Sequence variants in four genes underlying Bardet-Biedl syndrome in consanguineous families

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Purpose: To investigate the molecular basis of Bardet-Biedl syndrome (BBS) in five consanguineous families of Pakistani origin.

Methods: Linkage in two families (A and B) was established to BBS7 on chromosome 4q27, in family C to BBS8 on chromosome 14q32.1, and in family D to BBS10 on chromosome 12q21.2. Family E was investigated directly with exome sequence analysis.

Results: Sanger sequencing revealed two novel mutations and three previously reported mutations in the BBS genes. These mutations include two deletions (c.580_582delGCA, c.1592_1597delTTCCAG) in the BBS7 gene, a missense mutation (p.Gln449His) in the BBS8 gene, a frameshift mutation (c.271_272insT) in the BBS10 gene, and a nonsense mutation (p.Ser40*) in the MKKS (BBS6) gene.

Conclusions: Two novel mutations and three previously reported variants, identified in the present study, further extend the body of evidence implicating BBS6, BBS7, BBS8, and BBS10 in causing BBS.

Bardet-Biedl syndrome (BBS; OMIM 209900) is a rare genetically heterogeneous developmental disorder with primary features of retinitis pigmentosa, postaxial polydactyly, obesity, renal dysfunctions, hypogonadism, and intellectual disability. Additional features reported in cases of BBS include strabismus, nystagmus, brachydactyly, syndactyly, truncal obesity, hydrenephrosis, pyelonephritis, cryptorchidism, small penis/hypospadias, delay in reaching puberty, hypoplasic fallopian tubes, poor articulation, anxiety, depression, obsessive-compulsive disorder, autism spectrum disorder or psychosis, hypertension, anosmia, dental anomalies (micrognathia, malocclusion, and microdontia), ataxia, and Hirschsprung disease. Variation in the phenotypes within and among different families has been reported [4,5,7]. Facial similarities reported among patients with BBS include deep-set eyes, hypertelorism with downward slanting palpebral fissures, a flat nasal bridge with antverted nares and prominent nasolabial folds, a long philtrum, a thin upper lip, and apathetic facial appearance [6,8].

BBS belongs to the group of ciliopathies that share partial-overlapping phenotypes and common genes [9]. Genetic studies revealed different variations within the genotype and phenotype of the disease [10]. To date, 21 BBS genes (BBS1–21) have been identified, which includes BBS1 located on chromosome 11q13 [11], BBS2 on chromosome 16q21 [12], BBS3 on chromosome 3p13-p12 [13], BBS4 on chromosome 15q22.3-q23 [11,14], BBS5 on chromosome 2q31 [15], BBS6 on chromosome 20p12 [16], BBS7 on chromosome 4q27 [17], BBS8 on chromosome 14q32.11 [18], BBS9 on chromosome 7p14 [19], BBS10 on chromosome 12q21.2 [20], BBS11 (TRIM32) on chromosome 9q33.1 [21], BBS12 on chromosome 4q27 [22], BBS13 (MKSI) on chromosome 17q23 [23], BBS14 (CEP290) on chromosome 12q21.3 [23], BBS15 (C2ORF86) on chromosome 21p15 [24], BBS16 on chromosome 1q43 [25], BBS17 (LZTFL1) on chromosome 3p21.31 [26], BBS18 (BBIP1) on chromosome 1q25q2 [27], BBS19 (IFT27) on chromosome 22q12 [28], BBS20 (IFT172) on chromosome 2p23.3 [29], and BBS21 (C8ORF37) on chromosome 8q22.1 [30]. We report two novel mutations and three previously reported variants in the BBS genes in five consanguineous families of Pakistani origin segregating BBS in an autosomal recessive pattern.
**METHODS**

**Ethical approval:** Permission to conduct the present research work was obtained from the Institutional Review Board (IRB) of Quaid-i-Azam University, Islamabad, Pakistan and Technical University Munich, Germany. This study adhered to the ARVO statement on human subjects, all those who participated in the study signed informed written consent forms and the research followed the tenets of the Declaration of Helsinki.

**Family history and blood collection:** In total, five consanguineous families (A–E) segregating Bardet-Biedl syndrome in an autosomal recessive pattern of inheritance were investigated in the present study. Families A and C live in a remote area of the Khyber Pakhtunkhwa (KPK) province of Pakistan. Two other families (B and D) originate from a remote village in Azad Jammu and Kashmir, bordering India and Pakistan. Family E was recruited from a remote area of Nawab Shah City, Sindh province of Pakistan. Information provided by the family elders was used for the construction of the pedigrees (Figure 1, Figure 2A). All five pedigrees convincingly support the autosomal recessive inheritance pattern of the disease.

Affected members in the families underwnt clinical examinations at local government hospitals. Venous blood samples from 38 members were collected in vacutainers (BD Biosciences, Franklin Lakes, NJ) containing EDTA.

**Genomic DNA extraction:** Genomic DNA was extracted from the collected blood samples using the Nucleospin® Blood kit (Macherey-Nagel, Germany). A NanoDrop-1000 Spectrophotometer (Thermal Scientific, Wilmington, NJ) was used for DNA quantification, measuring optical density at 260 nm and diluted to 40–50 ng/μl for amplification with PCR. PCR was performed in 25 μl reaction volume containing 40 ng genomic DNA, 20 pmol of each primer, 200 mM of each deoxynucleoside triphosphate (dNTP), 2.5 μl reaction buffer (KCl 50 mM, Tris–HCl pH 8.3, and MgCl₂ 1.5 mM), and 1 U Taq DNA polymerase (MBI Fermentas, Life Sciences, York, UK). PCR was performed using the GeneAmp® PCR System 9700 obtained from Applied Biosystems (Applied Biosystems Inc.) according to the manufacturer’s instructions. To identify sequence variants, the BioEdit sequence alignment editor version 6.0.7 was used. PCR primers were designed using the Primer3 program [32] and checked for specificity using BLAST.

**Microsatellite markers:** To identify the causative genes, homozygosity mapping followed by Sanger sequencing was performed in four families (A–D). In family E, exome sequencing was used to search for the causative gene. Linkage in the four families (A–D) was tested using several microsatellite markers linked to 21 candidate genes (BBS1–BBS21) known for causing Bardet-Biedl Syndrome. After linkage was established in the families, the candidate regions were further saturated by typing additional markers linked to three genes (BBS7, BBS8, and BBS10).

In family E, DNA from a proband (IV-3) was subjected to whole exome sampling (WES; Figure 2A). Exome enrichment was accomplished using the SureSelect XT Human All Exon 50 Mb kit, version 5 (Agilent Technologies, Santa Clara, CA), and sequencing was performed on HiSeq 2500 systems (Illumina, San Diego, CA). All thereads were aligned against the human assembly hg19 (GRCh37), using Burrows-Wheeler Aligner (BWA v 0.7.5). Subsequently, the Exome Depth (v1.0.0), PINDEL (v 0.2.40), and SAM tools (v 0.1.18) were used for variant calling. Filtering of the variants was performed with the help of the SAM tools varFilter script and custom scripts. All the variants obtained after filtering were inserted into an in-house database for further analysis.

**Screening the BBS7, BBS8, and BBS10 genes:** After linkage was established in the four families (A–D), three genes (BBS7, BBS8, and BBS10) were Sanger sequenced. In family E, WES identified a pathogenic homozygous variant in the MKKS gene. All exons and flanking intrinsic sequences of the gene were PCR amplified using gene-specific primers. The primer sequences used to screen the BBS7 and BBS8 genes are listed in Table 1, while those used for BBS10 were the same as reported previously [31]. The PCR products were purified using the Rapid PCR Purification System 9700 (Axygen, Union City, CA) and sequenced following the dideoxy chain termination method using the BigDye Terminator v3.1 Cycle Sequencing Kit and the Applied Biosystems 310 DNA Analyzer (Applied Biosystems Inc.) according to the manufacturer’s instructions. To identify sequence variants, the BioEdit sequence alignment editor version 6.0.7 was used. PCR primers were designed using the Primer3 program () [32] and checked for specificity using BLAST.

**RESULTS**

**Clinical features:** Affected members, investigated in the five families (A–E), were 10–38 years of age at the time of the study (Figure 2B–I, Figure 3; Table 2).

**Family A:** All three affected members presented clinical features of obesity, retinitis pigmentosa, postaxial polydactyly (hexadactyly), mental delay, hypertension, and cutaneous syndactyly. Radiographs of the affected individual (IV-3) revealed normal carpal, metacarpal, and phalanges while the feet radiographs revealed postaxial polydactyly (PAP) type A, with an extra toe originating from a two-headed metatarsal. Tarsals and metatarsals were hypoplastic and...
Figure 1. Pedigrees and Sanger sequencing results for four families segregating BBS in an autosomal recessive pattern. A: Pedigree of family A. B: Sequence analysis of the BBS7 gene showing a 3 bp deletion at nucleotide position 580–582 (c.580_582delGCA). C: Pedigree of family B. D: Sequence chromatograms of 6 bp deleted variant (c.1592_1597delTTCCAG) in the BBS7 gene. E: Pedigree of family C. F: Sequence analysis of the variant (c.1347G>C) identified in the gene BBS8 in family C. G: Pedigree of family D. H: Sequence chromatogram of the frameshift mutation (c.271_272insT) found in the BBS10 gene in family D. The genotype of individuals for the mutation identified in the respective family, verified with segregation analysis, is written below each member tested. The upper panel shows the nucleotide sequence in the homozygous affected member, the middle panel in the heterozygous carrier, and the lower panel in the homozygous normal member in each sequence chromatogram.
distorted (Figure 3P,Q). Carpals were hypoplastic in the second affected member (IV-4) of the family (Figure 3R,S).

Fundus examination in two affected individuals (IV-3 and IV-4) showed typical features of sine pigmentosa, a variant of retinitis pigmentosa that have characteristic features, including atrophy of the RPE and vessel (arteriolar) attenuation. The vessels were thinner than normal, and the optic nerve had pallor. Features such as microaneurysms, edema, exudates, neovascularization, and hypo- and hyper-pigmentation of macula were not observed in either affected individual (Figure 3W,X).

**Family B:** Affected individuals showed mental delay, hypotension, retinitis pigmentosa, reproductive tract/organ anomalies, obesity, postaxial polydactyly, and cutaneous syndactyly.

**Family C:** Affected members displayed weak analytical ability, vision impairment, obesity, hypogonadism, and postaxial polydactyly. Radiographs of both hands of affected individual (IV-6) displayed complete PAP type A. An extra digit, originated from the fifth metacarpal, was present in the left hand. In the right hand, the extra digit had no connection with the fifth metacarpal. Both extra digits had fix flexion deformity. Feet radiographs of the affected individuals (IV-1 and IV-6) showed PAP type A, and an extra toe originated from the fifth metatarsal (Figure 3T,U).

**Family D:** Both affected individuals (V-1 and V-2) showed clinical features of cognitive impairment, obesity, vision impairment, polysyndactylism, and hypogonadism. Camp-todactyly was also observed in the right hand of affected

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**Figure 2. Pedigree drawing of family E showing autosomal recessive inheritance.**

- **A:** The red arrow indicates the affected individual for whom whole exome sequencing (WES) was performed.
- **B:** Affected individual IV-3 has typical features of BBS syndrome, including hypertelorism, deep-set eyes, a flat nasal bridge, a small mouth, retrognathia, malar hypoplasia, and curly hair.
- **C, D:** Dorsal and palmar view of hands that have postaxial polydactyly only in the left hand.
- **E:** Feet of affected individual IV-3 who has obesity and bilateral post axial polydactyly.
- **F:** Typical BBS facial features shown in affected individual IV-4: flat nasal bridge, poor eyesight, intellectual disability, and a small mouth.
- **G, H:** Dorsal and palmar view of the hands (IV-4) and postaxial polydactyly in the right hand.
- **I:** Feet of affected individual IV-4, showing obesity but no polydactyly.
- **J:** The gene structure of the MKKS gene. The arrow shows the mutation (c.119C>G) identified in exon 1 of the MKKS gene in the present study.
- **K:** Schematic representation of the MKKS protein domains (equatorial, intermediate, and apical domain); the red arrow shows the identified mutation (p.Ser40*) within the MKKS equatorial domain. Intrinsic regions are not drawn to scale.
- **L:** The upper panel shows the nucleotide sequence in the homozygous affected individual.
- **M:** The middle panel shows the nucleotide sequence in the heterozygous carrier.
- **N:** The lower panel shows the nucleotide sequence in the homozygous normal individual in each sequence chromatogram.
- **O:** Partial amino acid sequence comparison of the human MKKS protein with other orthologs showing serine residue in green conserved across different species.
| Gene | Exon | F/R | Sequence (3’) |
|------|------|-----|--------------|
| BBS8 | 1    | F   | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 2a   | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 2    | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 3    | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 4    | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 5    | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 6    | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 7    | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 8    | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 9    | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 10   | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 11   | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 12   | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 13   | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| 14   | F    |     | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |
| BBS7 | 1    | F   | CAGCTCTTCACCTCCACGC |
|      | R    |     | CAGCTCTTCACCTCCACGC |

**Table 1. Primer sequence used for amplification of different BBS genes.**
| Gene | Exon | F/R | Sequence (-3') |
|------|------|-----|----------------|
| MKKS | 1    | R   | TCCACTGGGATTCGACAACC |
Figure 3. Clinical features of affected members in four families.  

A, B: Affected individual IV-3 in family A, showing a thin upper lip, obesity, cutaneous syndactyly, and polydactyly in the left foot.  
C–E: Affected individual IV-4 in family A showing truncal obesity, postaxial polydactyly (PAP) in the hands, and polysyndactylyism in the feet.  
F, G: Clinical features of affected individual IV-7 in family B showing obesity and postaxial polydactyly in his feet.  

H, I: Affected individual (V-1) in family B showing postaxial polydactyly in his hands and feet.  

J: Affected individual (IV-1) in family C showing clinical features of obesity, prominent nasolabial folds, a long philtrum, a thin upper lip, postaxial polydactyly, and curving of the pinky toward the ring finger (clinodactyly) in his hands.  

K: Postaxial polydactyly in both hands and clinodactyly in the right hand in affected individual (IV-2) in family C.  

L: Postaxial polydactyly in the left foot in the affected individual (IV-6) of family C.  

M–O: Affected individuals (V-1 and V-2) of family D with clinical features of obesity and anteverted nares, postaxial polydactyly in the right hand of affected individual V-1, and postaxial polydactyly and camptodactyly in the right hand of affected individual V-2.  

P, Q: Hands and feet radiographs of affected individual IV-3 of family A, who has PAP only in both feet and normal hands.  

R, S: Hands and feet radiographs of affected individual IV-4 of family A, who has a skin tag on his right hand and PAP type A in both feet.  

T, U: Hands and feet radiographs of affected individual IV-6 of family C, who has PAP type A.  

V: Fundus photographs of affected individuals IV-3 and IV-4 representing sine pigmento (retinitis pigmentosa).  

W, X: Hand radiographs of affected individual IV-1 who has PAP type A.
**Table 2. Clinical manifestations observed in affected members in family A-E.**

| Family | Patient | Sex | Age (Years) | RP | Polydactyly (PAP) | Obesity | Hypogonadism | CI | RI | Syndactyly | Clinodactyly |
|--------|---------|-----|-------------|----|------------------|---------|--------------|----|----|------------|-------------|
| A      | IV-3    | M   | 21          | +  | Right feet       | +       | +            | +  | _  | Right foot, 2-3 toes | _            |
| A      | IV-4    | M   | 15          | +  | Both hands and feet | +       | +            | +  | _  | Both feet, 5-6 toes | _            |
| A      | IV-5    | M   | 12          | +  | Both hands       | +       | +            | +  | _  | Both feet, 2-3 toes | _            |
| B      | IV-2    | M   | 17          | +  | Both feet        | _       | +            | _  | _  | _          | _            |
| B      | IV-4    | M   | 16          | +  | Both hands       | +       | +            | _  | _  | _          | _            |
| B      | IV-7    | M   | 10          | +  | Both feet        | +       | +            | _  | _  | _          | _            |
| B      | V-1     | M   | 6           | ?  | Both hands and feet | +       | ND           | +  | _  | _          | _            |
| C      | IV-1    | M   | 13          | +  | Both hands and feet | +       | +            | _  | _  | _          | +            |
| C      | IV-2    | M   | 12          | +  | Both hands and left foot | +       | +            | ND | _  | _          | +            |
| C      | IV-6    | F   | 10          | +  | Left foot       | +       | ND           | +  | _  | _          | _            |
| D      | V-1     | M   | 15          | +  | Right hand      | +       | +            | _  | _  | _          | _            |
| D      | V-2     | M   | 14          | +  | Both hands      | +       | +            | +  | _  | _          | _            |
| E      | III-7   | F   | 38          | +  | Both hands      | +       | _            | +  | _  | _          | _            |
| E      | IV-3    | M   | 17          | +  | Left hand       | +       | +            | +  | _  | _          | _            |
| E      | IV-4    | M   | 15          | +  | Right hand only | +       | +            | +  | _  | _          | _            |

+=presence of feature, _=Absence, RP=Retinitis Pigmentosa, PAP=Postaxial Polydactyly, ND=No data available, CI=Cognitive Impairment, RI=Renal Impairment,
individual V-2. Clinical reports of an affected individual (V-2) suggested the right kidney is either missing or severely hypoplastic, and the affected individual had a small penis with cryptorchidism. Presence of cognitive impairment was assessed through interviews with parents of affected members. In affected members of all four families (A–D), retinitis pigmentosa debuted with night blindness started at the age of 5 years with progressive decreased visual acuity at the age of 7–10 years.

**Family E:** Clinical evaluation of all three affected individuals in family E demonstrated common BBS phenotypes, such as obesity, learning disability, speech difficulties, mild hearing problems, and slight mental retardation. PAP was observed in the left hand and feet of affected individual IV-3 (Figure 2C–E) and the right hand of affected individual IV-4 (Figure 2G,H). An extra digit was surgically removed from affected individual III-7.

**Linkage, WES, and Sanger sequencing:** Selected microsatellite markers, mapped on chromosomal regions harboring genes for BBS1–BBS21, were used to search genetic linkage based upon the homozygosity mapping technique in four families (A–D). Haplotype analysis showed linkage in two families (A and B) to chromosome 4q27 harboring **BBS7**, in family C to chromosome 14q32 harboring **BBS8**, and in family D to chromosome 12q21.2 harboring **BBS10**. All affected members were heterozygous with microsatellite markers linked to other BBS genes.

In family E, direct WES was performed using DNA from an affected individual (IV-3) at the Institute of Human Genetics, Helmholtz Zentrum Munchen, Germany [33]. All the variants were filtered and validated according to minor allele frequency (MAF) >0.001 in the Single Nucleotide Polymorphism database (dbSNP) and in the 1000 Genome Project, Exome Variant Server (EVS), and Exome Aggregation Consortium (ExAC), 7,000 in-house exome database (Appendix 1).

Sanger sequencing of the **BBS7** gene identified a novel 3 bp deletion (c.580_582delGCA) in family A (Figure 1B) and a previously reported 6 bp in-frame deletion (c.1592_1597delTTCCAG) in family B (Figure 1D). In family C, sequence analysis of the **BBS8** gene revealed a homozygous G to C transition at nucleotide position 1347(c.1347G>C) resulting in a missense variant (p.Gln449His) in all affected individuals (Figure 1F). In family D, sequence analysis of the **BBS10** gene revealed a homozygous frameshift mutation (c.271_272insT; Figure 1H). In family E, a novel nonsense mutation (c.119C>G, p.Ser40*) was detected in the **MKKS** gene (Figure 2L). Unaffected members in the respective families were either heterozygous for a mutant allele or had a wild-type allele. Sanger sequencing validated cosegregation of the variants with the disease phenotype in all five families (A–E). The frequency of the identified variants in the in-house 7,000 exomes (IHG; Germany) and ExAc is presented in Appendix 2. The nonpathogenic nature of the four variants (c.580_582delGCA and c.1592_1597delTTCCAG in the **BBS7** gene, c.1395G>C in the **BBS8** gene, c.271_272insT in the **BBS10** gene, and c.119C>G in the **MKKS** gene) were excluded in 175 ethnically matched control individuals.

**DISCUSSION**

Bardet-Biedl syndrome is a clinically pleiotropic disorder segregating in an autosomal recessive pattern [34]. However, in rare cases, the triallelic nature of BBS involving three mutated alleles in two genes have been reported as well [35]. In the study presented here, we identified disease-causing alleles in five consanguineous families (A–E) of Pakistani origin. The disease was inherited in an autosomal recessive pattern. Clinical features, observed in the five families, were similar to those reported previously [31]. Linkage and WES analysis followed by Sanger sequencing revealed five variants, including two novel mutations in **BBS6** (**MKKS**) and **BBS7** and three previously reported mutations in the **BBS7**, **BBS8**, and **BBS10** genes.

The **BBS7** gene spans a 60.06 kb genomic region on chromosome 4q27. It is composed of 19 exons encoding 715 amino acids for the **BBS7** protein. The **BBS7** protein shares structural features with the **BBS1** and **BBS2** proteins [17]. A group of seven BBS proteins (**BBS1**, **BBS2**, **BBS4**, **BBS5**, **BBS7**, **BBS8**, and **BBS9**) form a complex called the BBSome that promotes ciliogenesis. The cilia are microtubule-based structures that play important roles in the development of several tissues. Two of these proteins, **BBS7** and **BBS8**, although structurally different, function as intraflagellar transport (IFT) cohesion factors [36]. Three other proteins (**BBS6**, **BBS10**, and **BBS12**) function as chaperonins and arbitrate assembly of BBSome. Any sequence variant causing disruption in the seven genes results in defective ciliogenesis.

To date, 31 mutations, including 16 missense/nonsense, four splice sites, eight deletions, one small indel (insertion/deletion), and one complex rearrangement, have been reported in the **BBS7** gene [HGMD] [37]. The present study reported a ninth novel deletion mutation (c.580_582delGCA, p.Ala194del) in the **BBS7** gene. The 3 bp deletion removed an evolutionarily conserved alanine codon 194, which most likely altered the secondary structure of the **BBS7** protein. The pathogenic nature of the variant was validated using multiple online bioinformatics tools. The second deletion variant (c.1592_1597delTTCCAG, p.Val531_Pro532del),
identified in family B, was previously reported in a family of Iranian origin [37].

The **BBS8** gene is one of the 21 genes identified for Bardet-Biedl syndrome. This gene encodes the 515 amino acid BBS8 protein that interacts with PCM1 in ciliary biogenesis [18]. Thus far, 15 mutations (including four missense/nonsense, five splice sites, five small deletions, and one small insertion) have been reported in the **BBS8** gene (HGMD) [37]. Riazuddin et al. found a splice-site mutation (c.115–2A>G) in a retina-specific exon of **BBS8** that causes non-syndromic retinitis pigmentosa in a consanguineous family of Pakistani origin [38]. Goyal et al. reported another **BBS8** variant (p.Gln449His) that causes non-syndromic retinitis pigmentosa in a consanguineous family of Indian origin. The same variant (p.Gln449His) was detected in family C in the present study [39]. However, in addition to retinitis pigmentosa, the affected members in family C showed all the primary features and phenotypic abnormalities of BBS. Among the 15 mutations reported in the **BBS8** gene thus far, only two were found to cause non-syndromic retinitis pigmentosa while the other 13 cause BBS. It is possible that the allelic mutation or familial background of the families plays a pivotal role in causing different phenotypes.

**BBS10** with two exons, encoding a 723 amino acid protein, mapped to chromosome 12q21.2 [20]. Stoetzel et al. reported the most common mutation (271dupT, C91fsX95) in the **BBS10** gene in several families with Bardet-Biedl syndrome. The same mutation was detected in family D in the present study [20]. In a study involving five fetuses and a child, one fetus was found to be carrying homozygous 271dupT, 3 compound heterozygous with another mutation in the **BBS10**, and a fifth with homozygous 271dupT in addition to a truncating variant in the **BBS6** gene [40]. To date, 88 mutations, including 54 missense/nonsense, one splice site, 25 deletions, seven insertions, and one indel mutation, have been found in the **BBS10** gene (HGMD) [37], which account for 20% of BBS cases.

**MKKS/BBS6**, mapped on chromosome 20p12, encodes a 570 amino acid protein. **MKKS** has been reported to cause the phenotypically overlapping McKusick-Kaufmann syndrome. Mutations in critical ciliary-regulating proteins result in an increase in rhodopsin in the inner segment and cause eventual photoreceptor cell death [41]. This results in phenotypes that include tunnel vision, peripheral vision loss, and blindness. The BBS chaperonin complex is formed at the base of the primary cilia in the photoreceptor cell and comprises the three BBS proteins (MKKS/BBS6, BBS10, and BBS12) that form the BBSome complex [42,43]. Mice that lack Bbs6 and humans who have mutations in the **MKKS/BBS6**, **BBS10**, or **BBS12** phenotypically resemble defects caused by BBSome genes [44]. However, mutations in the **MKKS/BBS6** gene give rise to variable phenotypes, yet similar to other BBS syndromes [45]. The mutation (p.Ser40*) identified in family E resides within the predicted equatorial domain (Figure 2K) and most likely results in loss of function of the MKKS protein either through nonsense-mediated mRNA decay (NMD) or resulting in the production of a truncated MKSS protein. To date, 52 mutations have been identified in the **MKKS** gene, including 43 nonsense/missense, one splice site, six deletions, one insertion, and one indel (HGMD) [37].

**BBS** genes show expression in ciliated cells. Proteins, encoded by these genes, are divided into two groups, including BBSome, a complex formed by the assembly of BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, and BBS9, involved in promoting ciliogenesis by recruiting Rab8GTP to enter the cilium and type II chaperonin-like proteins, a complex formed by BBS6, BBS10, and BBS12, which plays a role in the regulation of BBSome assembly [46]. In conclusion, we have identified mutations in four **BBS** genes that cause BBS phenotypes. This study will support genetic testing of patients with BBS in Pakistan.

APPENDIX 1. FILTERING STEPS FOLLOWED TO SEARCH FOR THE CANDIDATE VARIANT IN AFFECTED INDIVIDUAL OF FAMILY E (IV-3).

To access the data, click or select the words “Appendix 1.”

APPENDIX 2. FREQUENCY OF THE IDENTIFIED VARIANTS IDENTIFIES IN FIVE FAMILIES (A-E) IN BOTH IN-HOUSE EXOMS AND EXAC.

To access the data, click or select the words “Appendix 2.”

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