Phenotypic Variability Due to a Novel Glu292Lys Variation in Exon 8 of the BEST1 Gene Causing Best Macular Dystrophy

Elliott H. Sohn, MD; Peter J. Francis, MD, PhD; Jacque L. Duncan, MD; Richard G. Weleber, MD; David A. Saperstein, MD; Donald F. Farrell, MD; Edwin M. Stone, MD, PhD

Objective: To study the phenotypic characteristics of patients with a novel p.E292K mutation in BEST1.

Methods: Affected individuals underwent ophthalmic examination and testing that included photography, autofluorescence, optical coherence tomography, and electro-physiological testing. Their DNA was analyzed for BEST1 mutations.

Results: Five patients aged 5 to 59 years who expressed the p.E292K mutation in BEST1 were identified in 3 families. Electro-oculographic light-rise was subnormal in all probands and carriers. Carriers had normal findings from fundus examination, multifocal electroretinography, and visual acuity, and were emmetropic or myopic. Only probands had hyperopia and fundus findings typical of Best macular dystrophy. Optical coherence tomography of vitelliform lesions demonstrated retinal pigment epithelium elevation without subretinal fluid; atrophic lesions exhibited disruption of the hyperreflective outer retina–retinal pigment epithelium complex. Intense hyperautofluorescence correlated with the vitelliform lesion.

Conclusions: Patients with the Glu292Lys variation in BEST1 exhibit intrafamilial and interfamilial phenotypic variability. A disproportionate fraction (26%) of Best disease–causing mutations occurs in exon 8, suggesting that the portion of protein encoded by this exon (amino acids 290-316) may be especially important to bestrophin’s function. Relatively good visual acuity with vitelliform lesions can be explained by preservation of the outer retina, demonstrated by optical coherence tomography.

Clinical Relevance: A novel mutation in this region of BEST1 carries implications for disease pathogenesis.

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Although the precise structure of the protein has yet to be elucidated, it has been hypothesized that bestrophin has 4 transmembrane domains with amino and carboxyl terminals located in the cytoplasm. Most of the described \textit{BEST1} mutations causing BMD have been missense mutations,\textsuperscript{1,2,22-40} and a disproportionate fraction of these mutations occurs in exon 8, suggesting that the portion of the protein encoded by this exon may be especially critical to its function. At the time of this writing, the Carver Nonprofit Genetic Testing Laboratory has observed 155 instances of 84 different \textit{BEST1} mutations in probands affected with Best disease, and 22 of these mutations (26%) lie within exon 8 (Tyler Kinnick, PhD, and E.M.S., unpublished data, 2008). Additional evidence of the functional importance of this portion of the protein is that exon 8 is highly conserved among the \textit{BEST1} orthologs of \textit{Caenorhabditis elegans}, \textit{Drosophila melanogaster}, and mice.\textsuperscript{2} In addition, a functional analysis of an exon 8 variant (Q293H) in human embryonic kidney cells revealed a severe reduction of chloride current that behaved in a dominant negative manner.\textsuperscript{27}

In the present study, we identified individuals from 3 apparently unrelated families with a missense mutation in \textit{BEST1} causing a change at position 292 of glutamic acid to lysine. Clinical characterization including AF, OCT, and ffERG and multifocal ERG (mfERG) of these individuals demonstrates highly variable expression between individuals as well as intrafamilial variability with nonpenetration of fundus findings in 2 consecutive generations.

### METHODS

Three probands (aged 5-59 years) from 3 unrelated families were identified by characteristic fundus findings and abnormal Arden ratio on EOG. Family members of the 5-year-old boy were also examined, and electrophysiology was performed. After informed consent was obtained, blood samples were taken for DNA extraction, and subsequent mutation screening of \textit{BEST1} was performed at the John and Marcia Carver Nonprofit Genetic Testing Laboratory in Iowa City, Iowa. The protocol adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review boards of the institutions involved.

A full medical history was taken and ophthalmic examination was performed. Patients underwent digital color fundus photography. The AF images of the 54-year-old subject were obtained using a TopCon 50EX digital fundus camera equipped with AF filters purchased from Ophthalmic Imaging Systems (Sacramento, California), similar to a system previously described.\textsuperscript{5,41} The OCT images were obtained using the Stratus III (Stratus OCT 4.0.2 software; Zeiss Instruments, Dublin, California). Electrophysiological assessment included ffERG and EOG, using recording methods laid out by International Society for Clinical Electrophysiology of Vision (ISCEV) standards and recommendations for electroretinography\textsuperscript{43,44} and EOG.\textsuperscript{45} The mfERG was performed in 4 cases according to guidelines that have been described elsewhere.\textsuperscript{41,45}

Mutation analysis revealed a missense variation resulting in a change of glutamic acid to lysine at amino acid position 292 in \textit{BEST1} in all probands and obligate carriers. Genotyping of 4 informative short tandem repeat polymorphisms at the \textit{BEST1} locus revealed a distinctly different disease-associated haplotype in family 1, strongly suggesting that the Glu292Lys mutation in that family occurred independently (data not shown). Summaries of the clinical findings for patients in this study are shown in the Table.

#### Table. Summary of Clinical Findings in 3 Families of Patients With the Glu292Lys \textit{BEST1} Variation

<table>
<thead>
<tr>
<th>State</th>
<th>Sex/Age, y</th>
<th>BCVA</th>
<th>Refraction</th>
<th>Fundus</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband 1</td>
<td>M/5</td>
<td>20/40 OU</td>
<td>+5.25 sphere OU</td>
<td>Green</td>
<td>Vitelliform, both eyes</td>
</tr>
<tr>
<td>Asymptomatic mother of proband 1</td>
<td>F/30</td>
<td>20/20 OD, 20/200 OD</td>
<td>+2.25 + 075@180 OD</td>
<td>Green</td>
<td>Normal</td>
</tr>
<tr>
<td>Proband 2</td>
<td>M/54</td>
<td>20/20 OD</td>
<td>+1.75 075@165 OS</td>
<td>Brown</td>
<td>Atrophic, right eye; Vitelliform, left eye</td>
</tr>
<tr>
<td>Proband 3</td>
<td>F/59</td>
<td>20/70 OD, 20/50 OD</td>
<td>+1.25 + 050@170 OD</td>
<td>Green</td>
<td>Atrophic, both eyes</td>
</tr>
</tbody>
</table>

Abbreviation: BCVA, best-corrected visual acuity.

#### RESULTS

The parents of a 5-year-old boy noted intermittent convergent strabismus in the child for 8 weeks. Refractive error of +5.25 diopter spheres OU resulted in orthophoria with best-corrected visual acuity of 20/40 OU. His irides were green. Anterior segment examination, including anterior chamber depth, was within normal limits. Fundus examination revealed bilateral central vitelliform lesions (Figure 1, A and B). The ffERG was normal and EOG revealed Arden ratios of 1.1 OD and 1.4 OS. The OCT showed a discrete elevation of RPE and widening of the hyperreflective signal from the outer retina–RPE complex (Figure 1, C and D).

The asymptomatic mother, who is of Scandinavian, Irish, and German descent, and the father, who is of French, German, and Norwegian descent, both had visual acuity of 20/20 without correction and normal fundus examination. However, EOG of the 30-year-old mother revealed Arden ratios of 1.2 OD and 1.3 OS. Her ffERG and mfERG scans showed no abnormalities. Genetic testing of the mother revealed that she had the same mutation in \textit{BEST1} Glu292Lys as her son.

This led to examination and testing of other family members (Figure 2) to further characterize the inheritance pattern. The proband’s maternal grandfather, aged 57 years, was asymptomatic, with best-corrected visual acuity of 20/20. He had Arden ratios of 1.3 OU, and his ffERG and
mfERG showed no abnormalities. Manifest refraction was −0.75/H11001 1.00@055 OD, −1.00/H11001 1.00@180 OS. His intraocular pressure was 14 mm Hg OU, and irides were hazel. His anterior chamber depth was normal. The crystalline lens was clear. Posterior segment examination revealed rare RPE changes but no vitelliform lesion or atrophy.

In addition, EOG testing of the above proband and asymptomatic carriers revealed prolonged light-peak slow oscillation, consistent with previously described findings in humans46 and animals47 with BMD.

PROBAND 2

The patient is a 54-year-old man of East Indian descent who was referred for reduced vision in the right eye for the past 10 years. His family history was unremarkable for similar vision problems. His best-corrected visual acuity was 20/200 (eccentric) OD and 20/20 OS. His refractive error was +2.25 + 0.75@180 OD, +1.75 + 0.75@165 OS. His intraocular pressure was 12 mm Hg OD and 14 mm Hg OS.

Anterior segment examination revealed brown irides and normal anterior chamber depth. Fundus examination revealed a central circumscribed area of atrophy in the right eye and a vitelliform lesion in the left eye (Figure 3, A and B). Both eyes exhibited deep fleck-like changes nasal to the disc and along the temporal arcades. The AF images of the right eye (Figure 3E) showed a central macular hypofluorescent lesion that corresponded to the area of atrophy on fundus examination with patches of increased AF, especially in the periphery of the lesion. Autofluorescence of both eyes

Figure 1. Fundus photographic and ocular coherence tomographic (OCT) images of proband 1. A and B. Fundus shows well-demarcated vitelliform lesions in the central macula. C and D. Corresponding OCT sections show elevation at the level of the retinal pigment epithelium, with preservation of the outer retinal layer.

Figure 2. Pedigree of proband 1 (indicated by shaded box) demonstrates lack of penetrant fundus findings and hyperopia in carriers. Wt indicates wild type; yo, years old.
allowed better visualization of the fleck-like lesions around the disc and arcades, exhibiting mixed hyperautofluorescence and hypoautofluorescence that was confluent in many areas. The AF images of the left eye (Figure 3F) showed a central area of homogenously increased autofluorescence corresponding to the vitelliform lesion. Optical coherence tomographic imaging (Figure 3C) revealed increased backscatter from the underlying sclera in the region of RPE atrophy with some irregular disruptions in the outer retina–RPE complex at the edges of the atrophic lesion in the right eye; OCT images in the left eye (Figure 3D) showed discrete elevation of the RPE without discontinuity of the hyperreflective outer retina–RPE complex.

Electrophysiological assessment revealed an Arden ratio of 1.5 OD and 1.3 OS. The fERG was normal. Fixation was not stable enough to permit reliable mfERG recording using a pupil camera in the right eye, but mfERG of the left eye demonstrated reduced responses from the central 1° to 5° OS (Figure 4) with relative preserva-
tion of the response amplitude and timing from the surrounding macula.

PROBAND 3

This 59-year-old woman of Norwegian and Jewish descent with green irises was found on routine examination 22 years earlier to have fundus findings suspicious for BMD. Two sisters and a nephew are thought to have BMD, and while her mother had vision problems, no diagnosis had been made at the time of her death. Ocular history was significant for hyperopia and laser peripheral iridotomy in both eyes for occludable angles. Medical history was significant for well-controlled diabetes mellitus type 2, polymyalgia rheumatica, reflux disease, and osteoarthritis.

Visual acuity was 20/70 OD with $+1.25 + 0.50 @ 170$ and 20/50 OS with $+1.00 + 0.25 @ 10$. On biomicroscopy, the laser peripheral iridotomies were patent and there was mild nuclear sclerosis in both eyes. Fundus examination revealed bilateral central atrophy with few drusen in the midperiphery (Figure 5, A and B). Corresponding to the area of atrophy seen on examination, OCT revealed attenuation of the outer retina and hyperreflectivity of the RPE with increased signal posterior to the RPE (Figure 5, C and D).

The Arden ratio was 1.2 OD and 1.1 OS. Her ffERG showed no abnormalities; mfERG (Figure 6) exhibited attenuation of amplitude and latency delay that was most prominent in the central macula with relative sparing of the peripheral macula.

**COMMENT**

The **BEST1** gene, together with **BEST2**, **BEST3**, and **BEST4**, are part of a closely related gene family characterized by several transmembrane-spanning domains and an invariant arginine-phenylalanine-proline motif. These 4 human genes are believed to be orthologous to a gene in *C. elegans* that shares a highly conserved 26–amino acid sequence beginning at position 289. Three lines of evidence support the idea that the novel bestrophin variation reported here is disease causing. First, the glutamic acid residue normally present at position 292 is highly conserved evolutionarily. Second, glutamic acid is negatively charged at neutral pH, while the lysine residue found in affected individuals is positively charged. This is the most extreme charge difference possible for a point mutation. Finally, presence of the mutation in 3 unrelated families with different bestrophin haplotypes suggests that the variation arose more than once. This makes it very likely that Glu292Lys is the disease-causing variation in the gene and not simply a non–disease-causing polymorphism in linkage disequilibrium with a true disease-causing mutation nearby. Mutations are common in the region of the human **BEST1** gene encoding amino acids 290 through 316, suggesting that this portion of the protein is critical to its function.

In the present study, we performed a detailed clinical and electrophysiological evaluation of 5 subjects from 3 unrelated families who share a previously undescribed missense mutation in **BEST1**, a change from glutamic acid to lysine at amino acid position 292. We observed variable expressivity in our cohort of patients. Only the reduced light peak on the EOG was completely penetrant. Hyperopia was also found in all probands but was distinctly absent in carriers. Though it is tempting to attribute this to reduced axial length from the elevated fundus hyperopia alone was also found in our patients with flat atrophic retinas, as demonstrated on OCT. Moreover, similar degrees of hyperopia were seen in proband 2 despite atrophy in one eye and a vitelliform lesion in the other. Hyperopia is a common finding in patients with BMD and is found in other conditions caused by mutations in **BEST1** such as autosomal dominant vitreoretinochoroidopathy and autosomal recessive bestrophinopathy. Findings of hyperopia in probands may be related to genetic modifiers such as microphthalmia-associated transcription factor (*MITF*). Interactions between *MITF* and **BEST1** have not been fully explored. While it is unclear whether our probands were hypersensitive before the development of lesions, it would be interesting to compare the prevalence of hyperopia in probands and carriers and determine whether hyperopia is a predictive factor for developing vitelliform lesions. Such a finding would have implications for prognosis and counseling of patients and families. The lack of hyperopia among carriers in our study could indicate a favorable prognostic sign in patients with this mutation.

While proband 1 had mildly diminished visual acuity despite the presence of vitelliform lesions, both his mother and his 57-year-old maternal grandfather had relatively normal fundi despite diminished EOG and the presence of the Glu292Lys variation. In addition to emmetropia, normal visual acuity, and lack of fundus lesions, both carriers also had normal ffERG and mfERG responses. This appears to be the first demonstration of nonpenetrant fundus findings in 2 consecutive generations with a confirmed **BEST1** mutation. Given the early age at which large vitelliform lesions are typically observed, it seems more likely that the variable clinical findings among patients carrying the same **BEST1** variation are due to modifying genes rather than environmental factors.
The results of OCT and AF performed in our patients allow us to speculate on the nature of the vitelliform lesion, for which histopathology in a human eye has not been performed. Models of BEST1 mutations in dogs and mice are not adequate for this purpose, as these animals do not develop typical vitelliform lesions. Human eyes with the scrambled egg appearance have abnormally high amounts of lipofuscin. Studies in elderly individuals homozygous or heterozygous for mutations in BEST1 have demonstrated modestly elevated levels of A2E compared with controls. However, it is unknown whether A2E is a by-product of the latter stages of disease or whether other components of lipofuscin contribute to the hyperautofluorescent appearance in early stages.

Intense hyperautofluorescence corresponding to the vitelliform lesion was seen in the left eye of proband 2, but...
a circumscribed patch of decreased AF corresponded to the atrophic lesion with speckled increased AF. In light of histopathological studies of late BMD,15,16 this loss of AF is likely due to irregularities in the RPE cell monolayer with secondary loss of photoreceptor outer segment turnover correlating with the poor level of vision in this eye. Relatively preserved visual acuity was seen in probands 1 and 2, with vitelliform lesions causing RPE elevations. Notably, OCT revealed no evidence of serious detachment in our patients, as recently reported in patients with BMD studied with spectral domain OCT.55 This contrasts with other studies of autofluorescent vitelliform lesions that showed fluid between the RPE and outer retina.21,56 One hypothesis accounting for our findings is that mutated bestrophin in RPE, which likely contains lipofuscin in the vitelliform stage, causes impaired transport of fluid to the choroid, resulting in separation of the RPE from the choroid. Progression causes defective pumping of subretinal fluid to the RPE, resulting in the detachment of the outer retina from RPE that was shown in previous studies of eyes with more advanced disease.

Patients with Glu292Lys variation in BEST1 exhibit intrafamilial and interfamilial phenotypic variability. Thus, it is important for clinicians to realize that identification of the variation by genetic testing does not always predict the eventual development of macular disease. Our findings support the idea that the portion of the protein consisting of amino acids 290 through 316 may be critical to the function of bestrophin. Relatively good visual acuity with vitelliform lesions can be explained by preservation of the outer retina demonstrated by OCT.

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Correspondence: Elliott H. Sohn, MD, Doheny Eye Institute, 1450 San Pablo St, Ste 3608, Los Angeles, CA 90033 (elliott.sohn@gmail.com).

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