BBS1 Mutations in a Wide Spectrum of Phenotypes Ranging From Nonsyndromic Retinitis Pigmentosa to Bardet-Biedl Syndrome

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Objective: To investigate the involvement of the Bardet-Biedl syndrome (BBS) gene BBS1 p.M390R variant in non-syndromic autosomal recessive retinitis pigmentosa (RP).

Methods: Homozygosity mapping of a patient with isolated RP was followed by BBS1 sequence analysis. We performed restriction fragment length polymorphism analysis of the p.M390R allele in 2007 patients with isolated RP or autosomal recessive RP and in 1824 ethnically matched controls. Patients with 2 BBS1 variants underwent extensive clinical and ophthalmologic assessment.

Results: In an RP proband who did not fulfill the clinical criteria for BBS, we identified a large homozygous region encompassing the BBS1 gene, which carried the p.M390R variant. In addition, this variant was detected homozygously in 10 RP patients and 1 control, compound heterozygously in 3 patients, and heterozygously in 5 patients and 6 controls. The 14 patients with 2 BBS1 variants showed the entire clinical spectrum, from nonsyndromic RP to full-blown BBS. In 8 of 14 patients, visual acuity was significantly reduced. In patients with electroretinographic responses, a rod-cone pattern of photoreceptor degeneration was observed.

Conclusions: Variants in BBS1 are significantly associated with nonsyndromic autosomal recessive RP and relatively mild forms of BBS. As exemplified in this study by the identification of a homozygous p.M390R variant in a control individual and in unaffected parents of BBS patients in other studies, cis- or trans-acting modifiers may influence the disease phenotype.

Clinical Relevance: It is important to monitor patients with an early diagnosis of mild BBS phenotypes for possible life-threatening conditions.


BARDET-BIEDL SYNDROME (BBS) (OMIM 209900) is a clinically and genetically heterogeneous disorder characterized by a wide spectrum of clinical features. It is considered a member of the group of conditions collectively called ciliopathies.1 Primary clinical features include retinitis pigmentosa (RP) (OMIM 268000), polydactyly, obesity, learning disabilities, hypogonadism in males, and renal abnormalities. Secondary clinical features include speech disorders, strabismus, brachydactyly or syndactyly, developmental delay, polydipsia-polyuria (diabetes insipidus), ataxia, mild spasticity, diabetes mellitus, dental crowding or hypodontia, congenital heart diseases, and hepatic fibrosis. The clinical diagnosis of BBS is based on the presence of at least 4 primary features or a combination of 3 primary plus at least 2 secondary features.2 The clinical phenotypic spectrum of BBS is wide, and genotype-phenotype correlations have not been clearly established.3-4 Some clinical features overlap those of other ciliopathies, such as McKusick-Kaufman (OMIM 604896), Alstrom (OMIM 203800), or Laurence-Moon (OMIM 245800) syndromes, which have been described in families with the BBS6, BBS10, or BBS12 mutation.5-7 The transmission of the disease is autosomal recessive,8-10 but a digenic inheritance in the form of triallelism has been reported in some families in which either 3 mutations in 2 BBS genes are required to manifest the phenotype11,12 or a third variant may modulate the expression of the clinical phenotype.13-15
The prevalence of BBS ranges from 1:125 000 to 1:160 000 in Europe\textsuperscript{16-18} and 1:65 000 in the Arab population\textsuperscript{19}, a higher incidence was observed in isolated populations of Newfoundland (1:13 000),\textsuperscript{2} Kuwait (1:17 000),\textsuperscript{20} and the Faroe Islands (1:3700).\textsuperscript{21}

To date, 15 genes have been associated with BBS.\textsuperscript{12,22} Most BBS proteins can be divided into 2 groups. One forms a complex called the BBsSome (BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, and BBS9), which is believed to recruit γ-glutamyl transpeptidase Rab8,\textsuperscript{21} and the second group comprises chaperonin-like proteins,\textsuperscript{24} including BBS6, BBS10, and BBS12. Mutations in known BBS genes are detected in approximately 73% of families, with BBS1 and BBS10 each accounting for 20% to 25% of families of European descent with BBS1-associated disease.\textsuperscript{25,26} Two widespread mutations resulting from founder effects have been described, p.M390R in BBS1\textsuperscript{8,13,27} and p.C91fsX95 in BBS10.\textsuperscript{26} The p.M390R variant is the most common BBS1 mutation and has been observed\textsuperscript{20} in approximately 80% of families with BBS1-associated disease.

Retinitis pigmentosa is the most common inherited retinal degeneration, with an estimated worldwide prevalence of 1:4 000,\textsuperscript{28} and is characterized by progressive photoreceptor dysfunction, followed by photoreceptor cell death. The disease is highly heterogeneous and displays all patterns of inheritance, that is, autosomal recessive, autosomal dominant, or X-linked. In addition, there are some cases with mitochondrial mutations and digenic inheritance.\textsuperscript{29,30} Mutations in 36 genes have been identified that cause autosomal recessive nonsyndromic RP, and mutations in 51 genes cause autosomal recessive syndromic retinal dystrophy, including BBS and Usher syndrome (RetNet, http://www.sph.uth.tmc.edu/RetNet/). These genes encode proteins involved in the phototransduction cascade, vitamin A metabolism, cellular or cytoskeletal structure, cell-to-cell signaling or synaptic interaction, gene expression, phagocytosis, and RNA splicing factors. Several RP proteins, including approximately half the BBS proteins, are part of the photoreceptor sensory cilium proteome, a large protein complex associated with and constituting the connecting cilium.\textsuperscript{21}

Homozygosity mapping has proven to be fruitful to identify defects in known and newly identified genes implicated in autosomal recessive retinal degenerations\textsuperscript{32-34} and to establish novel genotype-phenotype correlations.\textsuperscript{35,36} Using this approach, we identified a patient with RP with a large homozygous region encompassing the BBS1 gene. The fact that retinal degeneration is consistently seen in BBS patients with BBS1 mutations and previous observations that some mutations in BBS3/ARL6, BBS8/TTC8, BBS12, and BBS14/CEP290 cause nonsyndromic retinal degeneration or retinal degeneration with minimal systemic features\textsuperscript{37-40} prompted us to screen BBS1 in this patient and to further test the hypothesis that mutations in BBS1 are also a significant cause of nonsyndromic RP. In this study, we analyzed the p.M390R BBS1 mutation in 2007 patients with RP. We identified p.M390R BBS1 mutation in a homozygous or compound heterozygous manner in 14 patients with a wide clinical spectrum ranging from nonsyndromic RP to classic BBS, as well as in 1 putative healthy individual.

**METHODS**

**PATIENTS AND CLINICAL EVALUATION**

In Canada, Europe, and Israel, 2007 unrelated patients with isolated or autosomal recessive RP (80 from Ghent, 96 from Jerusalem, 245 from Madrid, 361 from Montpellier, 343 from Montreal, 143 from Naples, 215 from Nijmegen, and 524 from Tübingen) and 1824 ethnically matched individuals serving as controls (103 from Ghent, 100 from Jerusalem, 320 from Madrid, 56 from Montpellier, 361 from Montreal, 140 from Naples, 233 from Nijmegen, and 311 from Tübingen) were included in the study. The diagnosis of RP was based on ophthalmologic examination that included measurement of best-corrected visual acuity, Goldmann visual fields, slitlamp biomicroscopy, dilated indirect ophthalmoscopy, and fundus photography. In addition, full-field flash electroretinography (ERG), according to the guidelines of the International Society of Clinical Electrophysiology of Vision,\textsuperscript{41} was performed when Goldmann visual fields were measurable and indicated a preserved visual field with peripheral limits beyond the pericentral 10°. A broad definition of RP was used to avoid excluding patients with atypical features: progressive visual loss, night blindness, and abnormal ERG findings in a rod-cone pattern when recordable. Blood samples were obtained after informed consent forms were signed. Ethics approval was given to all participating institutions, and the study conformed to the tenets of the Declaration of Helsinki. Participants were evaluated again after the identification of the BBS1 gene defects, since they had previously received a diagnosis of nonsyndromic RP. Special attention was given to identifying systemic features associated with BBS,\textsuperscript{4} which include the primary features of RP, polydactyly, obesity, learning disabilities, hypogonadism in males, and renal abnormalities, and the secondary features of speech disorders, cataract, brachydactyly or syndactyly, developmental delay, polydipsia/polyuria (diabetes insipidus), ataxia, mild spasticity, diabetes mellitus, dental crowding or hypodontia, congenital heart diseases, and hepatic fibrosis. Where and when appropriate, patients were clinically reexamined or their records were again reviewed. Clinical evaluation included best-corrected visual acuity and slitlamp biomicroscopy of the anterior segment and retina. Additional examinations included kinetic and/or static perimetry and ERG according to the protocol of the International Society for Clinical Electrophysiology of Vision.

**HOMOZYGOSITY MAPPING AND BBS1 MUTATION ANALYSIS**

Genomic DNAs were isolated from lymphocytes by standard salting-out procedures.\textsuperscript{45} Only the DNA sample of patient 5 was genotyped, using a microarray that contains 500,000 single-nucleotide polymorphisms (SNPs) (GeneChip Genome-Wide Human SNP Array 3.0; Affymetrix). Array experiments were performed according to protocols provided by the manufacturer. The 5.0 array data were genotyped (Genotype Console, version 2.1; Affymetrix) and, subsequently, regions of homozygosity were identified using commercial software (Partek, version 6.1, Partek, Inc).

Primers for the amplification of the 17 coding exons and splice junctions of BBS1 were designed by Primer3\textsuperscript{46} and are available on request. Polymerase chain reaction (PCR) products were purified with 96-well filter plates (Multiscreen HTS-PCR; Millipore) or by gel extraction (Qia-Quick Gel Extraction Kit; Qiagen). Sequencing was performed with dye terminator chemistry (BigDye Terminator, version 3 on a 3100, 3730, or 3730XL DNA analyzer; Applied Biosystems, Inc).
SCREENING FOR THE BBS1 p.M390R AND MGC1203 c.403C>T VARIANTS

We developed a restriction enzyme test to screen the patients and controls for the recurrent BBS1 p.M390R mutation because the c.1169T>G variant in exon 12 introduces an AvaII recognition site (G/GWCC). The PCR analysis of exon 12 of BBS1 was performed with forward primer 5'-CTGTGATTCTGGTCTCCTC-3' and reverse primer 5'-CCAGAGCTCTTCGCTCTCC-3', using 50 ng of DNA and 10 pmol of each primer in a standard 25-μL reaction. The PCR amplification was performed (PTC-200 Thermo Cycler; MJ Research) under the following conditions: 3 minutes at 94°C, 35 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 45 seconds, and a final extension step at 72°C for 10 minutes. The PCR products were purified with 96-well filter plates (Multiscreen HTS-PCR; Millipore). Sequencing was performed with dye terminator chemistry (BigDye Terminator, version 3 on a 3100, 3730, or 3730XL DNA analyzer; Applied Biosystems, Inc.).

IN SILICO ASSESSMENT OF M NSSENSE SUBSTITUTIONS

We used PolyPhen-2144 (http://genetics.bwh.harvard.edu/pph2/index.shtml) and SIFT145 (http://sift.jcvi.org) to predict the functional effect of novel missense substitutions identified in this study. To assess the evolutionary conservation, amino acid sequences of orthologous BBS1 proteins (Pan troglodytes XP_001171950.1, Sus scrofa XP_540830.2, Mus musculus NP_001091721, Canis lupus familiaris XP_540830.2, Mus musculus NP_001028300, Xenopus tropicalis NP_001116276.1, Drosophila melanogaster NP_698080.1, and Caenorhabditis elegans NP_749033.1) were obtained from the NCBI protein database (http://www.ncbi.nlm.nih.gov/protein/) and were aligned with the protein sequence of human BBS1 (NP_078925.3) using Vector NTI software (version 11; Invitrogen).

RESULTS

IDENTIFICATION OF BBS1 MUTATIONS IN RP PATIENTS

Upon homozygosity mapping, several homozygous regions were identified in patient 5, who received a diagnosis of severe RP, the largest of which measured 19 Mb and encompassed the BBS1 gene on chromosome 11q13.2. Sequence analysis of all 17 coding exons of BBS1 revealed the homozygous c.1169T>G (p.M390R) missense mutation in exon 12. To determine whether the p.M390R variant could be a rather frequent cause of non-syndromic RP, we carried out a restriction fragment length polymorphism analysis for the p.M390R BBS1 mutation in 2006 patients with isolated or autosomal recessive RP in Canada, Europe, and Israel. We found 8 additional unrelated patients and a sibling pair with a homozygous p.M390R mutation (Table 1). Three patients carried the p.M390R allele combined with a second BBS1 variant in a compound heterozygous state. Two of these mutations (c.1163T>C [p.L388P] and c.803G>C [p.R268P], detected in patients 2 and 13, respectively) are novel, and 1 mutation (c.1130_1134del [p.C377WfsX36] in patient 4) was described previously.27 Alignment of the BBS1 amino acid sequences of various orthologs showed that the substituted amino acids (ie, leucine at position 388 and arginine at position 268) are highly conserved from human to C elegans. PolyPhen-2 predicted that p.L388P and p.R268P variants are probably damaging, and SIFT analysis revealed that neither is tolerated.

Analysis using restriction fragment length polymorphism revealed 3 patients with only 1 p.M390R variant; subsequent sequence analysis of the 17 BBS1 exons did not reveal a second allele. In 1824 ethnically matched control individuals, we found 6 heterozygous p.M390R carriers and 1 individual who was homozygous for this variant. Because these were anonymous healthy individuals, further clinical assessments could not be performed. Because Badano et al identified a modifier allele for BBS in MGC1203, we tested the presence of the c.403C>T variant in all cases and the 1 control individual with 2 BBS1 alleles. Using restriction fragment length polymorphism analysis, this modifier allele was not detected in any of these cases.

CLINICAL FINDINGS

The clinical findings of the 14 patients with BBS1 variants are reported in Table 1. Patients were divided into 4 groups. Group A contained the 3 patients who appeared to have a nonsyndromic form of RP. In these patients, cataract was the only feature that might be related to BBS. Group B consisted of 4 patients who may have a nonsyndromic form of RP. These patients showed few extraocular features that may be associated with the retinal phenotype. Three of these patients were obese, and, in 1 patient, routine ultrasound revealed a few mild renal cysts without renal dysfunction. The 6 patients in group C demonstrated definite extraocular features commonly associated with BBS but insufficient to warrant a diagnosis of classic BBS. Finally, group D included 1 patient who fulfilled the criteria for the diagnosis of classic BBS after reevaluation.

The results of the ophthalmologic examinations of the 14 patients with RP or (mild) BBS are summarized in Table 2. In most patients, night blindness was the first symptom of retinal degeneration. Early photoreceptor dysfunction, at or before the age of 10 years, was observed in 6 of the 14 patients (including 2 siblings, patients 3 and 7). A visual acuity level of counting fingers or less was observed in 8 patients, and none of the patients displayed visual acuity better than 20/40. Figure 1 (patient 4) clearly illustrates the severity of the phenotype in these patients. A relatively later age at onset did not necessarily reflect positively on the chance of encountering severe visual loss, as demonstrated by patient 14,
who experienced visual field loss at age 35 years, but at age 54 years, her visual acuity was reduced to light perception. In most patients there was extensive loss of the visual field. Four patients could not see the largest target or showed only a modest temporal region of remaining sensitivity; 6 patients demonstrated severe visual field constriction. When ERG responses could be elicited, a rod-cone pattern of photoreceptor degeneration was observed. In 10 of 13 patients, the ERG became nonrecordable during the course of their disease.

**COMMENT**

Mutations in the **BBS1** gene have been associated with BBS, and in one study they were associated with RP and variable mild systemic features. For some BBS families, a digenic-triplet inheritance model has been proposed in which interactions were suggested between BBS1 and BBS2 or BBS1 and BBS6, although this has not been replicated in other studies. We found that the **BBS1** variant p.M390R is significantly associated with nonsyndromic RP or mild BBS because we identified it in 21 of 4014 alleles in our probands vs 8 of 3648 alleles in the controls ($P = .02$, Fisher exact test). Similarly, the **BBS3/ARL6** variant p.A89V and the **BBS12** variant p.S701X have been implicated in both nonsyndromic RP and BBS. A splice mutation affecting a retina-specific exon of **BBS8/TTC8** also causes nonsyndromic RP. In other studies, considerable variations of systemic features have been described in patients with BBS.

The phenotype of the **BBS1**-associated retinal dystrophy in our patient group was more severe than that reported by Azari and coworkers. Eight of the 14 patients in the current study had visual acuity of counting fingers or lower, whereas Azari and coworkers observed only 1 patient with visual acuity below 20/200 in their 10 patients with principally classic BBS. In addition, the age at onset was relatively early in our patients and nystagmus was present in 3 patients. For the most part, the patients in the current study showed classic RP with a relatively homogeneous clinical presentation. In view of the early age at onset, low visual acuity, and extensive visual field loss, the photoreceptor dystrophy of the 14 patients in this study should be considered severe.

The disease spectrum of the patients in this study is broad, and any attempt at organization is arbitrary at best.

### Table 1. Spectrum of Ocular and Extraocular Features of Patients With **BBS1** Mutations

<table>
<thead>
<tr>
<th>Patient No./Sex</th>
<th>Country</th>
<th>Ocular</th>
<th>Extraocular</th>
<th>Primary Features</th>
<th>Secondary Features</th>
<th>No. of BBS Features</th>
<th>Diagnosis</th>
<th>Allele 1/Allele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M</td>
<td>NL</td>
<td>RP</td>
<td>None</td>
<td>None</td>
<td>Cataract</td>
<td>1/1</td>
<td>Nonsyndromic RP</td>
<td>M390R/M390R</td>
</tr>
<tr>
<td>2/M</td>
<td>FR</td>
<td>RP</td>
<td>None</td>
<td>None</td>
<td>Cataract</td>
<td>1/1</td>
<td>Nonsyndromic RP</td>
<td>M390R/L388P</td>
</tr>
<tr>
<td>3/Fa</td>
<td>IT</td>
<td>RP</td>
<td>None</td>
<td>None</td>
<td>Cataract</td>
<td>1/1</td>
<td>Nonsyndromic RP</td>
<td>M390R/M390R</td>
</tr>
<tr>
<td>4/M</td>
<td>NL</td>
<td>RP</td>
<td>Mild renal cysts without renal dysfunction</td>
<td>None</td>
<td>None</td>
<td>1/1</td>
<td>Possibly nonsyndromic RP</td>
<td>M390R/C377WfsX36</td>
</tr>
<tr>
<td>5/M</td>
<td>CA</td>
<td>RP</td>
<td>Obesity; not truncal</td>
<td>None</td>
<td>Cataract</td>
<td>2/1</td>
<td>Possibly nonsyndromic RP</td>
<td>M390R/M390R</td>
</tr>
<tr>
<td>6/M</td>
<td>FR</td>
<td>RP</td>
<td>Obesity</td>
<td>None</td>
<td>None</td>
<td>2/0</td>
<td>Possibly nonsyndromic RP</td>
<td>M390R/M390R</td>
</tr>
<tr>
<td>7/Ma</td>
<td>IT</td>
<td>RP</td>
<td>Obesity</td>
<td>None</td>
<td>Cataract</td>
<td>2/0</td>
<td>Possibly nonsyndromic RP</td>
<td>M390R/M390R</td>
</tr>
<tr>
<td>8/Mb</td>
<td>BE</td>
<td>RP</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>1/1</td>
<td>Mild BBS</td>
<td>M390R/M390R</td>
</tr>
<tr>
<td>9/F</td>
<td>GE</td>
<td>RP</td>
<td>Learning difficulties</td>
<td>None</td>
<td>Speech disorder</td>
<td>2/1</td>
<td>Mild BBS</td>
<td>M390R/M390R</td>
</tr>
<tr>
<td>10/Fc</td>
<td>FR</td>
<td>RP</td>
<td>Obesity</td>
<td>None</td>
<td>Cataract</td>
<td>2/1</td>
<td>Mild BBS</td>
<td>M390R/M390R</td>
</tr>
<tr>
<td>11/M</td>
<td>IT</td>
<td>RP</td>
<td>Nephronophthisis</td>
<td>None</td>
<td>None</td>
<td>2/1</td>
<td>Mild BBS</td>
<td>M390R/M390R</td>
</tr>
<tr>
<td>12/F</td>
<td>FR</td>
<td>RP</td>
<td>Obesity/learning difficulties</td>
<td>None</td>
<td>None</td>
<td>3/1</td>
<td>Mild BBS</td>
<td>M390R/M390R</td>
</tr>
<tr>
<td>13/F</td>
<td>NL</td>
<td>RP</td>
<td>Obesity/polydactyly</td>
<td>None</td>
<td>Cataract</td>
<td>3/1</td>
<td>Mild BBS</td>
<td>M390R/R268P</td>
</tr>
<tr>
<td>14/M</td>
<td>GE</td>
<td>RP</td>
<td>Obesity/learning difficulties</td>
<td>None</td>
<td>None</td>
<td>3/2</td>
<td>BBS</td>
<td>M390R/M390R</td>
</tr>
</tbody>
</table>

**Abbreviations:** BBS, Bardet-Biedl syndrome; BE, Belgium; CA, Canada; FR, France; GE, Germany; IT, Italy; NL, the Netherlands; RP, retinitis pigmentosa.

a Patients 3 and 7 are siblings.
b Sister with polydactyly.
c Sister with polydactyly and RP.
Cataract is commonly found in up to 50% of patients with RP and, in many patients, cataract surgery is performed at a relative early age. Consequently, in the absence of other primary or secondary signs, we believed that the condition in the 3 patients in group A should be diagnosed as nonsyndromic RP, despite the presence of

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>First Symptoms</th>
<th>Age at Examination, y</th>
<th>Visual Acuity</th>
<th>Anterior Segment</th>
<th>Ophthalmoscopy</th>
<th>Perimetry</th>
<th>Scotopic/Photopic ERG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Night blindness at age 3 y, VA loss at age 12-15 y</td>
<td>33</td>
<td>CF</td>
<td>CF</td>
<td>Polar subcapsular cataract</td>
<td>Typical RP with symmetric atrophy in the posterior pole</td>
<td>Ring scotoma at age 12 y; later in life, central islands, 15°</td>
</tr>
<tr>
<td>2</td>
<td>Night blindness at age 18 y</td>
<td>31</td>
<td>20/50</td>
<td>20/63</td>
<td>Moderate cortical cataract</td>
<td>Punctate pigment deposits and peripheral bone spicules; preserved maculae</td>
<td>Central islands, 10°</td>
</tr>
<tr>
<td>3a</td>
<td>Nystagmus and hemeralopia at age 1 y</td>
<td>55</td>
<td>LP</td>
<td>LP</td>
<td>Unknown</td>
<td>Typical RP with marked peripheral pigmentation</td>
<td>Modest temporal region of remaining sensitivity</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Night blindness at age 8 y</td>
<td>43</td>
<td>LP</td>
<td>LP</td>
<td>Posterior cortical opacity</td>
<td>Extensive atrophy, striking macular atrophy, attenuated vessels and irregular midperipheral pigmentations (Figure 1)</td>
<td>Patient could not observe targets</td>
</tr>
<tr>
<td>5</td>
<td>Night blindness at age 18 y</td>
<td>43</td>
<td>HM</td>
<td>HM</td>
<td>Pseudophakia</td>
<td>Severe maculopathy, leopard spots, diffuse pigmentation (Figure 1)</td>
<td>Patient could not observe targets</td>
</tr>
<tr>
<td>6</td>
<td>Night blindness at age 10 y</td>
<td>19</td>
<td>20/50</td>
<td>20/63</td>
<td>No abnormalities</td>
<td>Typical RP with atrophy at the fovea</td>
<td>Central islands, 5°</td>
</tr>
<tr>
<td>7a</td>
<td>Nystagmus and hemeralopia at age 1 y</td>
<td>55</td>
<td>LP</td>
<td>LP</td>
<td>Pseudophakia</td>
<td>Typical RP with macular involvement</td>
<td>Modest temporal region of remaining sensitivity</td>
</tr>
<tr>
<td>Group C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Night blindness at age 12 y</td>
<td>16</td>
<td>20/80</td>
<td>20/160</td>
<td>Unknown</td>
<td>Typical RP</td>
<td>Severely constricted (30°)</td>
</tr>
<tr>
<td>9</td>
<td>Night blindness at age 5 y</td>
<td>43</td>
<td>20/200</td>
<td>20/200</td>
<td>Posterior cortical opacity</td>
<td>RP with diffuse pigment clumping and depigmentation</td>
<td>Relative central scotoma of 60°, with relative and absolute regions</td>
</tr>
<tr>
<td>10</td>
<td>VA loss at age 23 y, moderate night blindness</td>
<td>41</td>
<td>HM</td>
<td>HM</td>
<td>Pseudophakia at age 41 y</td>
<td>Profound macular atrophy, bone spicules, and attenuated vessels</td>
<td>Large central scotoma of 40°</td>
</tr>
<tr>
<td>11</td>
<td>Nystagmus since infancy</td>
<td>21</td>
<td>LP</td>
<td>LP</td>
<td>Unknown</td>
<td>Typical RP with macular involvement</td>
<td>Patient could not observe targets</td>
</tr>
<tr>
<td>12</td>
<td>VA loss at age 10 y</td>
<td>36</td>
<td>20/63</td>
<td>20/63</td>
<td>Subcapsular cataract</td>
<td>Typical RP, normal aspect of macula</td>
<td>Severely constricted (30°)</td>
</tr>
<tr>
<td>13</td>
<td>Night blindness at age 18 y</td>
<td>38</td>
<td>20/50</td>
<td>20/40</td>
<td>Cortical cataract</td>
<td>Typical RP with severe atrophy</td>
<td>Severely constricted (30-35°)</td>
</tr>
<tr>
<td>Group D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Visual field loss at age 35 y</td>
<td>54</td>
<td>LP</td>
<td>LP</td>
<td>Cataract</td>
<td>RP without pigmentation, severe atrophy central and in midperiphery</td>
<td>Severely constricted (20°-45° OD)</td>
</tr>
</tbody>
</table>

Abbreviations: CF, counting fingers; ERG, electroretinography; HM, hand motion; LP, light perception; NR, nonrecordable; RP, retinitis pigmentosa; VA, visual acuity.

a Patients 3 and 7 are siblings.
secondary feature cataract. The same line of reasoning more or less holds with the primary feature of obesity in the patients in group B. Obesity is a rather nonspecific finding in current Western society and perhaps even more so in patients with retinal degeneration who are prone to physical inactivity. In addition to RP, the patients in group B had few primary and/or secondary features that could be associated with BBS but may be coincidental. The isolated presence of moderate obesity, especially without truncal distribution and in combination with parental obesity, is not necessarily an indication of extraocular abnormalities due to BBS1 mutations, so that nonsyndromic RP is at least a possibility. Following this approach, 3 of our 14 patients (group A) exhibited nonsyndromic RP and 4 patients (group B) demonstrated a form of RP that may be nonsyndromic.

Despite the inclusion criterion of autosomal recessive and/or isolated RP, 7 patients showed syndromic features that seem to be associated with the retinal phenotype. In one patient (group D), a previously undiagnosed classic form of BBS was discovered. The extra digits were removed shortly after birth in this patient, emphasizing the importance of obtaining a complete history in patients with RP. In the remaining 6 patients (group C), extraocular features were present, but these abnormalities were insufficient to warrant the diagnosis of classic BBS. Extraocular features in these 6 patients were highly divergent, making correct identification of this milder form of BBS challenging. In patient 11, RP was associated with nephropathesis, a combination known as the Senior-Løken syndrome. This syndrome has been associated with mutations in the nephronophthisis genes and not, to our knowledge, with BBS1 mutations. The observation that BBS1 mutations may cause milder BBS phenotypes has been reported in a case history of 2 brothers. The danger of a misdiagnosis in patients with milder BBS phenotypes is important in view of the potentially severe consequences of life-threatening conditions associated with BBS1 mutations, as well as the accuracy of genetic counseling.

The presence of a presumed unaffected individual with 2 p.M390R variants in our study is not unprecedented; Badano and coworkers reported on 2 families with 2 p.M390R alleles in BBS patients and their unaffected fathers. The differential penetrance of BBS1 alleles in one of these families was subsequently explained by the detection of a heterozygous trans-acting epistatic allele (MGC1203; c.430C>T) in the patient with BBS but not in the unaffected father. The MGC1203 gene encodes a pericentriolar protein that interacts with BBS1, BBS2, BBS4, BBS5, BBS6, BBS7, and BBS8 and colocalizes with BBS1 and BBS4. The MGC1203 variant c.430C>T enhances the use of a cryptic splice acceptor site, causing the introduction of a premature stop codon and the reduction of MGC1203 mRNA levels. An alternative explanation for the variable phenotypes associated with BBS1 alleles could be cis-acting regulators of expression. We did not find this modifier in our case and control patients with 2 BBS1 alleles. Different enhancer or promoter variants may result in higher messenger RNA expression levels of the BBS1 p.M390R alleles in unaffected individuals, which may compensate for the reduced activity of the p.M390R-carrying BBS1 protein (Figure 2A, cis-acting modifier). Alternatively, as shown in Figure 2B, mild BBS or BBS can also be caused by a combination of 2 BBS1 variants and a negatively acting trans-acting modifier, such as the MGC1203 variant c.430C>T.

Figure 1. The right eye of patient 4 at age 33 years. This color fundus photograph illustrates the severity of the retinitis pigmentosa phenotype in many of the patients in this study. In addition to the midperipheral bone spicules and attenuated vessels, there is widespread chorioretinal atrophy. The atrophy is especially severe at the posterior pole. The visual acuity in this patient was only light perception; electroretinographic responses were absent.

Figure 2. Cis- and trans-acting Bardet-Biedl syndrome (BBS) gene BBS1 expression regulator model. A, Enhancer/promoter variants (cis-acting) may modulate the messenger RNA expression levels of BBS1 and thereby determine the penetrance or expression of disease. Unaffected individuals carry hypomorphic variants (v1 and v2, such as the p.M390R variant) on 1 or 2 highly (+++) expressed BBS1 alleles; moderately (++) expressed BBS1 alleles result in nonsyndromic retinitis pigmentosa (RP) or mild BBS, and low (+−) expressed BBS1 alleles result in BBS. B, A trans-acting third variant (v3) present in an interactor of BBS1 or a transcription factor negatively regulating the expression of BBS1 may contribute to the phenotype.
In conclusion, by analyzing a very large cohort of patients with isolated or autosomal recessive RP, we identified BBS1 variants in individuals with a wide clinical spectrum, ranging from nonsyndromic RP (mild BBS) to classic BBS.

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