

Molecular Analysis of Bardet-Biedl Syndrome Families: Report of 21 Novel Mutations in 10 Genes

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PURPOSE. Bardet-Biedl syndrome (BBS) is genetically heterogeneous with 15 *BBS* genes currently identified, accounting for approximately 70% of cases. The aim of our study was to define further the spectrum of *BBS* mutations in a cohort of 44 European-derived American, 8 Tunisian, 1 Arabic, and 2 Pakistani families (55 families in total) with BBS.

METHODS. A total of 142 exons of the first 12 *BBS*-causing genes were screened by dideoxy sequencing. Cases in which no mutations were found were then screened for *BBS13*, *BBS14*, *BBS15*, *RPGRIP1L*, *CC2D2A*, *NPHP3*, *TMEM67*, and *INPP5E*.

RESULTS. Forty-three mutations, including 8 frameshift mutations, 10 nonsense mutations, 4 splice site mutations, 1 deletion, and 20 potentially or probably pathogenic missense variations, were identified in 46 of the 55 families studied (84%). Of these, 21 (2 frameshift mutations, 4 nonsense mutations, 4 splice site mutations, 1 deletion, and 10 missense variations) were novel. The molecular genetic findings raised the possibility of triallelic inheritance in 7 Caucasian families, 1 Arabian family, and 1 Tunisian patient. No mutations were detected for *BBS4*, *BBS11*, *BBS13*, *BBS14*, *BBS15*, *RPGRIP1L*, *CC2D2A*, *NPHP3*, *TMEM67*, or *INPP5E*.

CONCLUSIONS. This mutational analysis extends the spectrum of known BBS mutations. Identification of 21 novel mutations highlights the genetic heterogeneity of this disorder. Differences in European and Tunisian patients, including the high frequency of the M390R mutation in Europeans, emphasize the population specificity of BBS mutations with potential diagnostic implications. The existence of some BBS cases without

mutations in any currently identified *BBS* genes suggests further genetic heterogeneity. (*Invest Ophthalmol Vis Sci.* 2011; 52:5317-5324) DOI:10.1167/iov.11-7554

Bardet-Biedl syndrome (BBS; MIM 209900) is a genetically heterogeneous autosomal recessive disorder characterized by progressive retinal degeneration, truncal obesity, cognitive impairment, polydactyly, hypogonadism, and renal anomalies.¹⁻⁶ Among the nonconsanguineous populations of Northern Europe and America, the prevalence ranges from 1 in 100,000 in North America⁷ to 1 in 160,000 in Switzerland.⁸ BBS incidence increases within populations of high consanguinity or those that are geographically isolated.^{4,9,10} To date, 15 genes (*BBS112*, Meckel syndrome 1 [*MKS1*, *BBS13*], centrosomal protein 290 kDa/nephronophthisis 6 [*CEP290/NPHP6*, *BBS14*], and *C2ORF86* [*BBS15*]) have been implicated in BBS.^{11,12} Mutations in these genes account only for approximately 70% of BBS patients, suggesting that there are additional genes to be found for BBS.¹³ *BBS1* and *BBS10* are major pathogenic genes in European populations, each accounting for at least 20% of cases in most series.^{14,15} However, most other genes are rare contributors. For example, there is only a single documented family with mutations in each of *BBS11* (*TRIM32*), *BBS13* (*MKS1*), *BBS14* (*CEP290*), and *BBS15* (*C2ORF86*).^{12,16,17} A number of recent publications have expanded the spectrum of known BBS mutations,¹⁸⁻²¹ and suggested that mutations in some genes, including *BBS13* and *TMEM67/MKS3*, can modify the expression of BBS phenotypes in patients who have mutations in other genes.

BBS is also characterized by profound interfamilial and intrafamilial clinical variability, which is possibly explained in part by the presence of second-site modifiers. In some families, three mutated alleles in two *BBS* genes have been implicated (triallelic inheritance). *BBS1*, *BBS2*, *BBS3*, *BBS4*, *BBS5*, *BBS6*, *BBS7*, *BBS8*, and *BBS10* have been implicated in triallelic inheritance of BBS,²²⁻²⁵ but the specific contribution of each allele is difficult to ascertain. Moreover, it has been suggested that there might be a connection between triallelism and the phenotypic severity of the disease.²⁶ However, some studies have not identified a complex inheritance mode in this syndrome.^{27,28}

In this study, we analyzed 55 families for known *BBS* genes (*BBS1-BBS15*) and identified 43 mutations in 46 families, 21 of which are novel. We also identified 7 Caucasian, 1 Arabic, and 1 Tunisian family in whom the molecular genetic findings raised the possibility of triallelic inheritance involving *BBS1*, *BBS2*, *BBS3*, *BBS6*, *BBS7*, *BBS8*, *BBS9*, *BBS10*, or *BBS12*. Although some sequence variations were identified in additional genes, no homozygous potential mutations were identified in previously unidentified *BBS* genes.

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MATERIALS AND METHODS

Appropriate informed consent was obtained from all participants in the study, in accord with the tenets of the Declaration of Helsinki. This study was approved by the Massachusetts Eye and Ear Infirmary and Harvard Medical School Institutional Review Boards, the ethics review board of Charles Nicolle Hospital, Tunis, and the CNS Institutional Review Board at the National Institutes of Health.

Study Subjects

We studied 70 persons with Bardet-Biedl syndrome (BBS) from 55 unrelated families including 8 recruited in Tunisia, 2 recruited in Pakistan, and 44 of European and 1 of Arabian descent recruited in the United States. In this study, the 8 BBS families recruited from Tunisia did not overlap with participants from a previous study.²⁸ Among the 55 families, 44 were sporadic cases and the remaining had at least 2 affected members. BBS was diagnosed on the basis of the established criterion that 4 primary features or 3 primary plus 2 secondary features are necessary to make the diagnosis.²⁹ The control group consisted of 96 persons of European origin, 96 persons of Tunisian origin, and 96 persons of Pakistani origin.

Mutation Screening

We carried out a mutation screen of the first 12 identified *BBS* (*BBS1-BBS12*) genes in 55 families (for a total of 70 patients) collected from the United States, Tunisia, and Pakistan. For *BBS1* to *BBS12*, all genes were screened in all cases. Cases in which no mutations were found in *BBS1* to *BBS12* were then screened for mutations in *BBS13* (*MKS1*), *BBS14* (*CEP290*, *NPHP6*), and *BBS15* (*C2ORF86*) as well as in *RPGRIP1L*, *CC2D2A*, *NPHP3*, *TMEM67*, and *INPP5E* because they have been implicated in other ciliopathies. Genomic DNA was isolated from blood leukocytes using standard protocols.³⁰ Coding regions and exon-intron boundaries of the 20 genes were amplified by PCR using standard methods. Amplifications were carried out as previously described,²⁸ and the PCR products were analyzed on 2% agarose gels and purified by vacuum filtration manifold plate (Millipore, Billerica, MA). The PCR primers for each exon were used for bidirectional sequencing using a reaction mix (BigDye Terminator Ready; Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Sequencing was performed on a gene analyzer (ABI PRISM 3130xl Genetic Analyzer; Applied Biosystems), and sequence traces were analyzed (MutationSurveyor [Soft Genetics Inc., State College, PA] and the SeqMan program of DNASTAR Software [DNASTAR Inc., Madison, WI]).

Criteria to Determine the Pathogenicity of New *BBS* Mutations

A mutation was considered novel if it was not present in the Human Mutation Database (<http://www.hgmd.cf.ac.uk/ac>) or the National Center for Biotechnology Information dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/index.html>) and not published. A sequence variation was considered pathogenic when it segregated with the disease in the family and, when parental origin could be determined, double heterozygous mutations acted in trans; it was not present in 96 randomly selected controls from the ethnically matched population; it altered a well-conserved amino acid, preferably in a conserved region (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>); and it was judged significant in a computational test for novel mutations. The neural network splice site scoring program (http://www.fruitfly.org/seq_tools/splice.html) was used to evaluate the effect of mutations affecting splice sites. Splice site scores were calculated with Splice site Score Calculation software (http://rulai.cshl.edu/new_alt_exon_db2/HTML/score.html). PolyPhen analysis (<http://genetics.bwh.harvard.edu/pph/>) was used to predict whether missense variations could impact the protein structure and function. The prediction is based on the position-specific independent counts score derived from a combination of available structural information and multiple

sequence alignments of observations. PolyPhen scores >2.0 indicate the variant is probably damaging to protein function; scores from 1.5 to 2.0 indicate the variant is possibly damaging; and scores <1.5 indicate the variant is Likely benign. In the present study, both "probably damaging" and "possibly damaging" changes were classified as suspected pathogenic mutations.

RESULTS

General Overview of *BBS* Mutations

Mutations were detected in 46 (of 55; 84%) families, and no mutations were detected for the remaining 9 (of 55; 16%) families. We identified 43 different mutations in the 46 families, including 39 families from the United States, including 1 of Saudi Arabian derivation, 5 from Tunisia, and 2 from Pakistan. Of the 43 different mutations, 21 were novel (Fig. 1, Table 1^{13-15,24-26,28,31-34}), confirming the genetic heterogeneity of BBS. Seven Caucasian, 1 Arabian, and 1 Tunisian family (20% of families with mutations detected) carry 3 mutations in 2 different genes, raising the possibility of triallelic inheritance (Table 1). Thirty-seven families (83% of patients with mutations detected) carry only 2 mutations in the same gene (Table 1): 15 for *BBS1* (13 homozygotes and 2 compound heterozygotes), 4 for *BBS2* (3 homozygotes and 1 compound heterozygote), 2 homozygotes for *BBS3*, 2 homozygotes for *BBS5*, 3 homozygotes for *BBS7*, 11 for *BBS10* (5 homozygotes and 6 compound heterozygotes), and 1 compound heterozygote for *BBS12* (*C4ORF24*). None of the novel mutations or third-allele mutations was observed in 192 ethnically matched control chromosomes, and none is recorded in the dbSNP database.

BBS1 Mutations

BBS1 mutations were present in 19 of the 46 families with identified mutations and represented the primary pathogenic locus in 18 families (33%) and a possible third mutant allele in 1 family (Fig. 1, Table 1). In 15 families (13 homozygotes and 2 compound heterozygotes), only 2 mutations were identified in the *BBS1* gene (Table 1). Eighteen affected members of 12 Caucasian families and 1 Tunisian family were homozygous for the M390R allele, predicted to be pathogenic with a PolyPhen score of 2.7. The proband of family D597 is a compound heterozygote for M390R and c.518+1G>A, which was not found in controls and showed calculated splice site scores of 11.0 for the normal splice site and 0.2 for the variant site, suggesting that it affects splicing. The proband of family 7348 was a compound heterozygote for the M390R and E549X alleles. All affected members carrying the M390R mutation shared a common SNP haplotype across the *BBS1* gene (Supplementary Table S1, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7554/-/DCSupplemental>).

Possible triallelic inheritance with involvement of mutations at two distinct loci was seen in 3 European-American families (K550, J118, and E521) and 1 Tunisian family (057015) (Table 1), including the proband of family 057015, who was heterozygous for the F337L allele in *BBS8* and homozygous for the c.1473+4A>G *BBS1* allele, which showed a reduction from 7.3 for the natural splice site to 4.3 for the variant site, indicating that this alteration might affect splicing.

BBS2 Mutations

BBS2 mutations were identified in 6 of the 46 families; they were the primary pathogenic mutations in 5 families (9%) and a potential triallelic contributor in 1 family. Two were novel (Fig. 1, Table 1). The proband of family E325 was a

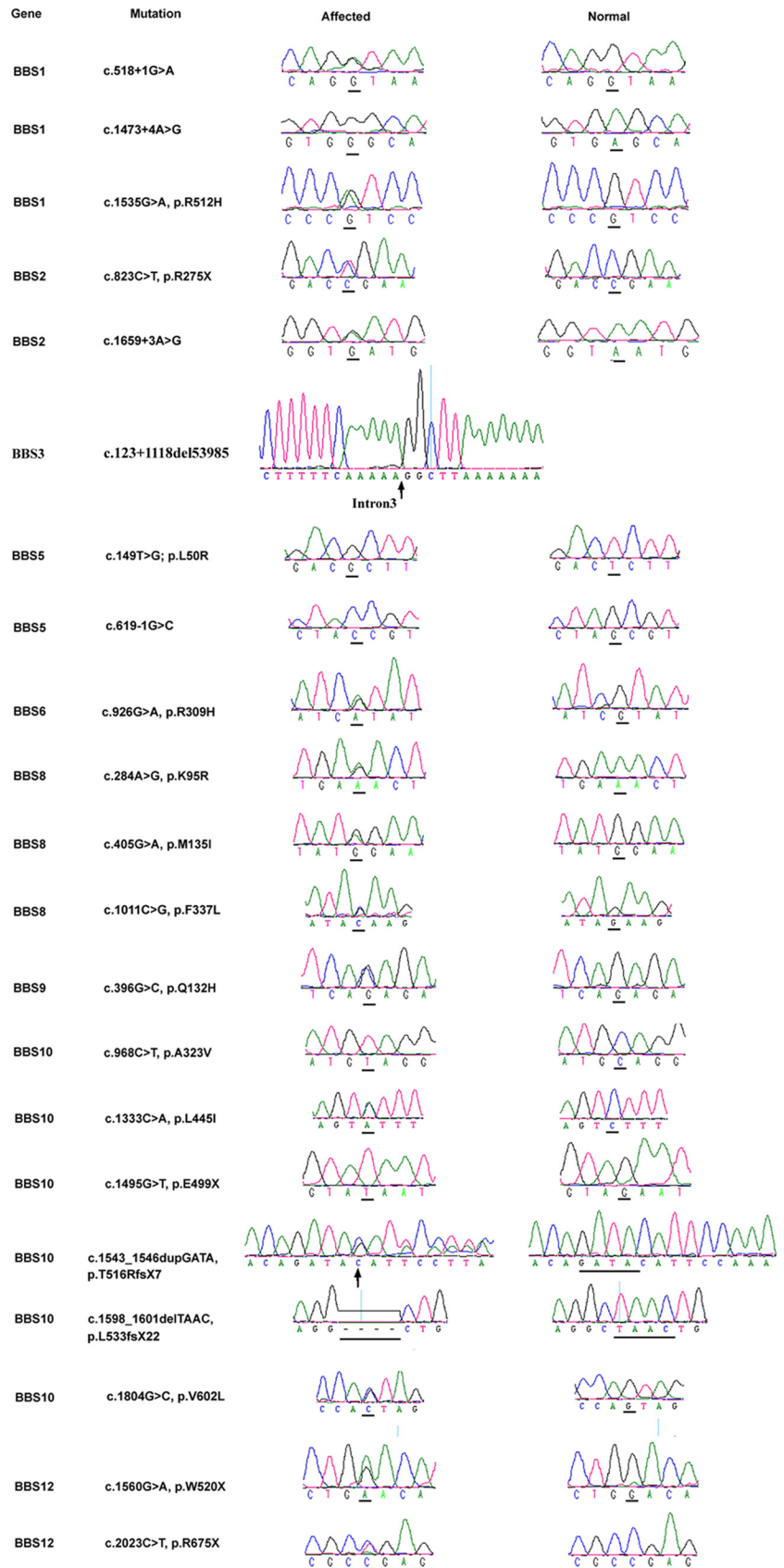


FIGURE 1. The 21 novel *BBS* mutations identified in this study. Three novel mutations were identified in *BBS1*: a heterozygous c.518+1G>A allele, a homozygous c.1473+4A>G allele, and a heterozygous R512H allele. Two novel mutations were identified in *BBS2*: a heterozygous R275X allele and a heterozygous c.1659+3A>G allele. A novel homozygous deletion was identified in *BBS3*: c.123+1118del53985. Two novel mutations were identified in *BBS5*: a homozygous L50R allele and a homozygous c.619-1G>C allele. One novel mutation was identified in *BBS6*: a heterozygous R309H allele. Three novel mutations were identified in *BBS8*: a heterozygous K95R allele, a heterozygous M135I allele, and a heterozygous F337L allele. One novel mutation was identified in *BBS9*: a heterozygous Q132H allele. Six novel mutations were identified in *BBS10*: a homozygous L533fsX22 allele, a homozygous A323V allele, a heterozygous L445I allele, a homozygous E499X allele, a heterozygous T516RfsX7 allele, and a heterozygous V602L allele. Two novel mutations were identified in *BBS12*: a heterozygous W520X allele and a heterozygous R675X allele. Sequence tracings of novel mutations identified in this study compared to normal sequences from controls.

compound heterozygote for R275X and D104A and had a score of 2.44 when analyzed with PolyPhen, suggesting pathogenicity.

Possible triallelic inheritance with involvement of mutations at two distinct loci was suspected in two European-derived families (E729 and K550) (Table 1). The proband of

TABLE 1. Mutations Identified in this Series of 46 Families (43 Mutations Found)

Family	Ethnic Origin	Gene	Exon	Nucleotide Change	Predicted Amino Acid Change	State	Report/Date
K550	Caucasian	<i>BBS1</i>	16	c.1645G>T	p.E549X	Homozygous	Ref. 14/2002
		<i>BBS2</i>	13	c.1659+3A>G†	splice mutation	Heterozygous	This study
057015	Tunisian*	<i>BBS1</i>	14	c.1473+4A>G†	splice mutation	Homozygous	This study
		<i>BBS8</i>	11	c.1011C>G†	p.F337L	Heterozygous	This study
J118	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Heterozygous	Ref. 27/2003
		<i>BBS1</i>	16	c.1645G>T	p.E549X	Heterozygous	Ref. 14/2002
		<i>BBS9</i>	5	c.396G>C†	p.Q132H	Heterozygous	This study
5360	Caucasian	<i>BBS10</i>	2	c.1598_1601delTAAC†	p.L533fsX22	Homozygous	This study
		<i>BBS12</i>	1	c.355G>A	p.G119S	Heterozygous	Ref. 34/2010
D257	Caucasian	<i>BBS10</i>	2	c.1090del A	p.N364TfsX5	Homozygous	Ref. 40/2007
		<i>BBS6</i>	3	c.926G>A†	p.R309H	Heterozygous	This study
E729	Caucasian	<i>BBS2</i>	2	c.311A>C	p.D104A	Heterozygous	Ref. 25/2001
		<i>BBS2</i>	15	c.1895G>C	p.R632P	Heterozygous	Ref. 26/2009
		<i>BBS10</i>	2	c.1333C>A†	p.L445I	Heterozygous	This study
E059	Arabic	<i>BBS3</i>	3	c.92C>T	p.T31M	Homozygous	Ref. 41/2004
		<i>BBS8</i>	5	c.405G>A†	p.M135I	Heterozygous	This study
9409	Caucasian	<i>BBS7</i>	7	c.632C>T	p.T211I	Homozygous	Ref. 42/2003
		<i>BBS8</i>	5	c.284A>G†	p.K95R	Heterozygous	This study
E521	Caucasian	<i>BBS12</i>	1	c.2023C>T†	p.R675X	Heterozygous	This study
		<i>BBS12</i>	1	c.476C>T	p.P159L	Heterozygous	Ref. 13/2007
		<i>BBS1</i>	15	c.1535G>A†	p.R512H	Heterozygous	This study
7367	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
7833	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
0775	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
7881	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
6579	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
1502	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
9390	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
5943	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
9437	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
2532	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
3163	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
F248	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
057016	Tunisian*	<i>BBS1</i>	12	c.1169T>G	p.M390R	Homozygous	Ref. 14/2002
D597	Caucasian	<i>BBS1</i>	6	c.518+1G>A†	splice mutation	Heterozygous	This study
		<i>BBS1</i>	12	c.1169T>G	p.M390R	Heterozygous	Ref. 27/2003
7348	Caucasian	<i>BBS1</i>	12	c.1169T>G	p.M390R	Heterozygous	Ref. 27/2003
		<i>BBS1</i>	16	c.1645G>T	p.E549X	Heterozygous	Ref. 14/2002
1087	Caucasian	<i>BBS10</i>	2	c.272 insertion T	p.C91LfsX5	Homozygous	Ref. 15/2006
E084	Caucasian	<i>BBS10</i>	2	c.272 insertion T	p.C91LfsX5	Homozygous	Ref. 15/2006
Q119	Caucasian	<i>BBS10</i>	2	c.272 insertion T	p.P.C91LfsX5	Homozygous	Ref. 15/2006
9407	Caucasian	<i>BBS10</i>	2	c.1495G>T†	p.E499X	Homozygous	This study
F931	Caucasian	<i>BBS10</i>	2	c.968C>T†	p.A323V	Homozygous	This study
5111	Caucasian	<i>BBS10</i>	2	c.272 insertion T	p.C91LfsX5	Heterozygous	Ref. 15/2006
		<i>BBS10</i>	2	c.907_910delAGTC	p.S303RfsX3	Heterozygous	Ref. 15/2006
9438	Caucasian	<i>BBS10</i>	2	c.272 insertion T	p.C91LfsX5	Heterozygous	Ref. 15/2006
		<i>BBS10</i>	2	c.907_910delAGTC	p.S303RfsX3	Heterozygous	Ref. 15/2006
F699	Caucasian	<i>BBS10</i>	2	c.272 insertion T	p.C91LfsX5	Heterozygous	Ref. 15/2006
		<i>BBS10</i>	2	c.1677C>A	p.Y559X	Heterozygous	Ref. 34/2010
2834	Caucasian	<i>BBS10</i>	2	c.687delT†	p.P229fsX8	Heterozygous	Ref. 15/2006
		<i>BBS10</i>	2	c.907_910delAGTC	p.S303RfsX3	Heterozygous	Ref. 15/2006
E352	Caucasian	<i>BBS10</i>	1	c.145C>T	p.R49W	Heterozygous	Ref. 15/2006
		<i>BBS10</i>	2	c.1804G>C†	p.V602L	Heterozygous	This study
9398	Caucasian	<i>BBS10</i>	1	c.164T>C	p.L55P	Heterozygous	Ref. 34/2010
		<i>BBS10</i>	2	c.1543_1546dupGATA†	p.T516RfsX7	Heterozygous	This study
E325	Caucasian	<i>BBS2</i>	2	c.311A>C	p.D104A	Heterozygous	Ref. 25/2001
		<i>BBS2</i>	8	c.823C>T†	p.R275X	Heterozygous	This study
057012	Tunisian*	<i>BBS2</i>	5	c.565C>T	p.R189X	Homozygous	Ref. 28/2006
M-679	Caucasian	<i>BBS2</i>	8	c.823C>T	p.R275X	Homozygous	Ref. 25/2001
PK01	Pakistani*	<i>BBS2</i>	11	c.1658C>T	p.R413X	Homozygous	Ref. 24/2003
M-002	Caucasian	<i>BBS5</i>	8	c.619-1G>C†	splice mutation	Homozygous	This study
057010	Tunisian*	<i>BBS5</i>	3	c.149T>G†	p.L50R	Homozygous	This study
J585	Caucasian	<i>BBS7</i>	7	c.710_713delAGAG	p.K237fsX60	Homozygous	Ref. 42/2003
B-489	Caucasian	<i>BBS7</i>	7	c.632C>T	p.T211I	Homozygous	Ref. 42/2003
J183	Caucasian	<i>BBS7</i>	7	c.632C>T	p.T211I	Homozygous	Ref. 42/2003
057013	Tunisian*	<i>BBS12</i>	1	1560G>A†	p.W520X	Heterozygous	This study
		<i>BBS12</i>	1	c.355G>A	p.G119S	Heterozygous	Ref. 34/2010
061049	Pakistani*	<i>BBS3</i>	3	c.123+1118del53985†		Homozygous	This study

* DNA samples from the Tunisian and Pakistani families were obtained through the National Eye Institute; all others were obtained through the Berman-Gund Laboratory, Harvard Medical School, Massachusetts Eye and Ear Infirmary.

† Novel mutations.

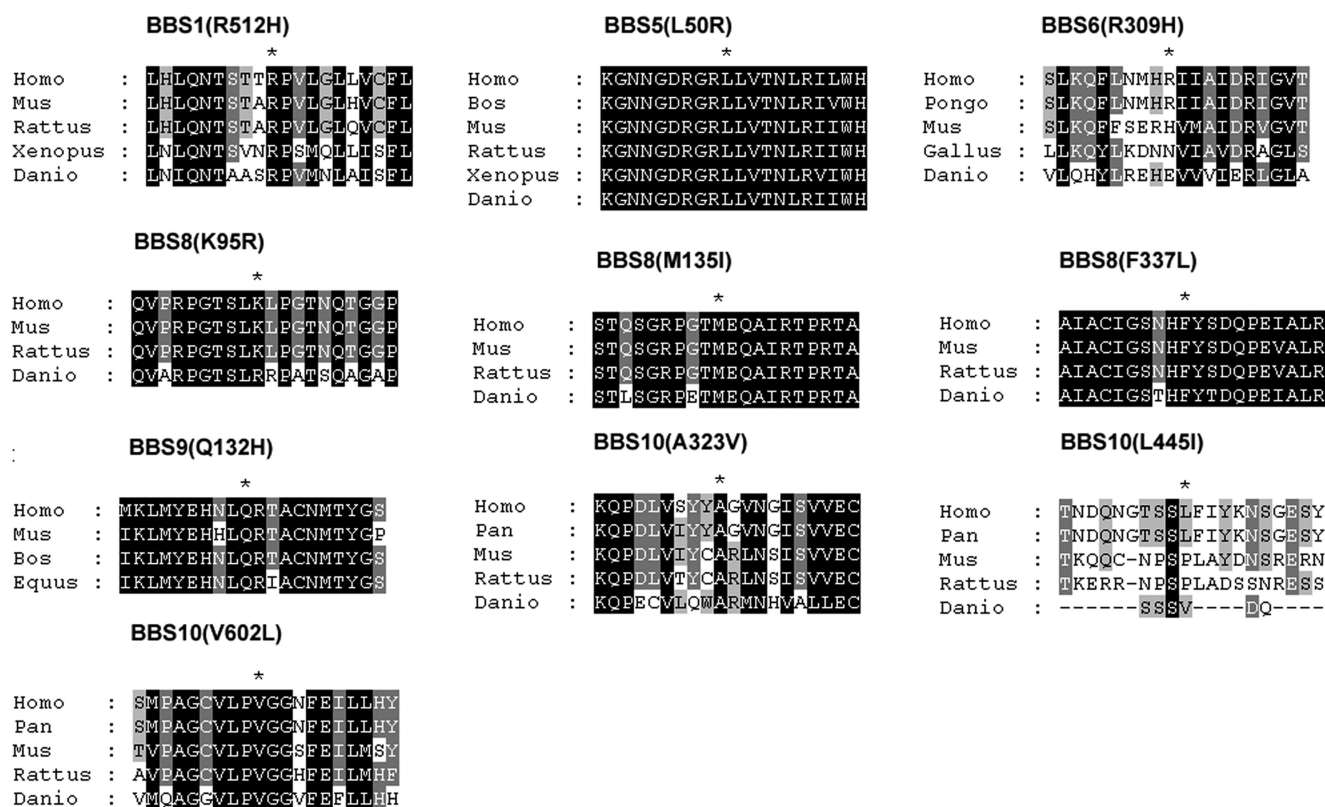


FIGURE 2. Amino acid sequence conservation around residues affected by novel missense mutations in *BBS1*, *BBS5*, *BBS6*, *BBS8*, *BBS9*, and *BBS10* identified in this study. *Asterisk*: position of the missense mutation. The sequences of BBS proteins or predicted translation products from several species have been compared and aligned. The relevant amino acids are in **bold**, and they are underlined when different from *Homo sapiens* reference; other identities are represented by a gray background. *Homo*, *H. sapiens*; *Mus*, *Mus musculus*; *Rattus*, *Rattus norvegicus*; *Xenopus*, *Xenopus (silurana) tropicalis*; *Danio*, *Danio rerio*; *Bos*, *Bos taurus*; *Pongo*, *Pongo abelii*; *Gallus*, *Gallus*; *Pan*, *Pan troglodytes*; *Equus*, *Equus caballus*.

family E729 was a compound heterozygote for the D104A and R632P (PolyPhen scores of 2.44 and 2.7, respectively) alleles and also carried a heterozygous L445I allele in *BBS10*. The proband of family K550 was heterozygous for the c.1659+3A>G *BBS2* allele (decreasing the splice site score from 0.52 to 0, consistent with loss of the donor site) and also carried a homozygous E549X allele in *BBS1*, the principal “pathogenic” mutation.

BBS3 Mutations

BBS3 mutations were found in 2 of the 46 families (4%), including a consanguineous Pakistani family (061049) in which affected members were homozygous for a novel 54-kb deletion beginning in intron 3, c.123+1118del 53,985, and extending beyond the end of the gene. In an Arabian family (E059), affected members were homozygous for a previously reported T31M allele in *BBS3*, predicted to be pathogenic (PolyPhen score 2.78). One member of this family also had a heterozygous M135I allele in *BBS8* predicted to be benign with a PolyPhen score of 1.09.

BBS5 Mutations

Novel *BBS5* mutations were found in 2 of the 46 families (4%), including a homozygous c.619-1G>C allele in European family M-002, decreasing the splice site score from 5.6 to -5.4 (Fig. 1, Table 1). Tunisian family 057010 showed a homozygous L50R (probably pathogenic) sequence change with a PolyPhen score of 2.17. Both mutations reported here affected the highly conserved DM16 domains,³⁵ supporting their pathogenicity.

BBS6 Mutations

A novel heterozygous sequence change was present in a single family of European origin (D257), which showed the R309H *BBS6* allele and carried a homozygous N364TfsX5 allele in *BBS10* as well (Table 1), suggesting the possibility of triallelic inheritance. However, the R309H residue was not highly conserved during evolution (Fig. 2), and PolyPhen analysis predicts R309H to be benign (Table 2), indicating that its pathogenic potential is low.

BBS7 Mutations

Previously described *BBS7* mutations were present in 4 of the 46 families (7%), all of European origin. Possible triallelic inheritance is suspected in 1 family (9409; Table 1), in which 2 affected family members are homozygous for the T211I *BBS7* allele and heterozygous for the K95R sequence change in *BBS8*. However, the K95R residue was not highly conserved during evolution (Fig. 2), and PolyPhen analysis predicts K95R to be benign with a score of 0.15 (Table 2).

BBS8 Mutations

Novel heterozygous *BBS8* sequence changes were identified in 3 of the 46 families (6%; M135I in E059, K95R in Arabian family 9409, and F337L in Tunisian family 057015), all of which may be compatible with triallelic inheritance. The M135I residue is highly conserved during evolution, but PolyPhen predicts M135I to be benign with a score of 1.09 (Fig. 2, Table 2). In contrast, the F337L residue is highly conserved during evolu-

TABLE 2. Evaluation of Novel *BBS* Missense Variants

Gene-Exon	Predicted Amino Acid Change	Nucleotide Change	State	Third Mutated Allele	Con-served	Computational Prediction		Present in Controls	Report
						PolyPhen Prediction	Remark		
BBS1-EX15	R512H	c.1535G>A	Heter	No	Yes	Possibly damaging	Pathogenic	No	This study
BBS10-EX2	A323V	c.968C>T	Homo	No	Yes	Likely benign	Neutral	No	This study
BBS10-EX2	L445I	c.1333C>A	Heter	Yes	No	Likely benign	Neutral	No	This study
BBS10-EX2	V602L	c.1804G>C	Heter	No	Yes	Likely benign	Neutral	No	This study
BBS5-EX3	L50R	c.149T>G	Homo	No	Yes	Probably damaging	Pathogenic	No	This study
BBS6-EX3	R309H	c.926G>A	Heter	Yes	No	Likely benign	Neutral	No	This study
BBS8_EX5	K95R	c.284A>G	Heter	Yes	No	Likely benign	Neutral	No	This study
BBS8_EX5	M135I	c.405G>A	Heter	Yes	Yes	Likely benign	Neutral	No	This study
BBS8-EX11	F337L	c.1011C>G	Heter	Yes	Yes	Probably damaging	Pathogenic	No	This study
BBS9-EX5	Q132H	c.396G>C	Heter	Yes	Yes	Possibly damaging	Pathogenic	No	This study

Heter, heterozygous; Homo, homozygous.

tion (Fig. 2), and PolyPhen analysis indicates that F337L is expected to be pathogenic with a score of 2.43 (Table 2).

BBS9 Mutations

A novel heterozygous *BBS9* sequence change was present in 1 European-derived family (J118), possibly consistent with triallelic inheritance. The Q132H residue is highly conserved during evolution (Fig. 2), and PolyPhen analysis predicts that Q132H would be expected to be pathogenic with a score of 1.92 (Table 2).

BBS10 Mutations

BBS10 mutations were present in 14 of the 46 families, all European derived, with 2 pathogenic alleles present in 11 (20%), 2 changes of uncertain significance in 2 more (4%), and a single heterozygous change in 1 family. Six are novel (Fig. 1, Table 1). The proband of family F931 was homozygous for the A323V allele, which is highly conserved during evolution, but it is a conservative change that PolyPhen analysis predicts to be benign, (Fig. 2, Table 2). Similarly, the proband of family E352 was a compound heterozygote for the R49W (previously reported¹⁵ with a probably damaging PolyPhen score of 2.46) and V602L alleles. Although the V602L residue is highly conserved during evolution, PolyPhen analysis predicts V602L to be benign. The proband of family 9398 was a compound heterozygote for the T516RfsX7 and L55P alleles. The L55P residue is highly conserved during evolution (Fig. 2), with a probably damaging PolyPhen score of 2.07 (Table 2).

Possible triallelic inheritance was found in probands of 3 families: 5360, which, in addition to homozygous L533fsX22, carries a heterozygous G119S allele in *BBS12*; D257 is homozygous for the N364TfsX5*BBS10* allele with a heterozygous R309H allele in *BBS6*; and E729; Table 1). The proband of family E729 was heterozygous for the L445I *BBS10* allele and also was a compound heterozygote for the D104A and R632P alleles in *BBS2*, the principal pathogenic mutations. The L445I residue is not highly conserved during evolution (Fig. 2), and PolyPhen predicts L445I to be benign (Table 2), suggesting low pathogenic potential.

BBS12 (C4ORF24) Mutations

BBS12 mutations (4 different alleles) were present in three families, including 2 families of European and 1 of Tunisian origin (Table 1). Possible triallelic inheritance was found in probands of 2 European-derived families. E521 carries a compound heterozygote for the previously reported¹³P159L and R675X alleles and a heterozygous R512H allele in *BBS1*. The proband of family 5360 is homozygous L533fsX22 allele in

BBS10 and heterozygous for the *BBS12* G119S allele, which is highly conserved during evolution (Fig. 2) and gives a probably damaging PolyPhen score (Table 2).

BBS4, BBS11 (TRIM32), BBS13, BBS14, BBS15 (C2ORF86), RPGRIP1L, CC2D2A, NPHP3, TMEM67, and INPP5E Mutations

No mutations were detected for *BBS4*, *BBS11* (*TRIM32*), *BBS13*, *BBS14*, *BBS15* (*C2ORF86*), *RPGRIP1L*, *CC2D2A*, *NPHP3*, *TMEM67*, or *INPP5E* in any of the 55 families studied.

DISCUSSION

In agreement with previous studies in which mutations were identified in 70% to 75% of *BBS* families,^{19–21,36,37} in our study, mutations were identified in 38 of 45 patients of European ethnic origin (84%) and 5 of 8 Tunisian families (62.5%), giving an overall detection rate of 73%.

Our results are consistent with previous reports that *BBS1* and *BBS10* mutations are frequent, contributing 30% and 21% in previous studies^{15,22,23} (Supplementary Fig. S1, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7554/-/DCSupplemental>). In this series *BBS1* mutations contributed to disease in 35% (19/55) of the patients, representing the primary pathogenic locus in 18 families (33%). Overall, these data support the previous observation that *BBS1* and *BBS10* are responsible for most of the disease in Europeans. In contrast, *BBS2* and *BBS8* are prime candidates in Tunisian-derived patients, in whom no *BBS10* mutations were identified.

Examination of the distribution of *BBS* mutations in European and Tunisian individuals shows a number of differences (Supplementary Fig. S2, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7554/-/DCSupplemental>). In agreement with previous reports, in which p.M390R has been reported to cause 18% to 32% of *BBS* cases,^{22,27} 16 of 55 (29%) of families with *BBS* were found to carry the M390R mutation (13 as homozygous alleles and 3 as heterozygous alleles), making it the most common mutation found. It accounts for 24 of 32 (75%) *BBS1* alleles identified in Europeans, in which the M390R mutation is considered to be an ancient mutation, as suggested by haplotype analysis. The M390R mutation has been described exclusively in European-derived patients of French descent.²⁷ Interestingly, although 1 Tunisian patient in this study carries this mutation, he shares a common *BBS1* SNP haplotype with the European patients. The geographic position of Tunisia in North Africa has ensured an eventful population history in which Phoenicians, Romans, Vandals, Byzant-

tines, Arabs, Ottomans, and French have controlled or colonized the region at different historical times.²⁸ Taken together with the haplotype data, this suggests that this Tunisian patient might be of French descent, consistent with the absence of the M390R mutation in most non-European populations. *BBS2* and *BBS8* are the most common genes implicated in Tunisians, and R189X is the only *BBS2* mutation seen in Tunisians. Finally, though the Tunisian data set is relatively small, it is notable for the complete absence of *BBS10* mutations, the second most common gene affected in Europeans after *BBS1*.

BBS is inherited primarily as an autosomal recessive trait. However, in some patients, 3 mutations across 2 BBS genes appear to interact to modify the onset or severity, or both, of the phenotype.²²⁻²⁵ Seven Caucasian families, 1 Arabian family, and 1 Tunisian family (15% of patients with mutations detected) were found to carry 3 mutations in the 2 different genes, termed triallelic inheritance (Supplementary Fig. S3, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7554/-DCSupplemental>). We found only a single, heterozygous mutation in *BBS1* in 3 families, in *BBS10* in 2 families, and in *BBS2*, *BBS3*, *BBS7*, and *BBS12* in single families (Table 1). Although these data are compatible with a potential epistatic interaction, in which the effects of the primary gene are modified by a specific allele at a second gene, they might also represent the presence of undetected second mutations or the random occurrence of sequence variants in these genes.

Interestingly, 8 of the 9 third variant alleles were novel, except for BBS12 p.G119S, which was reported recently as a third allele variant by Billingsley et al.³¹ (Table 1, Supplementary Fig. S3, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7554/-DCSupplemental>), and 2 of them involve genes that, to our knowledge, have not previously been reported to be involved in triallelic inheritance of BBS (*BBS9* and *BBS12*). Given that most of these sequence changes are missense variants, the possibility that some are benign cannot be excluded, even though they were not identified in 96 healthy persons. Of the 8 missense variants, 4 (R512H in *BBS1*, F337L in *BBS8*, Q132H in *BBS9*, and G119S in *BBS12*) were estimated to be pathogenic changes, whereas the remaining four (R309H in *BBS6*, K95R in *BBS8*, M135I in *BBS8*, and L445I in *BBS10*) were estimated to be neutral by the PolyPhen program (Table 2). Five, including all those predicted to be pathogenic by PolyPhen analysis, involve residues that are highly conserved during evolution (Fig. 2). Thus, alternative explanations must be considered, including that the third allele detected in each of the families is a benign polymorphism or that the third allele is not causal in these patients but might exert a more subtle modifying effect. These observations are compatible with the previous suggestion that the severity of the disease phenotype and the clinical symptoms may vary as a result of the interaction of mutations in different BBS genes.^{22,23} However, the absence of these variants in 96 ethnically matched control subjects does not provide sufficient statistical power to demonstrate a causal relationship to BBS; functional analysis will be required to provide a definitive answer to this question.

Recent studies provide insight into how the BBS proteins mediate ciliary function in mammalian cells. Seven evolutionarily conserved BBS proteins (*BBS1*, 2, 4, 5, 7, 8, and 9) form a stable complex, the BBSome, mediating vesicular transport to the cilium.³⁸ The BBSome is transported to the basal body through interactions between the *BBS4* subunit of the BBSome and PCM-1 of the centriolar satellite. Inside the cilium, *BBS7* and *TTC8* may be involved in vesicular transport pathways, including functioning as an IFT cohesion factor.³⁸ The ability of each BBS locus to contribute alleles that can modify the effect of mutations in other BBS genes suggests a common pathway and potentially the existence of a macromolecular complex

under the "poison" hypothesis, stating the presence of mutant subunits in a heterodimer or multisubunit complex prevents proper functioning of the entire assembly.³⁹ Although *BBS6*, *BBS10*, and *BBS12* are not components of the BBSome, mutations in these genes also lead to similar phenotypes. Seo et al.⁴⁰ demonstrated that chaperonin-like BBS proteins (*BBS6*, *BBS10*, and *BBS12*) are required for BBSome assembly.

In addition to BBS, a number of related syndromes result from ciliary dysfunction, including *BBS14* mutated in nephronophthisis, Joubert syndrome, and BBS and Meckel-Gruber syndrome.^{17,41,42} Although this provided a rationale for screening families without identified mutations in known BBS genes for mutations in these genes, no mutations were found in our patients.

In summary, we have identified 43 mutations in 55 families affected with BBS, including 21 novel mutations. We found evidence compatible with triallelic inheritance in affected persons from 9 families. This mutational analysis has expanded the already extensive categorization of *BBS* mutations and contributed to our understanding of both the population genetics of BBS and to the molecular pathology of the genes implicated in these diseases, as well as suggesting distinct optimal sequential mutational analysis in Tunisian and European patients for diagnostic purposes. The identification of many novel mutations unique to individuals highlights the genetic heterogeneity of the disorder, and the existence of some BBS cases without identified mutations in known *BBS* genes suggests further genetic heterogeneity.

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