Association of Clinical and Genetic Heterogeneity With BEST1 Sequence Variations

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IMPORTANCE Detailed phenotypic information on the spectrum of fundus abnormalities and clinical variability of all phenotypes associated with sequence variations in BEST1 is limited.

OBJECTIVE To report a detailed phenotypic and genetic analysis of a patient cohort with sequence variations in BEST1.

DESIGN, SETTING, AND PARTICIPANTS This retrospective case series took place at the Oxford Eye Hospital in Oxford, UK. Thirty-six patients from a single center with disease-causing sequence variations in BEST1 from 25 different families were analyzed. Data were collected from November 2017 to June 2018, and analysis began April 2018.

MAIN OUTCOMES AND MEASURES Results of ocular phenotyping and genetic testing using targeted next-generation sequencing to identify BEST1 sequence variations.

RESULTS Thirty-six patients from 25 families with disease-causing sequence variations in BEST1 were included. Of 36 patients, 20 (55.6%) were female. Three distinct clinical phenotypes were identified: autosomal recessive bestrophinopathy (ARB), best vitelliform macular dystrophy (BVMD), and adult-onset vitelliform macular dystrophy. The ARB phenotype group comprised 18 patients from 9 families with age in years at symptom onset ranging from less than 10 to 40s. All patients showed a common phenotype of fundus autofluorescence abnormalities, and spectral-domain optical coherence tomography features were similar in all patients with schitic and cystoid changes. A phenotype of a beaten metallic retinal appearance extending from the mid periphery to the far periphery was identified in 8 patients. Four patients from 1 family with ARB were previously reported to have autosomal recessive retinitis pigmentosa but were reclassified as having ARB as part of this study. The BVMD phenotype group comprised 16 patients from 14 families with age at symptom onset ranging from less than 10 to 70s. Fundus features were localized to the macula and consistent with the stage of BVMD. In the adult-onset vitelliform macular dystrophy phenotype group, the age in years at symptom onset varied from 50s to 70s in 2 patients from 2 families. Fundus features included small vitelliform lesions. Where available, electro-oculogram results demonstrated a reduced or absent light rise in all patients with ARB and BVMD. Genetic testing identified 22 variants in BEST1.

CONCLUSIONS AND RELEVANCE These findings support the notion that ARB, BVMD, and adult-onset vitelliform macular dystrophy are clinically distinct and recognizable phenotypes and suggest that the association of autosomal recessive retinitis pigmentosa with sequence variations in BEST1 should be rereviewed.

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The range of phenotypes associated with sequence variations in *BEST1* includes 5 clinically distinct retinal phenotypes: autosomal dominant best vitelliform macular dystrophy (BVMD) (otherwise known as vitelliform macular dystrophy), adult-onset vitelliform macular dystrophy (AVMD) (OMIM 608161), autosomal dominant vitreoretinochoroidopathy (ADVIRC) (OMIM 193200), autosomal recessive bestrophinopathy (ARB) (OMIM 611809), and autosomal dominant and recessive retinitis pigmentosa (RP) (OMIM 268000).1

*BEST1* encodes the transmembrane channel protein bestrophin-1, which is primarily expressed in the retinal pigment epithelium (RPE)2 and highly conserved across species.3 Bestrophin-1 consists of 585 amino acids and contains 4 transmembrane-spanning domains (TMD) with a large hydrophobic cytoplasmic loop between TMD2 and TMD3 (eFigure 1 in the Supplement).4 It is localized in the RPE basolateral plasma membrane2 where it acts as a regulator of intracellular calcium signaling and as an ion channel.1 However, it is still unclear how sequence variations in *BEST1* lead to retinal degeneration and why they cause the clinically distinct phenotypes collectively known as bestrophinopathies. Various theories have been proposed including defects in protein trafficking (seen in other channelopathies); oligomerization or anion channel activity that may have a deleterious effect; or sequence variations that may cause protein activation and change the way the protein is regulated.1

Phenotypic data regarding the spectrum of bestrophinopathies are derived from single case reports and small case series, but detailed phenotypic characterization with genotype correlation is limited. Although BVMD and AVMD are well described, and to a lesser extent ARB and ADVIRC, to date and to our knowledge there have been only 2 published reports regarding the phenotypic features in RP associated with *BEST1* sequence variations amounting in a total of 9 patients (6 families).5,7 The association of RP with sequence variations in *BEST1* has already been questioned.4 It is not yet fully understood how different variants in *BEST1* lead to the phenotypically distinct spectrum of bestrophinopathies, and an in-depth evaluation of phenotypic similarities between distinct *BEST1*-associated retinal phenotypes has not been previously undertaken. We report a detailed phenotypic and genetic analysis of a cohort of patients with disease-causing sequence variations in *BEST1* from a single British center and examine genotype and phenotype correlations.

**Methods**

The case notes of all patients from the Oxford Eye Hospital identified with disease-causing sequence variants in *BEST1* were reviewed to obtain ophthalmic details and family pedigrees. Sixteen patients underwent genetic testing using the Oxford next-generation sequencing inherited retinal diseases phenotype-based gene panels, and 17 patients underwent a *BEST1* gene screen or a family test. Clinical assessment included best-corrected visual acuity, refraction, slitlamp biomicroscopy, widefield retinal imaging (Optomap AI0022; Optos Ltd), short-wavelength fundus autofluorescence (Spectralis; Heidelberg Engineering), spectral-domain optical coherence tomography (SD-OCT) (Spectralis; Heidelberg Engineering), Goldmann visual field analysis, and electrodiagnostic testing where available. Ethics committee approval was obtained from the Essex 2 Research Ethics Committee with written patient consent from adults and assent from minors, and this study was conducted in adherence to the tenets of the Declaration of Helsinki.9 Patients did not receive compensation or an incentive for participating in this study. Data were collected from November 2017 to June 2018, and analysis began April 2018.

Genetic analysis of patients has evolved with our increasing knowledge of disease-causing genes and the advancement of new screening technologies. At the time of writing, enrichment for the *BEST1* gene was achieved as part of a customized HaloPlex enrichment system kit (Agilent Technologies) designed to capture the coding exons and at least 10 bp of the flanking introns of 111 retinal genes in the Oxford next-generation sequencing inherited retinal diseases phenotype-based gene panel (eMethods and eTable 1 in the Supplement).10 However, as this is a retrospective review, not all patients were screened on this panel but would have been screened by the most appropriate method available at their time of presentation. In silico analysis using 3 different prediction methods, PolyPhen-2,11,12 Sorting Intolerant from Tolerant,13,14 and Mutation Taster,15,16 to determine the deleteriousness of the variants, was carried out on all variants identified.

**Key Points**

**Question** What is the phenotypic variability associated with sequence variations in *BEST1*?

**Findings** In this case series of 36 patients, substantial phenotypic variability was identified in family members with the same variants. A phenotype associated with autosomal recessive bestrophinopathy led to the reclassification of 4 patients previously reported to have autosomal recessive retinitis pigmentosa associated with sequence variations in *BEST1* as having autosomal recessive bestrophinopathy.

**Meaning** These results support the notion that patients with sequence variations in *BEST1* can present with a wide spectrum of phenotypes that can exhibit substantial clinical variability.
identified in 4 participants (eTable 2 in the Supplement). The genetic testing results and clinical phenotypes of patients 1.1, 1.2, 1.3, 1.4 (family 5 in Davidson et al⁶), and 9.1 (family 4 in Burgess et al⁷) have previously been reported.

**ARB**

Eighteen patients (6 male, 12 female) from 9 families with an ARB clinical phenotype were identified. Their clinical characteristics were described in Table 1. None of the patients had evidence of shallow anterior chambers. All patients showed a common phenotype of autofluorescence abnormalities, which generally included a band of increased autofluorescence signal surrounding an area of decreased autofluorescence signal and ranging in extent from inside the central macula to external to the arcades (eFigure 2A and eTable 4 in the Supplement). In some individuals, the band of increased autofluorescence was also studied with focal vitelliform deposits (eFigure 2B and eFigure 3 in the Supplement). The SD-OCT features were similar in all patients with scitic and cystoid changes involving the center of the fovea extending in some cases throughout the whole macula to the arcades and beyond (eFigure 2 and eFigure 3 in the Supplement). Widefield retinal imaging was performed in 11 patients, and 8 of these patients showed additional abnormalities of the retina with a beaten metallic appearance extending from the mid periphery to the far periphery primarily in the temporal region but in some cases involving 360° (Figure). One patient (1.1) demonstrated bilateral nummular choriotinal atrophy in the far periphery of the temporal region (eFigure 4 in the Supplement). In 2 patients (2.1 and 7.1), central subretinal yellow deposits were seen, similar to the vitelliform or vitelliruptive stage of BVMD (eFigure 3 in the Supplement). One patient (7.1) demonstrated focal choroidal excavation in their left eye, and 1 patient (1.3) had an exudative retinal detachment in their right eye that spontaneously resolved.

Full-field electroretinogram (ERG) results were normal in 4 patients and abnormal in 7 patients, and electro-oculogram (EOG) results demonstrated a reduced or absent light rise in all patients.
Abbreviations: BCVA, best-corrected visual acuity; CF, counting fingers; NA, not applicable; NP, not performed; NR, not reported.

The most common sequence variation was c.418C>G, p.(Leu140Val) (gnomAD frequency = 0.000035; homozygous in 5 families) and p.(Val317Met) and p.(Met325Thr), have previously been identified by Burgess et al.17 Insilico analysis predicted this change to be pathogenic and the minor allele frequency in gnomAD was 0 (eTable3 in the Supplement). Patients 8.1 and 9.1 were compound heterozygotes, and these 4 missense sequence variations have previously been reported as disease causing.17,20

tested (eTable5 in the Supplement). Preservation of peripheral visual fields was seen in all patients tested, with central visual field loss affecting 5 patients (eTable 5 in the Supplement).

All 9 families with an ARB phenotype had missense sequence variations in BEST1, 7 had homozygous changes, and 2 were compound heterozygotes (Table 1 and eTable 3 in the Supplement). The most common variant in this cohort was c.418C>G, p.(Leu140Val) (gnomAD frequency = 0.000035; homozygous frequency is 0), which was homozygous in 5 families of South Asian origin in our cohort (families 1-5). Two interrelated families with an ARB clinical phenotype were identified (family 2 and 4). The most common sequence variation reported in ARB is p.(Arg141His); both Leu140 and Arg141 are located within the large intracellular hydrophobic loop on bestrophin-1 (eFigure 1 in the Supplement).

We identified 1 novel variant in the ARB group in patient 7.1 who was homozygous for c.964G>A, p.(Val322Met). Two compound heterozygous variants located in a similar region, p.(Val317Met) and p.(Met325Thr), have previously been identified by Burgess et al.17 In silico analysis predicted this change to be pathogenic and the minor allele frequency in gnomAD was 0 (eTable 3 in the Supplement). Patients 8.1 and 9.1 were both compound heterozygotes, and these 4 missense sequence variations have previously been reported as disease causing.17,20 This was also confirmed by our analysis (eTable 3 in the Supplement).

Table 2. Clinical Characteristics of Patients With Best Vitelliform Macular Dystrophy

<table>
<thead>
<tr>
<th>Patient*</th>
<th>Ethnicity</th>
<th>Age at symptom onset, y</th>
<th>Presenting symptoms</th>
<th>BCVA, logMAR</th>
<th>Refraction</th>
<th>BEST1 genotype</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.1</td>
<td>European</td>
<td>10s</td>
<td>Reduced central vision, photopsia, slow dark adaptation</td>
<td>CF OD; 0.36 OS</td>
<td>NP</td>
<td>Heterozygous c.74G&gt;T p.(Arg25Leu)</td>
<td>NA</td>
</tr>
<tr>
<td>11.1</td>
<td>Southeast Europe</td>
<td>40s</td>
<td>Reduced central vision</td>
<td>0.20 OD; 1.02 OS</td>
<td>NP</td>
<td>Heterozygous c.937A&gt;G p.(Arg313Gly)</td>
<td>Hemophilia B</td>
</tr>
<tr>
<td>11.2</td>
<td>Southeast Europe</td>
<td>10s</td>
<td>Asymptomatic</td>
<td>0.00 OU</td>
<td>NP</td>
<td>Heterozygous c.937A&gt;G p.(Arg313Gly)</td>
<td>Hemophilia B</td>
</tr>
<tr>
<td>12.1</td>
<td>European</td>
<td>20s</td>
<td>Reduced central vision in the right eye and slow dark adaptation</td>
<td>0.40 OD; 0.10 OS</td>
<td>+0.75 OD; +1.00 OS</td>
<td>Heterozygous c.728C&gt;T p.( Ala243Val)</td>
<td>NA</td>
</tr>
<tr>
<td>13.1</td>
<td>European</td>
<td>10s</td>
<td>Photopsia</td>
<td>0.32 OD; 0.54 OS</td>
<td>NP</td>
<td>Heterozygous c.671T&gt;C p.(Leu224Pro)</td>
<td>NA</td>
</tr>
<tr>
<td>14.1</td>
<td>European</td>
<td>70s</td>
<td>Photosensitivity, slow dark adaptation, reduced central vision</td>
<td>0.34 OD; 0.74 OS</td>
<td>+4.75/−1.75 × 107 OD; +4.25/−2.75 × 085 OS</td>
<td>Heterozygous c.653G&gt;A p.(Arg218His)</td>
<td>NA</td>
</tr>
<tr>
<td>15.1</td>
<td>European</td>
<td>10s</td>
<td>Asymptomatic</td>
<td>−0.06 OD; −0.08 OS</td>
<td>+0.50 OU</td>
<td>Heterozygous c.49T&gt;A p.(Phe17Ile)</td>
<td>NA</td>
</tr>
<tr>
<td>15.2</td>
<td>European</td>
<td>40s</td>
<td>Asymptomatic</td>
<td>0.04 OD; −0.06 OS</td>
<td>NP</td>
<td>Heterozygous c.49T&gt;A p.(Phe17Ile)</td>
<td>Asymptomatic relative</td>
</tr>
<tr>
<td>16.1</td>
<td>European</td>
<td>&gt;10</td>
<td>Reduced central vision</td>
<td>0.52 OD; 1.64 OS</td>
<td>NP</td>
<td>Heterozygous c.652C&gt;T p.(Arg218Cys)</td>
<td>NA</td>
</tr>
<tr>
<td>17.1</td>
<td>European</td>
<td>30s</td>
<td>Reduced central vision, slow dark adaptation</td>
<td>0.76 OD; 0.16 OS</td>
<td>NP</td>
<td>Heterozygous c.295A&gt;T p.(Asn91Tyr)</td>
<td>NA</td>
</tr>
<tr>
<td>18.1</td>
<td>NR</td>
<td>20s</td>
<td>Reduced central vision</td>
<td>−0.14 OD; 0.16 OS</td>
<td>NP</td>
<td>Heterozygous c.728C&gt;T p.(Ala243Val)</td>
<td>NA</td>
</tr>
<tr>
<td>19.1</td>
<td>European</td>
<td>30s</td>
<td>Reduced central vision</td>
<td>0.20 OD; 0.90 OS</td>
<td>NP</td>
<td>Heterozygous c.914T&gt;C p.(Phe305Ser)</td>
<td>NA</td>
</tr>
<tr>
<td>20.1</td>
<td>European</td>
<td>20s</td>
<td>Asymptomatic</td>
<td>0.56 OD; 0.74 OS</td>
<td>+1.00/−1.25 × 100 OD; +1.50/−1.50 × 085 OS</td>
<td>Heterozygous c.900G&gt;C p.(Glu300Asp)</td>
<td>NA</td>
</tr>
<tr>
<td>21.1</td>
<td>European</td>
<td>10s</td>
<td>Reduced central vision</td>
<td>0.70 OD; 0.84 OS</td>
<td>NP</td>
<td>Heterozygous c.5C&gt;G p.(Thr25er)</td>
<td>NA</td>
</tr>
<tr>
<td>22.1</td>
<td>European</td>
<td>30s</td>
<td>Slow dark adaptation and reduced central vision</td>
<td>0.66 OD; 0.40 OS</td>
<td>+6.75/−1.75 × 119 OD; +6.75/−2.50 × 066 OS</td>
<td>Heterozygous c.74G&gt;T p.(Arg313Glu)</td>
<td>NA</td>
</tr>
<tr>
<td>23.1</td>
<td>European</td>
<td>60s</td>
<td>Reduced central vision</td>
<td>0.70 OD; 0.50 OS</td>
<td>+2.25/−2.00 × 090 OD; +2.50/−2.00 × 085 OS</td>
<td>Heterozygous c.1010A&gt;G p.(Thr337Cys)</td>
<td>Bilateral pseudophakia</td>
</tr>
</tbody>
</table>

Abbreviations: BCVA, best-corrected visual acuity; CF, counting fingers; NA, not applicable; NP, not performed; NR, not reported.

* The first number indicates the family, and the second number indicates the patient.

Table 3. Clinical Characteristics of Patients With Adult-Onset Vitelliform Macular Dystrophy

<table>
<thead>
<tr>
<th>Patient*</th>
<th>Ethnicity</th>
<th>Age at symptom onset, y</th>
<th>Presenting symptoms</th>
<th>BCVA, logMAR</th>
<th>Refraction</th>
<th>BEST1 genotype</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.1</td>
<td>European</td>
<td>70s</td>
<td>Photosensitive</td>
<td>0.56 OD; 0.52 OS</td>
<td>+1.50/−1.75 × 094 OD; +1.50/−1.50 × 085 OS</td>
<td>Heterozygous c.934G&gt;A p.(Asp312Asn)</td>
<td>NA</td>
</tr>
<tr>
<td>25.1</td>
<td>European</td>
<td>50s</td>
<td>Distortion, reduced central vision in right eye</td>
<td>0.26 OD; −0.12 OS</td>
<td>+0.75/−2.00 × 100 OD</td>
<td>Heterozygous c.1515_1518del p.(Ser506Leufs*16)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviation: BCVA, best corrected visual acuity.

* The first number indicates the family, and the second number indicates the patient.
Sixteen patients (8 male, 8 female) from 14 families with a clinical phenotype of BVMD were identified. Their clinical characteristics are described in Table 2. Ophthalmoscopy in all patients demonstrated disease localized to the macula (eTable 4 in the Supplement). A normal retinal appearance was seen in patient 15.2. Incidental findings of retinoschisis in the left eye of patient 10.1 (eFigure 5A in the Supplement) and cobblestone atrophy in the temporal far peripheral of both eyes of patient 20.1 (eFigure 5B in the Supplement) were noted. Patient 22.1 had multifocal BVMD lesions in their left eye (eFigure 5C in the Supplement). All patients showed retinal changes on fundus autofluorescence and SD-OCT findings consistent with their stage of BVMD. Appearances ranged from normal to fibrosis and retinal/RPE atrophy (eTable 4 in the Supplement). Focal choroidal excavation was seen in patients 16.1 and 20.1.

Full-field ERG results were normal in 4 patients and abnormal in 4 patients, and EOG results demonstrated a reduced or absent light rise in all patients tested (eTable 5 in the Supplement). Preservation of peripheral visual fields was seen.
in all patients tested, with central visual field loss affecting 2 patients (eTable 5 in the Supplement).

All 14 families had heterozygous missense sequence variations in BEST1, 4 of which were novel: c.497T>A, p.(Phe17lle); c.748G>T, p.(Arg25Leu); c.937A>G, p.(Arg313Gly); and c.1010A>G, p.(Tyr337Cys) (Table 2 and eTable 3 in the Supplement). In silico analysis predicted these changes to be disease causing and the minor allele frequencies in gnomAD was 0 for all of them (eTable 3 in the Supplement).

Patients 10.1 and 22.1 have the same novel change (c.748G>T, p.(Arg25Leu)), which is predicted to be deleterious to the protein and interestingly changes at positions c.73C>T and c.74G>A resulting in p.(Arg25Trp) and p.(Arg25Gln), respectively, have been previously reported in patients with BVMD.16,21 Patient 15.1 had a novel p.(Phe17lle) change in the same location as the variants p.(Phe17cys) and p.(Phe17Ser), which have been previously reported in patients with BVMD and AVMD, respectively.18,22

AVMD

Two male patients from 2 families had an AVMD clinical phenotype, and their clinical characteristics are described in Table 3. Retinal appearance demonstrated small central hyperpigmented lesions (patient 24.1) and an irregular foveal reflex (patient 25.1). Spectral-domain optical coherence tomography demonstrated small bilateral subretinal lesions, and fundus autofluorescence imaging showed the typical appearance of central increased autofluorescence signal surrounded by a ring of reduced autofluorescence signal (eFigure 6 and eTable 4 in the Supplement).

The patients diagnosed with AVMD had a missense sequence variation c.934G>A, p.(Asp312Asn) (patient 24.1) and a novel frameshift variant (patient 25.1) (eTable 3 in the Supplement). This frameshift variant, c.1515_1518del results in the sequence variation c.1515_1518del/p.(Ser506Leufs*16), which is located in the C-terminal region of bestrophin-1 (eFigure 1 in the Supplement). Deletions in the C-terminal region have previously been reported as disease causing.23,24

Discussion

This study of a multiethnic British cohort shows that ARB, BVMD, and AVMD are clinically distinct and recognizable phenotypes. Six novel BEST1 pathogenic variants were identified in this cohort (eTable 3 in the Supplement). A phenotype of a beaten metallic retinal appearance extending from the mid periphery to the far periphery was identified in 8 patients with ARB. Four patients from 1 family with ARB were previously reported to have autosomal recessive RP but were reclassified as having ARB as part of this study, and the beaten metallic retinal appearance was observed in 1 of these patients (patient 1.4). Fundus features in BVMD were localized to the macula and consistent with the previously described disease stages.1 In those in whom an EOG was available, a reduced or absent light rise was observed in all patients with ARB and BVMD.

The phenotypic characteristics of ARB include widespread RPE irregularity throughout the posterior pole with punctate scattered deposits and macular edema. In some cases, a central vitelliform lesion was seen.25-27 Patients with ARB in our study showed a common phenotype with a band of increased autofluorescence signal surrounding an area of decreased autofluorescence signal within the posterior pole with or without focal vitelliform deposits studded within the band of increased autofluorescence and schitic and cystoid changes on SD-OCT. A beaten metallic retinal appearance was observed in 8 patients with ARB, extending from the mid periphery to the far periphery primarily in the temporal region. To our knowledge, this phenotypic feature has not been previously reported in association with ARB. Electrophysiology findings usually demonstrate an abnormal full-field ERG with a severely reduced EOG light rise, but some cases may show normal full-field ERGs.28 Results of the full-field ERGs in this cohort of patients with ARB demonstrated a spectrum from abnormal to normal; for all those for whom an EOG was available, a reduced or absent light rise was observed. Refraction in this condition is usually hyperopic, and patients may develop angle-closure glaucoma.28-30 None of the patients with ARB in this study had any evidence of shallow anterior chambers, and 3 patients had myopia.

Missense sequence variations in BEST1 causing autosomal dominant and autosomal recessive RP were first reported by Davidson et al25 in 2009, with a further case report of a patient with RP associated with a deletion of 9348bp from chromosome 11, resulting in a frameshift sequence variation in BEST1.7 However, this patient also had heterozygous sequence variations in 4 genes associated with recessive retinopathy including RP (GUC1A1, GPR179, IQCBL, and TRIM32), which would be more consistent with the phenotype. It has been suggested that patients with autosomal dominant and autosomal recessive RP originally reported by Davidson et al25 may represent ADVIRC and ARB, respectively.8 Four patients from family 1 (patient 1.1, 1.2, 1.3, and 1.4), previously reported by Davidson et al6 with a diagnosis of autosomal recessive RP have been reclassified to ARB, as well as the additional affected individuals with the same homozygous sequence variation c.418C>G p.(Leu140Val) in BEST1 (families 1-5).

To date, more than 300 sequence variations in BEST1 have been described, most of which are missense changes.31 In this study of 25 families with ARB, BVMD, and AVMD, 22 pathogenic variants were identified (21 missense, 1 frameshift) of which 6 are novel (5 missense, 1 frameshift; eTable 3 in the Supplement). The variants identified in this study do not demonstrate a correlation between their position on the structure of bestrophin-1 and retinal phenotype (eFigure 1 in the Supplement), but this linear analysis does not take into account the 3-dimensional structure of the protein or the mode of inheritance.

BEST1 sequence variations inherited as an autosomal dominant trait are thought to cause a dominant negative effect rather than loss of function. This is supported by a recent study from Milenkovic et al5 who used cell lines transfected with wild-type BEST1 and sequence variations leading to BVMD to demonstrate that nonfunctional BEST1 chloride channels containing mutant and wild-type subunits are formed and by the observation that Best1−/− mice do not show a BVMD phenotype.32 Burgess et al33 in 2008 first described the distinct clinical phenotype seen in ARB and proposed that ARB...
is the null phenotype of \textit{BEST1}, ie, that 2 mutant copies of the protein cause loss of function. Using whole-cell patch-clamping to measure chloride channel activity, they showed that if wild-type \textit{BEST1} was cotransfected with an ARB-associated sequence variation, active wild-type bestrophin-1 channels were formed. However, this hypothesis does not explain why some \textit{BEST1} variants, such as p.(Arg141His), when present in a heterozygous state cause BVMD and in a homozygous state cause ARB,\textsuperscript{16,33} and additionally why the heterozygous parents of these patients with ARB display no disease phenotype.\textsuperscript{17}

It has been suggested by Boon et al\textsuperscript{28,34} that panretinal photoreceptor degeneration in ARB may be associated with the role that the RPE and possibly bestrophin-1 play in ocular development. In this study, patients 1.3, 1.4, 1.5, and 3.1, all carrying the same homozygous p.(Leu140Val) sequence variation, demonstrated extensive photoreceptor degeneration on full-field ERG. However, patients 1.6, 2.1, 4.1, and 5.1 with the same homozygous sequence variation demonstrated normal full-field ERG results; thus, the mechanism is not yet clear. Widespread photoreceptor degeneration in ARB may occur secondary to RPE dysfunction; however, this hypothesis does not explain the variability in clinical phenotype seen in patients with the same \textit{BEST1} sequence variations.

In a 2017 review Guziewicz et al\textsuperscript{35} hypothesize that the retinal diseases caused by sequence variations in \textit{BEST1} are due to a compromise of the RPE-photoreceptor interface and that increased sensitivity and hence vulnerability of cone-associated microvilli and insoluble cone matrix sheaths to subretinal biochemical changes explain the predilection of the cone dense and highly metabolically active macular region to disease. In patients with ARB and BVMD, the observation of a hyperreflective thickening on SD-OCT corresponding to photoreceptor outer segments in areas of subretinal fluid, causing loss of RPE-photoreceptor apposition suggests that phagocytosis of photoreceptor outer segments may be impaired, potentially supporting the hypothesis of an RPE-photoreceptor interface disease. Furthermore, if ARB is the null phenotype of \textit{BEST1},\textsuperscript{17} the beaten metallic appearance observed within the mid to far retinal periphery in ARB also potentially supports the hypothesis that bestrophinopathies are caused by a compromised RPE-photoreceptor interface; this could explain the extent of disease only seen in this particular phenotype of \textit{BEST1} sequence variations.

**Limitations**

This study was retrospective, and although all available data have been presented for all patients, patients seen before the introduction of new imaging modalities or testing standards will not have had them performed. This study reported detailed phenotypic and genetic analysis of patients from a single center. It is possible that this may limit the generalizability of these findings, but as these patients represent a large multiethnic cohort, spanning a large age range, and the full spectrum of clinical severity, we believe that this is less likely.

**Conclusions**

This study contributes to the phenotypic characterization of bestrophinopathies and provides a detailed phenotypic and genetic analysis of a British cohort of patients with sequence variations in \textit{BEST1}. Patients in this cohort previously reported with a diagnosis of autosomal recessive RP have features more consistent with ARB. The majority report slow dark adaptation, which hitherto has not featured as a distinctive symptom. We also describe a beaten metallic retinal appearance in 8 patients with ARB, a feature that has not previously been reported to be associated with this phenotype, to our knowledge, and a common phenotype on fundus autofluorescence and SD-OCT. We also postulate that the phenotypic spectrum of bestrophinopathies is due to compromise of the RPE-photoreceptor interface. There was no clear genotype-phenotype correlation in this cohort of patients with sequence variations in \textit{BEST1} with respect to the type or location of the sequence variation and the clinical phenotype or its severity. It is possible that unknown genetic modifiers or environmental factors could be contributing to the clinical heterogeneity in patients with the same \textit{BEST1} sequence variations. As more detailed phenotyping of bestrophinopathy patients is undertaken alongside genotyping studies, functional analysis, and evaluation of potential modifying factors, these may help elucidate the molecular mechanisms underlying this protean disorder.

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**ARTICLE INFORMATION**

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