFT172) in patients with BBS (Aldahmesh et al., 2014, Ansley et al., 2003, Badano et al., 2003, Bujakowska et al., 2015, Chiang et al., 2006, Chiang et al., 2004, Katsanis et al., 2000, Leitch et al., 2008, Lindstrand et al., 2014,

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Marion et al., 2012, Mykytyn et al., 2001, Mykytyn et al., 2002, Nishimura et al., 2001, Nishimura et al., 2005, Otto et al., 2010, Scheidecker et al., 2014, Slavotinek et al., 2000, Stoetzel et al., 2006, Stoetzel et al., 2007, Young et al., 1999). Mutations have been detected in known BBS genes in about 80% of affected individuals (Redin et al., 2012, Stoetzel et al., 2007). The majority of mutations have been found in the genes BBS1 and BBS10, each accounting for 20–25% (Beales et al., 2003, Stoetzel et al., 2006).

Next-generation sequencing (NGS) provides rapid and feasible molecular diagnosis for genetically heterogeneous diseases such as BBS. Recently NGS has been widely used in these kinds of diseases as a cost-effective technique providing higher coverage with a shorter turnaround time.

In this study, we performed mutational analysis of 17 known BBS genes in 15 patients with clinically-diagnosed BBS and described phenotypic features related to the specific mutations detected in the patients.

2. Methods

2.1. Patients

Fifteen BBS diagnosed clinically patients were included in the study. Clinical diagnosis was established by experienced clinical geneticists with only patients showing at least two main features of BBS being included in the study. After obtaining informed consent, blood samples were collected from all BBS patients and their unaffected parents. This study was approved by the Research Ethics Board of Ege University, Izmir, Turkey and supported by the Scientific Research Projects Directorate of Ege University with the project number of 14-TIP-012.

2.2. Targeted NGS analysis

DNA was isolated from a 200 ul blood sample using the QIAamp DNA Blood Mini QIAcube Kit with a QIAcube instrument (QIAGEN, Hilden, Germany) according to the manufacturer's specifications.

Illumina TruSight Exome Content Set (Catalog No: TG-141-1001) and Illumina TruSight Rapid Capture Kit (Catalog no: FC-140-1104. Illumina Inc., San Diego, CA, USA) were used for enrichment of the coding regions and the exon-intron boundaries of the 17 BBS genes (BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12, MKS1, CEP290, WDPCP, SDCCAG8, NPHP1) as well as sample preparation for the next generation sequencing reaction. Sequencing was performed on an Illumina MiSeq NGS System (Illumina Inc., San Diego, CA, USA) using Miseq Reagent Kit V3(600 cycles) (Catalog No: MS-102-3003. Illumina Inc., San Diego, CA, USA). Reads were aligned to the hg19 genomic sequence.

2.3. Data analysis

Sequencing data was analyzed using Illumina Variant Studio (Illumina Inc., San Diego, CA, USA) variant analysis software and IGV (Integrative Genomics Viewer) (Robinson et al., 2011, Thorvaldsdottir et al., 2013). For data filtering, first the homozygous or compound heterozygous variants in 17 BBS causing genes with a frequency of less than 0.5% in public databases (e.g. NCBI dbSNP build 141 (http://www.ncbi.nlm.nih.gov/SNP/), 1000 Genomes Project (http://www.1000genomes.org/), Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/)) were selected. The impact of the mutations on the protein structure was evaluated using several in silico prediction tools specifically Mutation Taster (Schwarz et al., 2010), Polyphen-2 (Adzhubei et al., 2010), and SIFT (Kumar et al., 2009). Conservation of residues across species was evaluated by PhyloP algorithm (Pollard et al., 2010) and GERP (Davydov et al., 2010).

2.4. Confirmation and segregation analysis

The mutations detected by the NGS analysis were verified through Sanger sequencing on ABI PRISM 3130 DNA analyzer (Applied Biosystems, Foster City, CA, USA) using Big Dye Terminator Cycle Sequencing V3.1 Ready Reaction Kit (Life Technologies, Carlsbad, CA). Mutations found in the proband were then analyzed in the parents again using Sanger sequencing.

3. Results

3.1. Targeted NGS data

On average, 15 probands had 98.70%, 93.33% and 87.17% of mappable bases represented by a coverage of at least 1X, 10X and 20X reads, respectively. An average depth of 140.89 reads was achieved.

3.2. Mutational analysis

The ages of 15 unrelated BBS patients (10 males and 5 females) ranged from 7 months to 21 years old (average age 16.6). Nine patients had consanguineous parents and family histories of four patients revealed that both parents were from the same small village.

Thirteen patients in the study were found to have mutations in one of the analyzed 17 BBS genes (86.6%). BBS10 gene was the most frequently involved affected 4 of the 13 (31%) patients. This was followed by BBS1 gene affecting 3 of the 13 (23%) patients. The frequencies of the remaining mutated genes were as follows: 15% (2/13) for BBS2 and BBS7; 8% (1/13) for BBS4 and BBS9 genes (ClinVar number SCV000255605 – SCV000255613). No mutations were found in ARL6, BBS5, MKKS, TTC8, TRIM32, BBS12, MKS1, CEP290, WDPCP, SDCCAG8 and NPHP1 genes (Table 1). Twelve different mutations in 13 patients have been identified in this study. Three mutations have been previously described while nine are novel. No compound heterozygosity was observed in the study group. All unaffected parents were heterozygous for mutations detected in affected homozygous children. Among 13 BBS families with mutations, 3 had two affected children. All of these siblings were found to be homozygous for related mutations. No evidence of triallelic inheritance was detected in this study.

All of the six cardinal features of the syndrome were observed in 4 (30.7%) mutation positive BBS patients. Five patients (38.4%) had 5 major features, 2 patients (15.3%) had 4 major features, 1 patient (7.6%) had 3 major features and 1 patient (7.6%) had 2 major features. All patients over 1 year old showed intellectual disability. The most common features detected in all mutation positive patients, were truncal obesity and 2–3 toe syndactyly. Retinal dystrophy was the second most common major feature and observed in 11 of the 13 mutation positive patients (84%). This was followed by polydactyly in 11/13 (76%). Genital anomalies were documented in 9/13 (69.0%) and renal anomalies in 7/13 (54%) patients. Additionally, 22 minor features were also documented in the group studied. Clinical features of patients are shown in Table 2.

4. Discussion

4.1. Genotype

This is the first study from Turkey investigating mutation spectrum and phenotype–genotype correlation in BBS patients. In this study, 17 of 21 genes known to cause BBS were analyzed molecularly using targeted next-generation sequencing in this study. It has been reported that mutations in these selected BBS

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Table 1
Characteristics of the identified variants in the study.

Gene	Transcript ID	cDNA	Protein	Mutation type	MT	Polyphen2 score	SIFT	ExAC* (OAF)	Mean coverage depth	Reference
BBS1	NM_024649	c.48-3C > G	–	S	DC	NA	NA	–	27,2	Novel
BBS1	NM_024649	c.851delA	p.Y284SfsX5	FS	DC	NA	NA	–	53,7	Known[a]
BBS1	NM_024649	c.1012C > T	p.Q338X	M	DC	NA	NA	–	10,5	Novel
BBS2		c.263delG	p. G88AfsX6	FS	DC	NA	NA	–	86,2/33,9	Novel
BBS4	NM_001252678	c.406-2A > G	–	S	DC	NA	NA	–	89	Novel
BBS7	NM_176824	c.712_715delAGAG	p. R238EfsX59	FS	DC	NA	NA	0.00005768	85	Known[b]
BBS7	NM_176824	c.949C > G	p. L317V	M	DC	0.999	D	–	26,6	Novel
BBS9	NM_198428	c.102_112 + 2delAAATGGACAAGGT	p. N35X	FS	DC	NA	NA	–	58,8	Novel
BBS10	NM_024685	c.931T > G	p.S311A	M	DC	0.999	D	–	65,1	Known[c], rs137852837
BBS10	NM_024685	c.1856_1865delAAAAATGCCA	p.K619IfsX10	FS	DC	NA	NA	–	97,4	Novel
BBS10	NM_024685	c.1024_1025insA	p. I342NfsX20	FS	DC	NA	NA	–	64,1	Novel
BBS10	NM_024685	c.1547delC	p. T516NfsX8	FS	DC	NA	NA	–	51,2	Novel

*Exome Aggregation Consortium (http://exac.broadinstitude.org).
M: Missense, S: Splice site, F: Frameshift, NS: Nonsense, MT: MutationTaster, DC: Disease causing, D: Damaging, T: Tolerated, NA: Not available, OAF: Overall Allele Frequency [a]: (Mykytyn et al., 2002), [b]: (Bin et al., 2009), [c]: (Stoetzel et al., 2006).

genes are responsible for 70–80% of clinically diagnosed BBS patients (Forsythe and Beales 2013). In our study slightly higher level of diagnostic rate (86.6%) was achieved using NGS panel covering the same genes.

Family history of 9 mutation positive patients showed consanguinity between the parents. No consanguinity was detected in four other mutation positive patients. However both parents were found to come from the same small village. A higher rate of consanguinity in our study is considered to be the principal reason for the presence of the same mutations on both alleles in all patients.

In line with the previous studies, we found that BBS1 and BBS10 were the most commonly involved genes in BBS patients (Forsythe and Beales 2013). The frequency of BBS10 gene mutations has varied between 21 and 31% in previous studies (Deveault et al., 2011, Janssen et al., 2011). Regarding our study and previous studies from different populations, it has been considered that these two gene regions are more sensitive to mutational effects. The frequency of pathogenic mutations in other genes in BBS patients varies across different ethnic groups. BBS4 and BBS5 gene mutations have been reported more frequently in the Middle East (Billingsley et al., 2010). We found a BBS4 mutation in only one case and no BBS5 mutation was found. The patient carrying BBS4 mutation was from the eastern part of Turkey. The frequency of BBS7 gene mutations (15%) were higher in our study group when compare to the frequencies (1.5–3%) reported in previous studies from different populations (Deveault et al., 2011, Katsanis 2004).

4.2. Phenotype

A wide range of phenotypes were observed in our BBS patients. Truncal obesity, a cardinal feature of the syndrome, was observed in all our patients. Truncal obesity rate has been reported to be 72–92% in BBS (Forsythe and Beales 2013). Patient's age may affect obesity rate because truncal obesity has been commonly reported among adults with BBS while general obesity, involving the whole body, is more common during the childhood period (Beales et al., 1999, Forsythe and Beales 2013).

In line with previous studies, retinitis pigmentosa and rod-cone dystrophy were also common in our study group with a frequency of 84%. In a previous study, rod-cone dystrophy has been reported as the most common cardinal features with the frequency of 90% in BBS patients (Forsythe and Beales 2013). Retinal involvement appears to worsen with age in BBS and it is not usually observed in patients under 3 years old. A study by Beales et al. noted the mean age for retinal involvement in BBS patients was reported to be 8.5 years (Beales et al., 1999).

Postaxial polydactyly is another common finding in BBS patients, its frequency ranging from 63% to 81% (Forsythe and Beales 2013). Polydactyly was detected in 76% of our patients. In addition to this, both syndactyly of 2nd and 3rd toes, and brachydactyly were noted in all our patients. The frequencies of these abnormalities have varied from 15 to 100 percent across different studies (Deveault et al., 2011, Moore et al., 2005).

Genital abnormalities are commonly seen in BBS, particularly in male patients (Forsythe and Beales 2013). In total, 69% of our patients had genital abnormalities. All male patients had hypogonadism and micropenis, and one female patient had hydrometrocolpos. Hydrometrocolpos has been described as the most common genital abnormality seen in female BBS patients (Deveault et al., 2011).

Renal involvement and associated complications are the most prominent causes of morbidity and mortality in BBS (O'Dea et al., 1996). Its frequency has been reported to be 53–82% and severity appears to increase with age (Deveault et al., 2011, Imhoff et al., 2011, O'Dea et al., 1996). We found renal abnormalities in 54% of our study group. End stage renal disease is primarily found in older BBS patients but also affects approximately 10% of children with the syndrome (O'Dea et al., 1996). A 13 year-old patient in our study showed end stage renal disease.

Developmental delay and learning disabilities were present in 92% of our study group. Of these, 67% were classified as mild. Frequencies of learning disabilities and developmental delay have been reported at around 60% with the majority being mild (Deveault et al., 2011).

4.3. Genotype-phenotype correlation

BBS1 gene mutations have been reported in association with metabolic complications such as diabetes mellitus, hyperinsulinism, fatty liver, lipidemia and hypercholesterolemia (Deveault et al., 2011) as well as various psychiatric problems. In line with this, all three patients with BBS1 mutations in our study group showed changes in lipid and carbohydrate metabolism, and psychiatric problems (Deveault et al., 2011).

Minor eye abnormalities such as myopia, strabismus and cataract are also occasionally observed in BBS patients. Heon et al.

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Table 2
Summarized clinical features of patients.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13
Age (years)	16	2,5	16	13	20	8	15	13	19	8	19	6	0,6
BMI (kg/m^2)	24,1	22,6	31,6	22,2	27,9	26,4	27,2	37,3	29,5	25,5	33,6	29	29,5
Eye anomalies													
Rod-cone dystophy	+	−	+	+	+	+	+	+	+	+	+	+	−
Other				Myo				Cat		Myo, Str	Nys		
Hearing loss	−	−	−	−	−	−	−	−	−	−	−	+	−
Speech delay/disorder	+	−	+	+	+	+	+	+	+	+	−	+	NE
High arched palate	+	+	+	+	+	+	−	−	+	+	+	+	−
Dental anomalies	GI, Mal	ND	ND	TC,EWA, GI, Mal	TC, GI, Mal	TC,EWA, Mal	ND	ND	TC	Ma	ND	EWA, Mal	NE
Limb abnormalities													
Polydactyly	+	+	+	+	+	−	−	+	+	+	−	+	+
Other	BD, SD	BD, SD	BD, SD	BD, SD	BD, SD	BD, SD	BD, SD	BD, SD	BD, SD	BD, SD	BD, SD	BD, SD	BD, SD
Genital abnormalities													
Hypogonadism/Small genitalia	+	+	+		+		+	+	+			+	
Other	−	Cry	−	−	−	−	−	−	−	HMC	−	−	−
Kidney abnormalities	−	HN	−	RC, HN	RC	−	−	CKD	−	HN	HN, KS	HN	−
Liver abnormalities	−	−	FL, HSM	−	FL, HM	−	−	FL, HM	−	−	FL	HM	−
High transaminases	−	−	+	−	−	+	−	−	−	−	+	+	−
Cardiac abnormalities	AAA	−	−	−	−	−	−	−	−	ASD	−	−	−
Intellectual disability[a]	Mod	Mild	Mod	Mild	Mod	Mild	Mild	Mild	Mild	Mild	Mod	Mild	−
Developmental delay	+	+	+	+	+	+	+	+	+	+	+	+	−
Psychiatric problems	Dep	AB	Dep, Anx	−	Dep, Anx	AB	AB	EL	−	−	EL, Anx	AB	−
Seizure	+	−	−	−	−	Febril	−	−	−	−	−	−	+
Diabetes mellitus	−	−	−	−	+	−	+	−	−	−	−	−	−
Hyperinsulinemia	−	−	+	−	+	+	+	+	−	−	+	−	−
High triglycerides	−	+	−	−	−	−	−	+	−	+	+	+	−
High cholesterol	−	−	+	−	−	−	−	+	−	+	−	+	−
Sleep apnea	+	−	−	+	−	+	−	−	−	+	−	+	−
Asthma	+	−	+	−	−	−	−	−	−	−	+	+	−
BBS gene	BBS10	BBS1	BBS1	BBS10	BBS1	BBS4	BBS10	BBS2	BBS7	BBS2	BBS7	BBS10	BBS9
Nucleotide change	c.931T > G	c.48-3C > G[b]	c.851delA	c.1856_1865del AAAAATGCCA[b]	c.1012C > T[b]	c.406-2A > G[b]	c.1024_1025insA[b]	c.263delG[b]	c.712_715delAGAG	c.263delG[b]	c.949C > G[b]	c.1547delC[b]	c.102_112 + 2del AAATGGACAAGGT[b]
Amino acid change	S311A	Splice site	Y284Sfs*5	K619Ifs*10	Q338X	Splice site	I342Nfs*20	G88Afs*6	R238Efs*59	G88Afs*6	L317V	T516Nfs*8	N35*

AAA: Arcus aorta abnormality, AB: Aggressive behavior, Anx: Anxiety, ASD:Atrial septal defect, BD: Brachydactyly, BMI: Body mass index, Cat: Cataract, CKD: Chronic kidney disease, Cry: Cryptochidism, Dep: Depression, EL: Emotional lability, EWA: Enamel wearing away, FL: Fatty liver, GI: Gum infections, HM: Hepatomegaly, HMC: Hydrometrocolpos, HN: Hydronephrosis, HSM:Hepatosplenomegaly, KS: Kidney stone, Mal: Malocclusion, MO: Morbidly obese, Mod: Moderate, Myo: Myopia, ND: No data, NE: Not evaluate because of age, Nys: Nystagmus, OW: Overweight, RC: Renal cysts, SD: Syndactyly, Str: Strabismus, TC: Teeth crowding, [a]: <6 age: Stanford−Binet Intelligence Scale, >6 age: WISC-R were used to assess intellectual ability, [b]: Novel mutation.

suggested an association between two BBS genes, BBS3 and BBS4, and myopia (Heon et al., 2005). In our study group, four BBS patients had minor eye abnormalities and their mutations were either in BBS2 or BBS10 genes. Daniels et al. suggested that BBS1 mutations could actually lead to milder eye abnormalities in BBS patients (Daniels et al., 2012). In contrast to this, we observed severe eye abnormalities causing blindness in 2 of 3 patients with BBS1 mutations. The patient not having symptomatic eye involvement was 2 years old. All 3 BBS1 mutations observed in our patients were severe mutations (splice site, frameshift and nonsense mutations). Daniels et al. also reported a correlation between milder eye abnormalities and M390R mutation in at least one allele of BBS1 gene (Daniels et al., 2012). We have considered that a correlation between type of mutation and severity of eye abnormality exists but further cases should be evaluated to establish any clear association between eye abnormalities and BBS1 mutations.

A female patient with BBS2 mutation in our group had hydrometrocolpos. The BBS2 gene has previously been described as one of 3 BBS genes causing hydrometrocolpos (Deveault et al., 2011). Our case supports this association.

Deveault et al. found hyperinsulinism in all patients with BBS4 gene mutations. Sleep apnea and high transaminase levels were also observed in 50% of their study group with BBS4 gene mutations (Deveault et al., 2011). Only one patient from our study group had a BBS4 mutation. High transaminase levels and hyperinsulinism were also noted suggesting a possible connection between BBS4 mutations and certain metabolic changes.

Bin et al. described nystagmus in some patients with BBS7 mutations (Bin et al., 2009). One patient in our study group had a BBS7 mutation and also had nystagmus. Due to the number of cases showing this association being very limited in the literature to date, further evaluations are needed before any definitive conclusion can be drawn.

Janssen et al. reported obesity, rod-cone dystrophy and polydactyly in all patient with BBS10 mutations (Janssen et al., 2011). Four of our study group had BBS10 mutations and likewise, we observed obesity and rod-cone dystrophy in all four; three also had polydactyly. Additionally, sleep apnea was also detected in 3 of these 4 patients, supporting the report by Deveault et al.

Metabolic abnormalities associated with BBS10 mutations were found to be less frequent in our study group (50%) when compared to the findings of previous studies. (Deveault et al., 2011). Respectively younger ages and/or different mutations in our study group presents as a likely explanation for this.

Gastroesophageal reflux disease (GERD) and Hirschsprung disease have been reported sporadically in patients with the syndrome but to date no BBS gene has been linked specifically with these findings (Beales et al., 1999, de Pontual et al., 2009, Moore et al., 2005). One of our patients with a BBS10 mutation had both GERD and Hirschsprung disease.

In conclusion, molecular diagnosis could be established in 86.6% of BBS patients using a NGS panel which included 17 known BBS genes in this study. This shows NGS is a feasible and effective method for molecular analysis of this type of disorder. Although there have been a number of associations between certain genes and phenotypic features, additional studies are needed to better characterize the genotype–phenotype correlation of BBS patients.

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