Genotype-Phenotype Correlations in Bardet-Biedl Syndrome

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Objective: To determine whether mutations in different Bardet-Biedl syndrome (BBS) genes result in different ocular phenotypes.

Methods: Thirty-seven patients from 31 families were enrolled who met the clinical criteria for BBS and for whom a BBS mutation had been identified. Seventeen patients harbored mutations in BBS1, 10 in BBS10, and 10 in other genes (BBS2, BBS3, BBS5, BBS7, and BBS12). All the patients underwent ocular examination; 36 patients had computerized full-field electroretinograms (ERGs).

Results: Visual acuity was significantly better in BBS1 patients than in patients with other BBS mutations (P = .01), and a larger proportion of BBS1 patients had good (≥20/50) visual acuity (P = .01). The ERG amplitudes were significantly higher in BBS1 patients than in patients with other BBS mutations in response to 0.5-Hz and 30-Hz flashes (P < .001 for both). All the BBS1 patients harbored at least 1 missense mutation compared with only 45% of patients with mutations in other BBS genes (P < .001); the rest harbored only null alleles. However, multivariate analysis demonstrated that visual acuity or ERG amplitude did not depend on the type of mutation present (missense or null) when controlling for BBS gene. Prevalences of bone spicule pigmentation and cataract were comparable in BBS subtypes.

Conclusions: Patients with BBS1 mutations had a milder phenotype than did patients with mutations in other BBS genes. Clinically, this manifested as significantly better visual acuity and larger ERG amplitudes.

Clinical Relevance: These phenotypic differences can help guide genetic testing and genetic counseling for patients with this syndrome.


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THE BARDET-BIEDL SYNDROME (BBS) IS AN AUTOSOMAL RECESSIVE DISORDER WITH A PREVALENCE OF APPROXIMATELY 1:100 000 IN NORTH AMERICA.1 IT IS A MULTISYSTEM SYNDROME COMPRISING OCULAR AND NONOCULAR MANIFESTATIONS.2,4 OCULAR FINDINGS CLASSICALLY INCLUDE SIGNIFICANT RETINAL DEGENERATION (>90% PENETRANCE IN PATIENTS WITH BBS), POSTERIOR SUBCAPSULAR CATARACT, AND VARIABLE BONE SPICULE PIGMENTATION.3 RETINAL FUNCTION IS CHARACTERIZED BY ROD-CONE OR CONE-ROD DEGENERATION, LEADING TO PROGRESSIVE VISUAL LOSS BEGINNING EARLY IN LIFE AND POTENTIALLY LEADING TO LEGAL BLINDNESS BY THE SECOND DECADE OF LIFE.3 NONOCULAR FINDINGS ARE VARIABLE AND INCLUDE OBESITY, POLYDACTLY, RENAL DYSFUNCTION, HYPOGONADISM, AND COGNITIVE IMPAIRMENT.4-8,7 THE GENETICS OF BBS (OMIM 209900) ARE LIKELY COMPLEX. AT LEAST 15 KNOWN BBS GENES (BBS1-BBS15) ARE IMPLICATED IN THIS DISEASE.8,9 WHEREAS BBS1 AND BBS10 ARE RELATIVELY COMMON,10-12 OTHERS (BBS11, BBS13, BBS14, AND BBS15) HAVE BEEN REPORTED ONLY IN SINGLE FAMILIES.9,14,15 ALL THESE GENES ARE INVOLVED IN CODING PROTEINS IN THE BASAL BODY OF THE CILIUM, AND, THEREFORE, PHOTORECEPTORS, AS MODIFIED CILIATED CELLS, ARE AFFECTED.8,16

Given that mutations in multiple genes can cause BBS, it is not surprising that the phenotypes are variable. In fact, there is much variability not only between families that harbor the same genotype but also within individual families.8 Some studies17 have attempted to describe the phenotypes of patients with mutations in a given individual BBS gene. Other researchers18 have attempted to characterize the phenotype of BBS across genotypes more broadly. However, to our knowledge, no study has demonstrated a statistically significant difference in ocular phenotype between genotypes. In this study, we evaluated the clinical and electroretinographic (ERG) findings of patients with mutations in a variety of...
BBS genes. Whereas clinical features such as bone spicule pigmentation and cataract did not differentiate among genotypes, patients with mutations in the BBS1 gene had significantly higher ERG amplitudes than did patients with mutations in other BBS genes studied, and BBS1 patients were also more likely to have good visual acuity.

**METHODS**

**PATIENTS**

Thirty-seven patients from 31 families who met the clinical criteria for BBS based on the presence of 4 of the 6 primary criteria (retinal degeneration, polydactyly, obesity, learning disabilities, renal anomalies, and hypogonadism), or 3 primary and 2 secondary criteria (reviewed in detail elsewhere), were enrolled. These patients, all seen in the Berman-Gund Laboratory, represent a subset of patients whose mutations were described in a previous study by our group. The clinical findings in this subset of patients have not been previously described. Examination findings at the time of the first visit to the Berman-Gund Laboratory and of the first ERG were used in these analyses. This study was approved by the Harvard Medical School and Massachusetts Eye and Ear Infirmary institutional review boards; all work reported herein is compliant with the Health Insurance Privacy and Accountability Act, and the protocol was consistent with the Declaration of Helsinki.

**MUTATION SCREENING**

We previously performed a mutation screen of all the exons, and the intron-exon boundaries/splice sites, of the 15 identified BBS genes (BBS1-BBS15) in these patients. The determination of mutation status and the potential pathogenicity of all the mutations found in this cohort were described in a previous article. Briefly, the coding regions and intron-exon boundaries of the 15 genes were amplified by polymerase chain reaction using standard methods. Polymerase chain reaction products were analyzed on 2% agarose gel and were purified by vacuum filtration, and direct bidirectional sequencing was conducted, as previously described. Sequence variation was considered pathogenic when it (1) segregated with the disease in the family, (2) was not present in 96 controls, (3) altered a well-conserved amino acid, and (4) was judged significant in a computational test for novel mutations (the Neutral Network Splice Site Scoring Program for splice site mutations and PolyPhen for missense variations).

**OPHTHALMOCUTIC EXAMINATION**

All the patients underwent full ophthalmologic examination by 1 of us (E.L.B.), including determination of best-corrected Snellen visual acuities, Goldmann visual fields (V4e white test light), slitlamp biomicroscopy, Goldmann applanation tonometry, and dilated funduscropy. Full-field ERGs were recorded from 36 patients as previously described. Responses less than 10 µV in amplitude or responses that were variable owing to nystagmus were computer averaged. The lower limit of detectability is 1 µV to 0.5-Hz white flashes with computer averaging (the lower limit of normal is 350 µV). The lower limit of detectability is 0.05 µV to 30-Hz white flashes with narrow bandpass filtering and computer averaging (the lower limit of normal is 30 µV). For the purposes of statistical analysis, nondetectable values were coded as 1 µV to 0.5-Hz white flashes or as 0.05 µV to 30-Hz white flashes. The nonocular phenotypes of these patients were not assessed for this study.

**STATISTICAL ANALYSIS**

Visual acuities were converted to decimals and then to normalized ranks using the van der Waerden transformation. Visual fields were converted to areas by scanning and custom software, and visual field areas and ERG amplitudes were transformed to natural logarithms to better approximate normal distributions. Multiple regression was performed using PROC MIXED in SAS, version 9.1.3 (SAS Institute, Inc), with ocular function as the dependent variable and mutation (BBS1 vs other) as a class variable and age as a potentially significant covariate. The patient's age at the time of the first evaluation was used in all the analyses. This procedure adjusts for the correlation between eyes of the same patient and takes into account missing data. With the better eye as the unit of analysis, the relation between 2 class variables was assessed by the Fisher exact test and between a class-dependent variable and a continuous independent variable by logistic regression using JMP, version 6.0 (SAS Institute, Inc). Receiver operating characteristic curves were generated by an option in the logistic regression platform to find the best combination of specificity and sensitivity for predicting genotype by ocular function.

**RESULTS**

**DISTRIBUTION OF PATIENTS**

Of 37 patients, 17 (46%) harbored paired mutations in BBS1, 10 (27%) harbored paired mutations in BBS10, and 10 (27%) harbored paired mutations in another BBS gene. Of the 10 patients who harbored mutations in genes other than BBS1 or BBS10, 3 unrelated patients (8% of the total) had mutations in BBS2, 3 unrelated patients (8%) had mutations in BBS7, 2 siblings (5%) had mutations in BBS3, 1 patient (3%) had mutations in BBS5, and 1 patient (3%) had mutations in BBS12 (Table). For the purpose of assigning patients to a particular BBS locus, each patient was confirmed to have paired mutations in the particular BBS gene, whether homozygous for a single specific mutation or compound heterozygous for that gene. In addition, 5 patients displayed triallelism in that they were also heterozygous for a single mutation in a second BBS gene. One patient with 2 mutations in the BBS1 gene also harbored a single mutant allele at BBS9, 1 BBS2 patient was also heterozygous at BBS10, 1 BBS3 patient was heterozygous at BBS8, 1 BBS10 patient was heterozygous at BBS12, and 1 BBS12 patient was heterozygous for a mutation at the BBS1 locus (Table).

The mean age of all the patients at the time of first evaluation was 24.8 years (age range, 7-51 years). The mean (SD) age of patients with mutations in BBS1 (24.9 [10.7] years) did not differ from that of BBS10 patients (24.7 [12.0] years) or patients with mutations in other BBS genes (24.6 [13.0] years; P = .74, analysis of variance). Nineteen patients were male (8 BBS1 patients) and 18 were female (9 BBS1 patients).

**CLINICAL OCULAR PHENOTYPES**

Best-corrected Snellen visual acuity was significantly better in patients with BBS1 mutations than in patients with other BBS mutations, adjusting for age (P = .01). When triallelic patients were excluded from the analysis, so as...
to remove any confounding that this might introduce, this comparison remained significant (P < .001). Moreover, 41% of BBS1 patients had binocular visual acuity of at least 20/50 compared with only 10% of all other BBS patients, adjusting for age (P = .01) (Figure 1). Acuities were generally symmetrical between fellow eyes.

There was no significant difference in the prevalence of bone spicule pigmentary retinopathy in BBS1 patients (53%) compared with patients with mutations in other BBS genes (60%; P = .61). Similarly, the prevalence of posterior subcapsular cataract (or pseudophakia) in BBS1 patients (24%) compared with other BBS genes (26%; P = .902) was not significantly different.

### Table. Clinical, Mutation, and ERG Data in 37 Patients With Bardet-Biedl Syndrome

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<tr>
<th>Patient No./Sex/Age, y</th>
<th>BBS Gene</th>
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<th>Mutation Type</th>
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Abbreviations: BCVA, best-corrected visual acuity; BSP, bone spicule pigmentary retinopathy; CF1, counting fingers at 1 ft; CF5, counting fingers at 5 ft; Del, deletion; ERG, electroretinographic; fs, frameshift; HM, hand motions; Ins, insertion; LP, light perception; Mis, missense; NA, not available; ND, nondetectable; PSC, posterior subcapsular cataract (or the presence of pseudophakia after cataract extraction); Spl, splice site mutation; Term, termination; +, present; −, not present.

aLower limit of detectability for ERG recordings is 1 µV to 0.5-Hz flashes and 0.05 µV to 30-Hz flashes. Visual acuities are expressed as decimals (eg, 20/20 = 1.0 and 20/50 = 0.4).
bCapital superscript letters refer to siblings (eg, A and A are siblings, B and B are siblings, etc).
cBBS gene with 2 mutated alleles.
dSecond gene involved with a (heterozygous) mutated allele.
patients (40%) was not significantly different \( (P=.11) \). Visual fields available from 14 patients (7 BBS1 and 7 other BBS) suggested a trend toward a larger field area in BBS1 patients compared with the other genotypes studied herein, although it was not significant \( (P=.11) \).

**ELECTRORETINOGRAPHY**

The ERG amplitudes (mixed cone-rod responses) to 0.5-Hz white flashes were significantly higher in BBS1 patients (geometric mean = 15.4 \( \mu \)V) than in patients with mutations in other BBS genes (geometric mean = 1.63 \( \mu \)V) adjusting for age \( (P<.001) \) (Figure 2A). With the 5 triallelic patients excluded from the analysis, 0.5-Hz amplitudes remained significantly larger in BBS1 patients than in other BBS patients \( (P<.001) \). Moreover, 60% of BBS1 patients had amplitudes greater than 10 \( \mu \)V, and fewer than one-quarter had nondetectable responses (ie, <1 \( \mu \)V). In contrast, 81% of patients with mutations in other BBS genes had nondetectable 0.5-Hz responses (Figure 3A).

Similarly, cone ERG amplitudes to 30-Hz white flashes were significantly higher in BBS1 patients (geometric mean = 4.86 \( \mu \)V) than in other BBS patients (geometric mean = 0.27 \( \mu \)V), adjusting for age \( (P<.001) \) (Figure 2B). We again found that excluding triallelic patients from the analysis did not affect the significance of this association \( (P<.001) \). Thirty-seven percent of BBS1 eyes had 30-Hz cone ERG amplitudes greater than 10 \( \mu \)V, and an additional 40% had amplitudes of 1 to 9.9 \( \mu \)V. Only 23% of BBS1 patients had amplitudes less than 1 \( \mu \)V. In comparison, only a single eye of 1 non-BBS1 patient had an average 30-Hz cone ERG amplitude greater than 1 \( \mu \)V (1.26 \( \mu \)V) (Figure 3B). Representative 30-Hz cone ERG responses are illustrated in Figure 4.

Receiver operating characteristic curves demonstrated that ERG amplitudes to 0.5- and 30-Hz flashes (using each patient’s better eye) could be used to efficiently differentiate patients with BBS1 mutations from those with other BBS mutations. The best discriminator between genotypes was a bandpassed, computer-averaged 30-Hz cone ERG amplitude greater than 1 \( \mu \)V \( (P<.001) \) in the better of the patient’s 2 eyes, which was associated with a sensitivity for BBS1 mutation of 82% and a specificity of 95%. None of the patients with BBS1 mutations and cone ERG amplitudes less than 1 \( \mu \)V had good acuities, and, thus, visual acuity added nothing to the predictive power of this model.

![Figure 1](https://archophthalmol.com/figure1.png)

**Figure 1.** Patients with mutations in the BBS1 gene were more likely to have good acuity \( (\geq 20/50) \) than were patients with mutations in other BBS genes.

![Figure 2](https://archophthalmol.com/figure2.png)

**Figure 2.** Electroretinographic amplitudes to 0.5-Hz (A) and 30-Hz (B) white flashes by eye. The \( P \) values are based on a comparison of log data. Error bars represent SD.

![Figure 3](https://archophthalmol.com/figure3.png)

**Figure 3.** Graph showing the distribution of eyes in BBS patients with 0.5-Hz (A) and 30-Hz (B) electroretinographic (ERG) amplitudes in each range.
MUTATION TYPES

All the BBS1 patients harbored at least 1 missense mutation compared with 45% of patients with mutations in other BBS genes, the rest of whom harbored exclusively null alleles (frameshift, termination, or splice site mutations; \( P < .001 \)). For BBS1 patients, the M390R missense mutation was most common (Table).

Multivariate analyses including mutation type (missense vs exclusively null alleles) along with age and the BBS gene involved demonstrated that the particular type of mutation was not an independent predictor of 0.5-Hz ERG amplitude \( (P = .02) \), 30-Hz ERG amplitude \( (P = .09) \), or visual acuity \( (P = .44) \), whereas BBS1 gene involvement remained a significant predictor of 0.5-Hz ERG amplitude \( (P = .008) \), 30-Hz ERG amplitude \( (P < .001) \), and visual acuity \( (P = .01) \).

The present study shows that patients with mutations in the BBS1 gene have a milder phenotype than do patients with mutations in other BBS genes, with significantly larger 0.5- and 30-Hz full-field ERG amplitudes and significantly better visual acuities. In contrast to these disparities in ERG function and visual acuity, this study shows that the presence of bone spicule pigmentary retinopathy or posterior subcapsular cataract did not vary among genotypes. Similarly, there was no significant difference in the retained visual field area among genotypes. Thus, ERG function and acuity, not ocular appearance or visual field, help distinguish BBS1 from other genotypes.

Since patients with BBS1 mutations seem to have a milder ocular phenotype, one might expect that BBS1 patients would have their first evaluation at an older age. However, since the average age in each genotype category was remarkably similar in this study, and all statistical modeling was adjusted for age, these differences in ERG amplitudes and visual acuity cannot be attributed to the age at which these patients were first seen.

Of the 15 known genes involved in BBS, 4 (BBS11, BBS13, BBS14, and BBS15) are extremely rare, often reported in only a single family each.\(^9,14,15\) Of the remaining 11 genes found in multiple families, 7 are represented in the present cohort (BBS1, BBS2, BBS3, BBS5, BBS7, BBS10, and BBS12). Similar to most other studies,\(^10,11\) the BBS1 and BBS10 genes were the most frequently mutated in the present cohort (46% and 27%, respectively), and among these, the M390R mutation (in BBS1) and the C91fsX5 mutation (in BBS10) were the most common alleles, with prevalences similar to those reported elsewhere.\(^12\) Prevalences of homozygosity were also comparable with those previously reported.\(^12\)

The present distribution of BBS gene involvement is typical for a population that is mostly of European ancestry. The clinical ocular phenotypes for many of the genotypes not represented in this study (BBS4, BBS6, BBS8, and BBS9) have been described elsewhere in the literature.\(^17,18,21-23\) Generally, these genotypes have shown a severe phenotype, with early blindness and severely reduced or nondetectable ERGs (for studies in which ERG data are provided). No researchers, to our knowledge, have identified a mild ocular phenotype among any of the other genotypes (BBS4, BBS6, BBS8, and BBS9) not included in the present cohort. A severe ocular phenotype has been described by Moore and colleagues\(^21\) for the BBS6 genotype, which has a high prevalence in Newfoundland, Canada. Billingsley and colleagues\(^22\) likewise found that the phenotype in patients with BBS6 mutations was severe and was similar to that observed in patients with BBS10 and BBS12 mutations, without any statistically significant differences between genotypes. The phenotype of Norwegian patients with BBS4 mutations, described by Riise and colleagues,\(^17\) was noted to consist of severe retinitis pigmentosa with onset in early childhood. The BBS2, BBS3, and BBS4 mutations are common in the Bedouin population in the Negev Desert in Israel, and their phenotype was described by Heon and colleagues,\(^23\) who noted that retinitis pigmentosa was severe and early in all cases, with moderate to high myopia in BBS4 patients especially. Recently, Deveault and colleagues\(^18\) described the clinical ocular phenotypes of a heterogenous population of patients, including some with BBS8 and BBS9 mutations. Patients with mutations in these genes had ERGs that were nondetectable and had very poor acuities. None of the BBS genes not represented in the present study harbored mild ocular phenotypes. Therefore, although we can state definitively only that the BBS1 phenotype is milder than the other genotypes included in this study, it seems that this conclusion also applies to genotypes not included in this cohort because they have all been reported to have severe ocular phenotypes as well.

Previous studies have not identified any correlation between individual genotypes and particular ocular phenotypes in a way that might allow for clinical differentiation between genotypes. In a study\(^22\) focusing on patients with mutations in BBS6, BBS10, and BBS12, no ocular phenotype-genotype correlations were found, consistent with the present findings of a similar phenotype in BBS10 and BBS12. In a recent study of 105 patients,\(^18\) which included patients with BBS1 mutations, no clear

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**COMMENT**

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genotype-phenotype correlation was reported; the inability to identify a difference in ERG amplitudes between genotypes might relate to the fact that the study describes nondetectable responses for 80% of their patients. By using narrow bandpass filtering and computer-averaging of 30-Hz flicker cone ERGs, we could quantify amplitudes in nearly all the patients.

Recent studies24,25 have identified the various BBS gene products as components of a complex (termed the BBSome) that localizes to the cilium basal body, where it is involved in protein trafficking into and along the cilium. The core BBSome complex comprises BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, and BBS9.24 BBS6, BBS10, and BBS12 form a chaperone-type complex that mediates BBSome assembly.26 Thus, the apparent polygenic and pleiotropic syndrome results from mutation in a component of the same complex, thus causing the same overall clinical syndrome. BBS1 plays a unique role in this complex in serving as the direct interacting partner of Rabin8, which helps recruit the BBSome to the centromere/basal body from the neighboring centriolar satellites.24,27 This might provide a molecular basis for the clinical observation that patients with BBS1 mutations are not as severely affected as patients with mutations in other BBS genes. One could hypothesize that rather than being a structurally integral component of the BBSome itself, BBS1 effects its role as an adapter protein to Rabin8. Therefore, mutations of BBS1 might affect only this adapter function rather than result in dissolution of the entire core complex, as might result from mutations in other BBS genes.

Alternatively, one could hypothesize that the relatively mild phenotype of BBS1 patients might be more related to the type of mutations found in this gene, rather than the particular cellular functions of the particular protein that is mutated. In our study, all BBS1 patients were found to have at least 1 missense mutation, and often both mutations were predicted to be of the missense type (namely, a substitution of one amino acid for another, resulting in a dysfunctional protein). In contrast, only 45% of patients with mutations in other BBS genes had at least 1 missense mutation; the rest harbored nonsense, deletion, frameshift, or splice site mutations, which would be expected to cause a gene defect that leads to a completely nonfunctional or truncated protein or, perhaps, to nonsense-mediated decay. These prevalences of different mutation types are consistent with previous studies.13 However, including the type of mutation in multiple logistic regression modeling along with the BBS gene involved and age demonstrated that the type of mutation (whether the patient harbored missense mutations or exclusively null alleles) was not an independent predictor of visual acuity or ERG amplitude, whereas involvement of the BBS1 gene remained a significant predictor of phenotype.

In the present study, we classified patients according to the gene within which they harbored 2 mutated alleles (ie, they were homozygous or compound heterozygous within that particular gene). However, there has been much debate recently about the presence of nonmendelian inheritance in BBS, including suggestions that triallelism might lead to more severe phenotypes.28 Indeed, 5 patients had 3 mutations (ie, they were homozygous null in 1 BBS gene and heterozygous in a second BBS gene). All 5 patients with 3 mutations had severe phenotypes, with poor acuity and cone ERG amplitudes of less than 1 µV. However, all but 1 of these patients would have been predicted to have a severe phenotype based on their primary, homozygous gene (in BBS2, BBS3, BBS10, or BBS12), and 2 of these 4 additional heterozygous mutations should cause neutral (nonpathogenic) amino acid sequence changes.19 Supporting this, one of the BBS3 patients had an affected brother who did not harbor this additional third mutation yet manifested just as severe a phenotype, suggesting that the primary gene with 2 mutated alleles was sufficient to cause the phenotype (Table). Overall, we could not find compelling evidence for an effect of triallelism in this study population, although the study does not provide sufficient numbers of patients to address this issue.

The clinical applicability of these findings for genetic counseling should be evident. Many of our patients have come to the clinic asking for genetic testing to determine which gene mutation they harbor, and often family members who are potential carriers would like to be tested. Of course, this is not always feasible given the large number and size of these genes. To determine the probability of a patient having a mutation in the most common BBS1 gene, we created receiver operating characteristic curves. The ERG amplitudes to 0.5- and 30-Hz flashes (using each patient’s better eye) could be used to efficiently differentiate patients with BBS1 mutations from patients with other BBS mutations. The best discriminator between these 2 genotypes was the bandpassed, computer-averaged, 30-Hz cone ERG amplitude in the better of the patient’s 2 eyes. If the amplitude was greater than 1 µV (using methods previously described20), then the sensitivity for BBS1 mutation was 82% and the specificity was 95%.

In summary, we demonstrated that patients with mutations in the BBS1 gene have a milder phenotype than do patients with mutations in other BBS genes. Clinically, this manifests as significantly better acuity and larger ERG amplitudes. Multivariate analysis excluded the possibility that this phenotypic difference was attributable to the fact that the BBS1 mutations tended to be missense mutations, whereas mutations in other BBS genes were mostly null. Clinical findings such as bone spicule pigmentary retinopathy and posterior subcapsular cataract are poor predictors of genotype, whereas ERG measures of retinal function were the strongest predictors of genotype. This difference in clinical phenotypes can help guide genetic testing and genetic counseling for patients using the provided clinical prediction guideline.

Submitted for Publication: August 23, 2011; final revision received December 2, 2011; accepted December 8, 2011.

Published Online: March 12, 2012. doi:10.1001/archophthalmol.2012.89

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Author Contributions: Dr Berson had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Financial Disclosure: None reported.

Funding/Support: This work was supported in part by a center grant from the Foundation Fighting Blindness (Dr Berson).

Role of the Sponsors: This sponsor supported the collection of data but was not involved in the design or analysis of the study.

Previous Presentations: A portion of this work was presented at the Association for Research in Vision and Ophthalmology annual meeting: May 3, 2010; Fort Lauderdale, Florida; and the Aegean Retina Meeting: July 10, 2011; Rethymno, Crete, Greece.

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