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

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Abstract

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, OCT, and electrophysiological testing. DNA was analyzed for *BEST1* mutations.

Results—Five patients (aged 5–59) expressing the p.E292K mutation in *BEST1* were ascertained from three families. EOG light-rise was subnormal in all probands and carriers. Carriers had normal fundus examination, mfERG, visual acuity, and were emmetropic or myopic. Only probands had hyperopia and fundus findings typical of Best macular dystrophy. OCT of vitelliform lesions demonstrated RPE elevation without subretinal fluid; atrophic lesions exhibited disruption of the hyper-reflective outer retina-RPE complex. Intense hyperautofluorescence correlated to the vitelliform lesion.

Conclusions—Patients with Glu292Lys variation in *BEST1* exhibit intra- and interfamilial phenotypic variability. A disproportionate fraction (26%) of Best-disease-causing mutations occur 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 OCT.

Clinical relevance—We demonstrate findings that can be seen with novel mutation in this region of *BEST1* that carries implications for disease pathogenesis.

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Best macular dystrophy (BMD) is an autosomal dominant condition caused by mutations in the *BEST1* gene.[1, 2] Mutations in this gene result in a defective protein product, bestrophin, which localizes to the basolateral membrane of the retinal pigment epithelium (RPE)[3] and has been associated with conductance abnormalities in a family of chloride channels[4, 5] and voltage-gated calcium channels.[6–8] These alterations may account for the diminished light peak-dark trough ratio (Arden ratio typically ≤ 1.5) of the electro-oculogram (EOG) characteristic of BMD; full-field ERG is typically normal.[9, 10]

The fundus findings associated with *BEST1* mutations are quite varied and include a normal fundus appearance with an abnormal EOG; vitelliform lesions with a 'sunny side up' egg yolk appearance in the central macula; a 'pseudohypopyon' in which the yellow material gravitates inferiorly in the sub-RPE space; a 'scrambled egg' characterized by yellowish subretinal deposits admixed with patches of hyperpigmentation and atrophy of the retinal pigment epithelium; geographic atrophy; nodular subretinal gliosis centered on fixation; and rarely, choroidal neovascularization.[11] Visual acuity is often preserved in at least one eye throughout life, with more substantial visual loss occurring when BMD is complicated by choroidal neovascularization[12–14] or extensive geographic atrophy.

Newer diagnostic techniques have refined our understanding of the anatomy of the macular lesions seen in patients with Best disease.[15–20] For example, one of the first optical coherence tomography (OCT) studies in these patients showed that the vitelliform material may lie between the outer retina and the RPE.[21] Increased fluorescence of vitelliform fundus lesions on fundus autofluorescence (AF)[22, 23] images may be due to enhanced accumulation of fluorophores such as A2E.[18]

Although the precise structure of the protein has yet to be elucidated, it has been hypothesized that bestrophin has four transmembrane domains with amino and carboxyl terminals located in the cytoplasm. The majority of reported *BEST1* mutations causing BMD have been missense mutations,[1, 2, 22, 24–40] and a disproportionate fraction of these mutations occur 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 *BEST1* mutations in probands affected with Best disease, and 22 of these mutations (26%) lie within exon 8 (Kinnick and Stone, unpublished data). Additional evidence of the functional importance of this portion of the protein is that exon 8 is highly conserved among the *BEST1* orthologs of *C.elegans*, *D. melanogaster*, and mice.[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.[27]

In the present study, we identified individuals from three apparently unrelated families with a missense mutation in *BEST1*, causing a change at position 292 of glutamic acid to lysine. Clinical characterization, including AF, OCT, and full-field and multi-focal electroretinography (ERG; ffERG, mfERG) of these individuals, demonstrates highly variable expression between individuals, as well as intrafamilial variability with nonpenetrance of fundus findings in two consecutive generations.

METHODS

Three probands (aged 5–59) from three unrelated families were identified by characteristic fundus findings and abnormal Arden ratio on electro-oculography (EOG). Family members of the 5-year-old male were also examined, and electrophysiology was performed. After informed consent was obtained, blood samples were taken for DNA extraction, and subsequent mutation screening of *BEST1* was performed at the John and Marcia Carver Nonprofit Genetic Testing

Laboratory in Iowa City, IA. The protocol of the study 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 OIS similar to a system previously described.[41, 42] The OCT images were obtained utilizing the Stratus III (Stratus OCT 4.0.2 software; Zeiss instruments, Dublin, CA). Electrophysiological assessment included ffERG and EOG, using recording methods laid out by ISCEV standards and recommendations for electroretinography[43, 44] and electro-oculography. [43] The mfERG was performed in four cases according to guidelines that have been reported elsewhere.[41, 45]

RESULTS

Mutation analysis revealed a missense variation resulting in a change of glutamic acid to lysine at amino acid position 292 in *BEST1* in all probands and obligate carriers. Genotyping of four informative short tandem repeat polymorphisms at the *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 Table 1.

PROBAND 1 AND FAMILY MEMBERS

The parents of a 5-year-old male noted intermittent convergent strabismus in the child for 8 weeks. Refractive error of +5.25 diopter sphere (DS) OU resulted in orthophoria with best-corrected visual acuity (BCVA) of 20/40 OU. Irides were green. Anterior segment examination, including anterior chamber depth, was within normal limits. Fundus examination revealed bilateral central vitelliform lesions (Figures 1A 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 hyper-reflective signal from the outer-retina-RPE complex (Figures 1C and D).

The asymptomatic mother, who is of Scandinavian/Irish/German descent, and father, who is of French/German/Norwegian descent, both had VA of 20/20 without correction and normal fundus examination. However, EOG of the 30-year-old mother revealed Arden ratios of 1.2 OD, 1.3 OS. Her ffERG and mfERG were normal. Genetic testing of the mother revealed that she had the same mutation in *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, was asymptomatic, with BCVA of 20/20. He had Arden ratios of 1.3 OU, and his ffERG and mfERG were normal. Manifest refraction was −0.75 +1.00@055 OD, −1.00 +1.00@180 OS. Intraocular pressure (IOP) was 14 mm Hg OU. Irides were hazel colored. Anterior chamber depth was normal. 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, in keeping with previously reported findings in humans[46] and animals[47] with BMD.

PROBAND 2

The patient is a 54-year-old male of East Indian descent who was referred for reduced vision in the right eye for the past 10 years. Family history was unremarkable for similar vision

problems. His BCVA was 20/200 (eccentric) OD and 20/20 OS. Refractive error was +2.25 +0.75@180 OD, +1.75 +0.75@165 OS. His IOP was 12 mm Hg OD and 14 mmHg OS.

Anterior segment exam revealed brown irides and normal anterior chamber depth. Fundus examination revealed a central circumscribed area of atrophy OD and a vitelliform lesion OS (Figures 3A 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 hypoautofluorescent lesion that corresponded to the area of atrophy on fundus examination with patches of increased AF, especially in the periphery of the lesion. AF of both eyes allowed better visualization of the fleck-like lesions around the disc and arcades, exhibiting mixed hyper- 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. OCT 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 Arden ratio of 1.5 OD and 1.3 OS. The ffERG was normal. Fixation was not stable enough to permit reliable mfERG recording using a pupil camera in the right eye; but mfERG OS demonstrated reduced responses from the central 1–5 degrees OS (Figure 4) with relative preservation of the response amplitude and timing from the surrounding macula.

PROBAND 3

This 59-year-old woman of Norwegian/Jewish descent with green irides 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 OU for occludable angles. Past medical history was significant for well-controlled diabetes mellitus type II, 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@010. On biomicroscopy, the laser peripheral iridotomies were patent and there was mild nuclear sclerosis OU. Fundus examination revealed bilateral central atrophy with few drusen in the mid-periphery (Figures 5A and B). Corresponding to the area of atrophy seen on examination, OCT revealed attenuation of the outer retina and hyper-reflectivity of the RPE with increased signal posterior to the RPE (Figures 5C and D).

The Arden ratio was 1.2 OD and 1.1 OS. Her ffERG was normal; 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.

DISCUSSION

BEST1, 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 (RFP) motif. These four 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.[48] 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, the fact that the mutation was observed in three 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 290–316, 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 five subjects from three unrelated families that 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 lesion, hyperopia 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[22, 49] and is found in other conditions caused by mutations in *BEST1*such as autosomal dominant vitreoretinochoroidopathy[50] and autosomal recessive bestrophinopathy.[51] Findings of hyperopia in probands may be related to genetic modifiers such as microphthalmia-associated transcription factor (*MITF*).[52, 53] Interactions between *MITF* and *BEST1* have not been fully explored. While it is unclear whether our probands were hyperopic 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 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 non-penetrant fundus findings in two 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 to 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[54] and mice[47] 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.[17] Studies in elderly individuals homozygous or heterozygous for mutations in *BEST1* have demonstrated modestly elevated levels of A2E compared to controls.[18] 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 serous detachment in our patients, as recently reported in BMD patients 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 RPE from choroid. Progression causes defective pumping of subretinal fluid to 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 intra- and interfamilial phenotypic variability. Thus, it is important for clinicians to realize that the identification of the variation by genetic testing does not always portend the eventual development of macular disease. Our findings support the idea that the portion of the protein consisting of amino acids 290–316 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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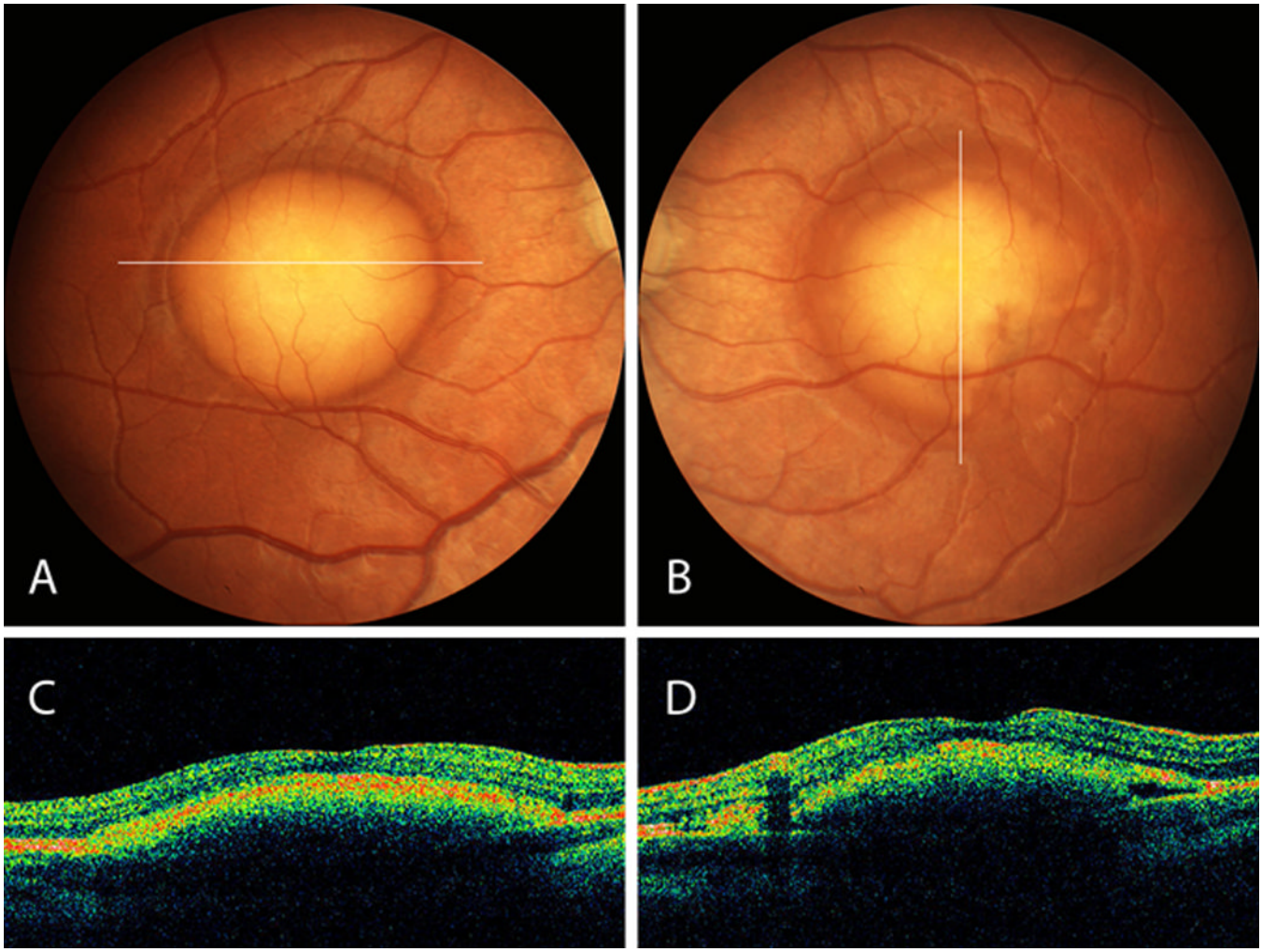


Figure 1.
A,B, Fundus of proband 1 shows well-demarcated vitelliform lesions in the central macula. C, D, Corresponding OCT sections shows elevation at the level of the RPE with preservation of the outer retina layer.

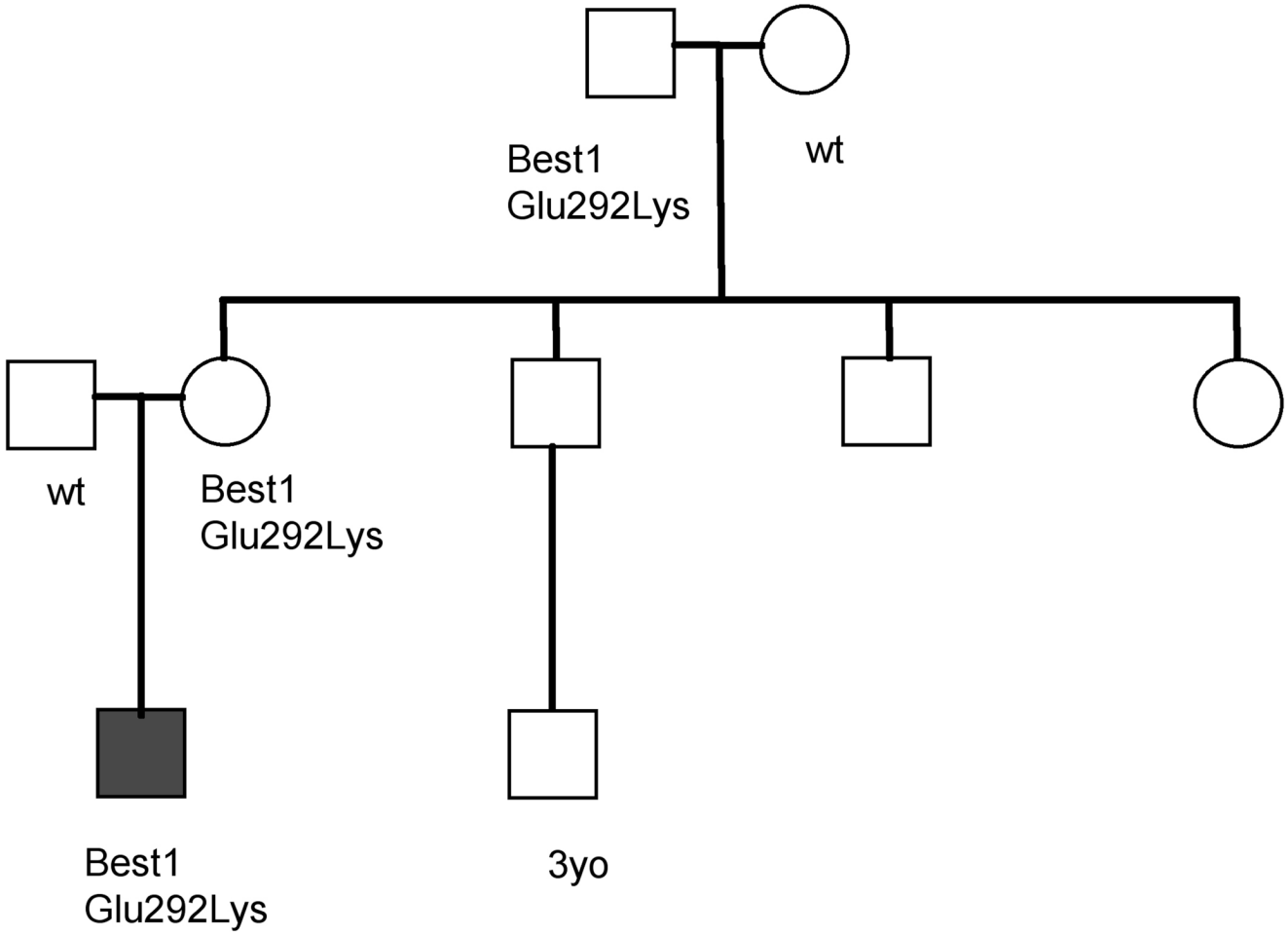


Figure 2.
Pedigree of proband 1 (indicated by shaded box) demonstrates lack of penetrant fundus findings and hyperopia in carriers.

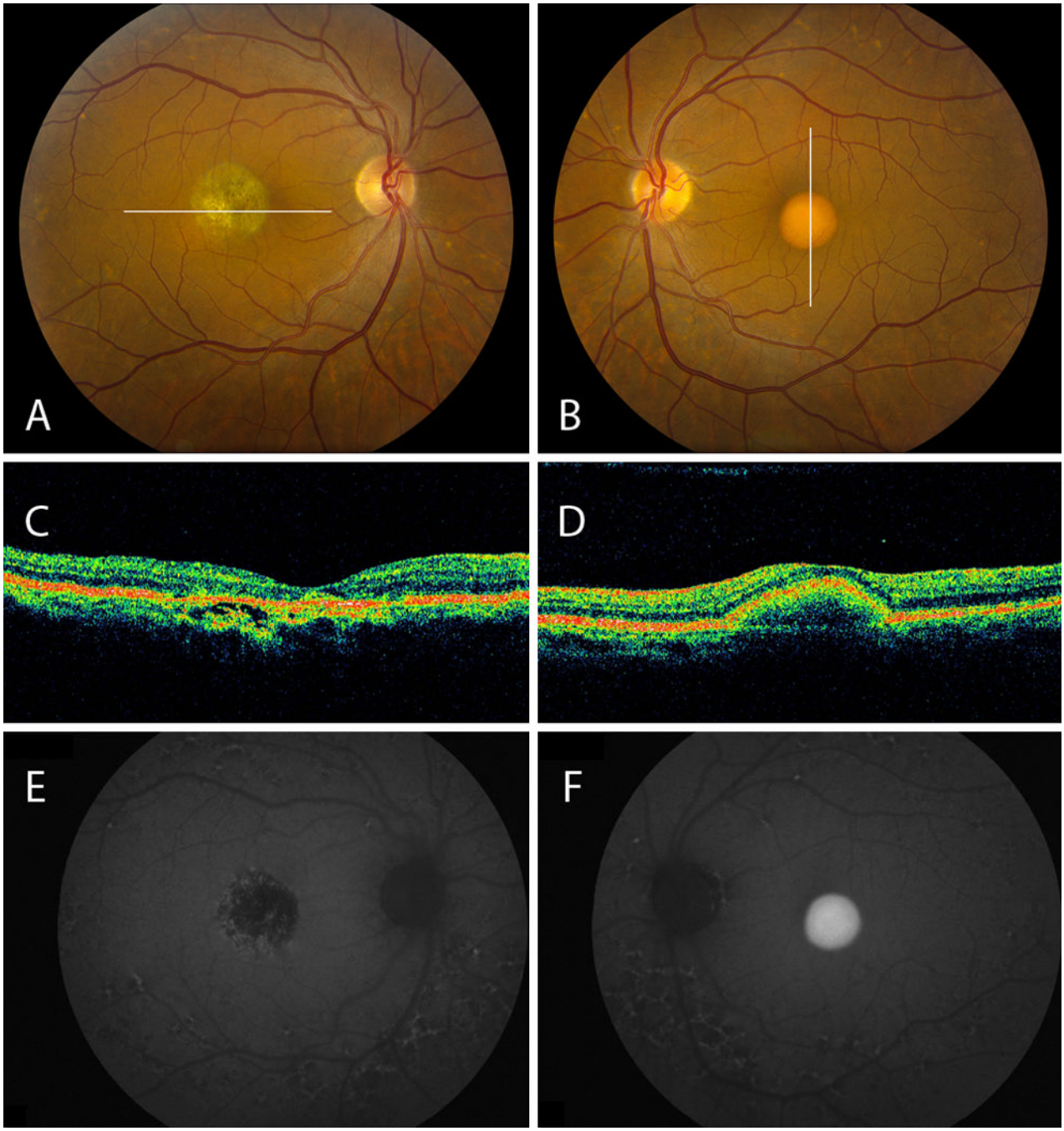


Figure 3.
A, B, Proband 2 fundus demonstrates central atrophy OD and a vitelliform lesion OS, respectively. Both exhibit fleck-like changes nasal to the disc and around the arcades. C, OCT OD reflects RPE atrophy with disruption in the outer retina-RPE complex. D, OCT OS shows discrete RPE elevation. E, AF OD demonstrates a central hypoautofluorescent lesion. Fleck-like lesions around the disc and arcades reveal mixed hyper- and hypoautofluorescence in both eyes. F, AF OS shows central hyperautofluorescence corresponding to the vitelliform lesion.

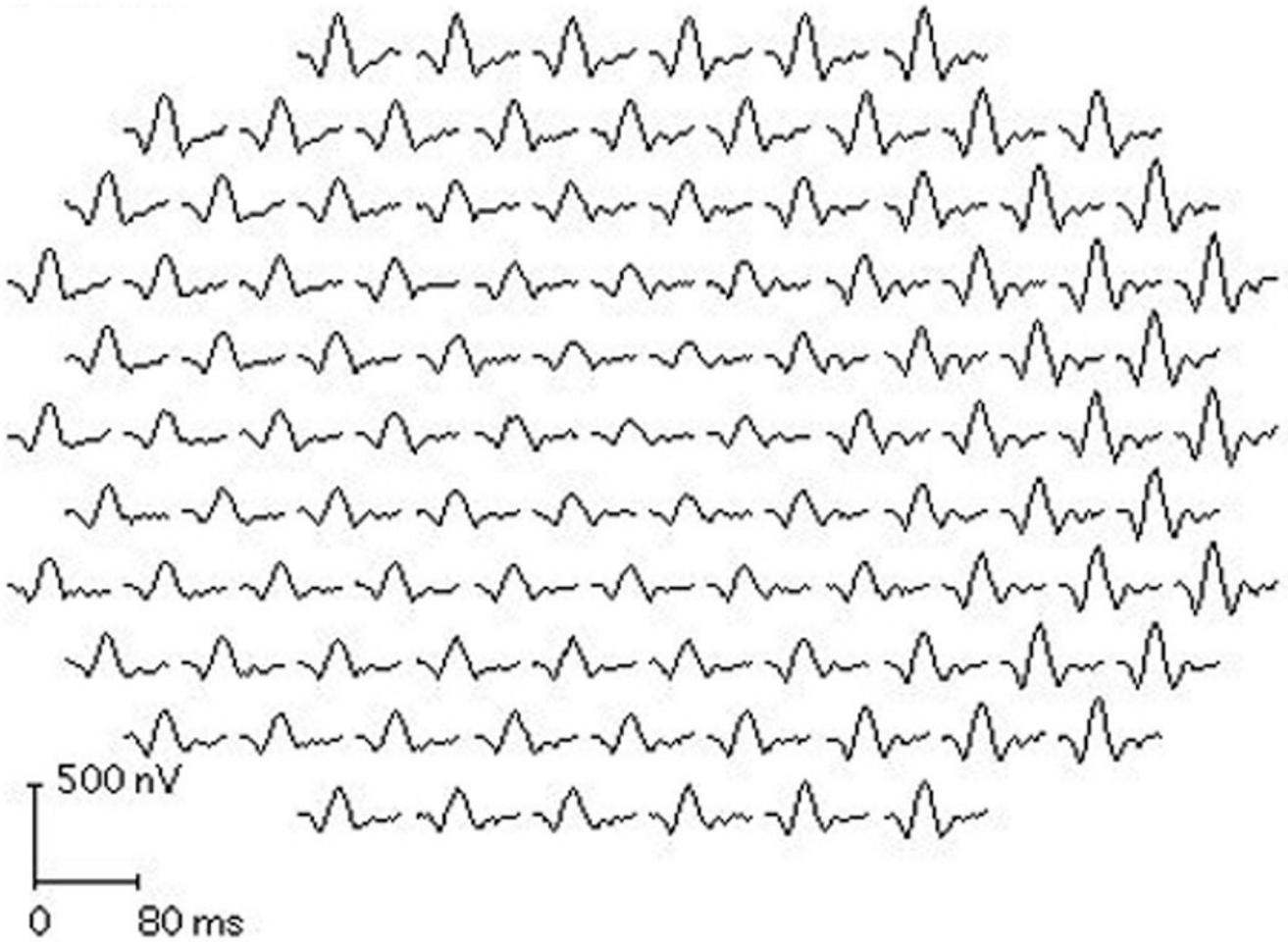


Figure 4.
mfERG OS from proband 2 demonstrates reduced responses from the central 1–5 degrees OS with relative preservation of the response amplitude and timing from the surrounding macula.

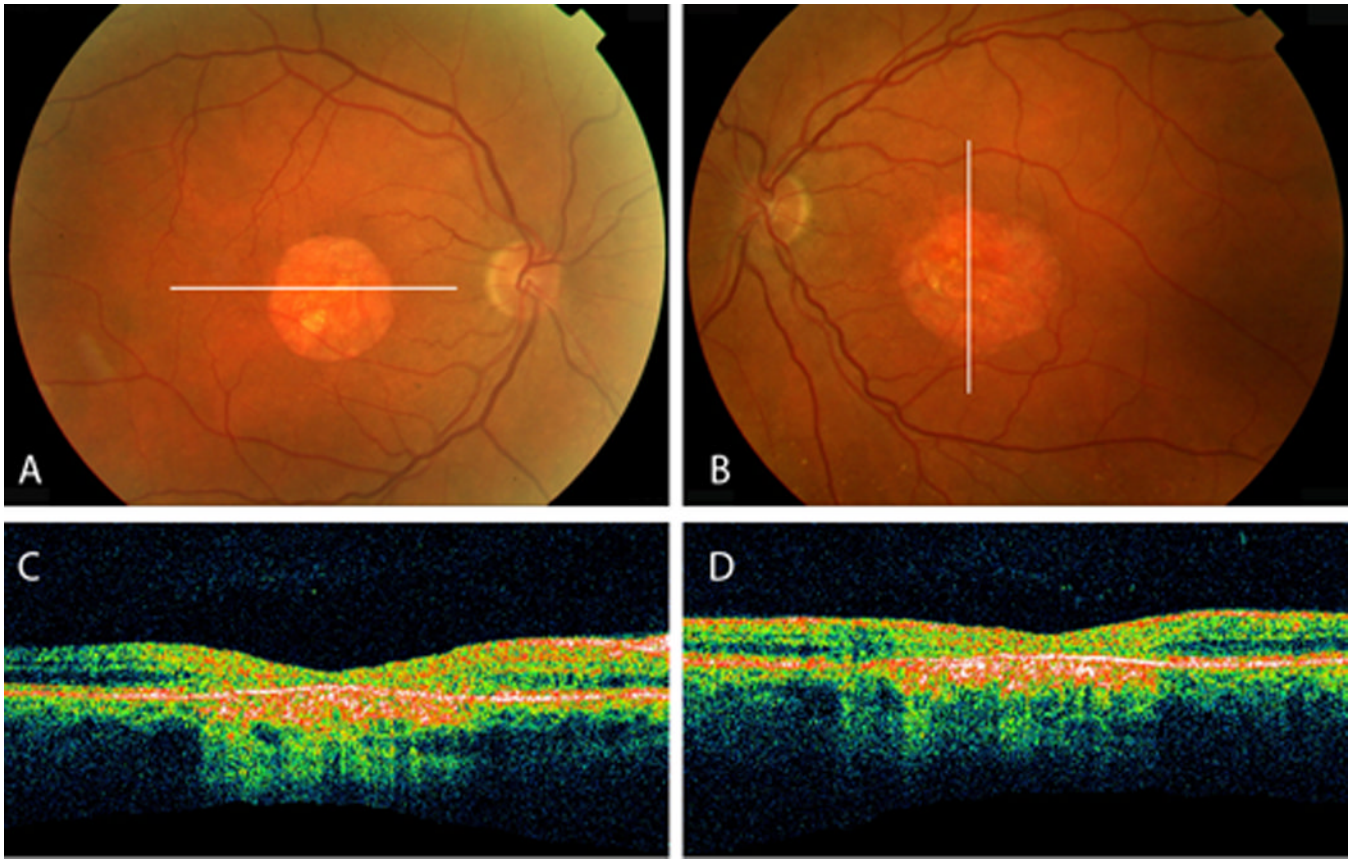
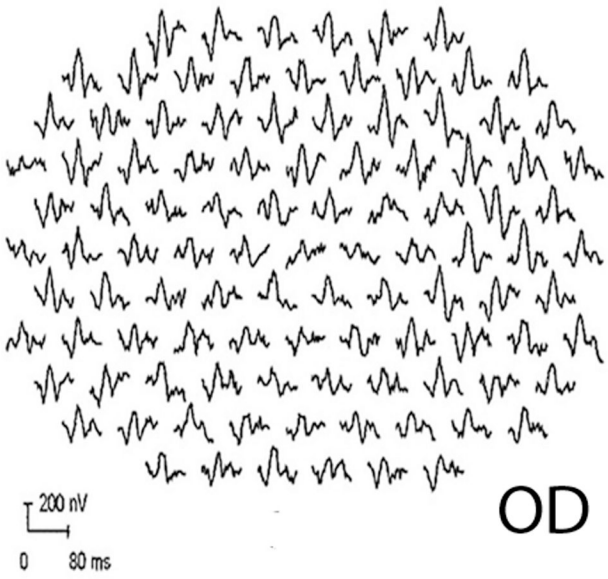


Figure 5.
A, B, Fundus of proband 3 reveals bilateral central atrophy with few drusen in the mid-periphery of both eyes. C, D, Optical coherence tomography indicates areas of atrophy seen with increased signal posterior to the retinal pigment epithelium and attenuation of the outer retina.



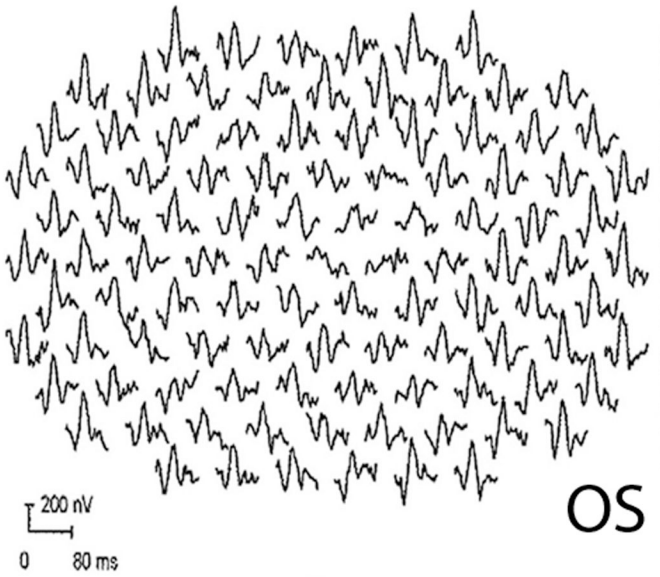


Figure 6.
mfERG of proband 3 demonstrates attenuation of amplitude and latency delay most prominent in the central macula with relative sparing of the periphery.

Table 1

Summary of clinical findings in 3 families of patients with the Glu292Lys *BEST1* variation.

State	Age	Sex	BCVA	Refraction	Irides	Fundus	Ethnicity
proband 1	5	M	20/40 OU	+5.25 sph OU	Green	vitelliform OU	Scandinavian Irish German French
asymptomatic mother of proband 1	30	F	20/20 OU	emmetropic	Green	normal	Scandinavian Irish German
proband 2	54	M	20/200 OD 20/20 OS	+2.25+075@180 OD +1.75+075@165 OS	Brown	atrophic OD vitelliform OS	East Indian
proband 3	59	F	20/70 OD 20/50 OS	+1.25+050@170 OD +1.00+025@010 OS	Green	atrophic OU	Norwegian Jewish