A Homozygous Frameshift Mutation in *BEST1* Causes the Classical Form of Best Disease in an Autosomal Recessive Mode

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**P**urpose. Best disease is a monogenic macular degeneration caused mainly by heterozygous mutations in the *BEST1* gene. The objective was to characterize the molecular and clinical features of patients with the classical form of Best disease that is inherited in an autosomal recessive mode.

**M**ethods. Clinical evaluation included detailed family history, a full ophthalmologic examination, electro-oculography (EOG), electroretinography, color vision testing, and ocular imaging. Mutation analysis was performed by direct sequencing of PCR products.

**R**esults. Two young siblings affected by Best disease, as confirmed by funduscopy, retinal imaging, and electrophysiologic assessment, were recruited for the study. Molecular analysis revealed a novel homozygous deletion (c.1415delT) in the *BEST1* gene leading to a frameshift followed by a premature stop codon, which cosegregated with the disease in a recessive mode. The heterozygous parents had normal visual acuity, retinal appearance, and function. The two heterozygous grandmothers, ages 61 and 62, also had normal Arden ratios on EOG, but one of them manifested moderate-to-severe dry non-vascular age-related macular degeneration.

**C**onclusions. We show here that the typical vitelliform phenotype of Best disease, usually transmitted in an autosomal dominant fashion, can be inherited as an autosomal recessive disease due to homozygosity for a frameshift mutation. (Invest Ophthalmol Vis Sci. 2011;52:5332–5338) DOI:10.1167/iovs.11-7174

Best vitelliform macular dystrophy (BVMD, MIM no. 153,700), also known as Best disease, is a progressive macular degeneration first described in 1905.1 On ophthalmoscopy, a characteristic sequence of changes often occurs: Early on, the fundus usually appears normal; a typical “egg-yolk” macular lesion may subsequently develop, reflecting abnormal accumulation of lipofuscin within and beneath the retinal pigment epithelium (RPE), with a distinguishing appearance on optical coherence tomography (OCT).2,3 Disruption of this lesion follows, usually accompanied by a drop in visual acuity and culminating in macular atrophy. Some patients may also develop cicatrical changes, with subretinal fibrosis and/or choroidal neovascularization.4 RPE function as measured by electro-oculography (EOG) is markedly affected from very early on, manifesting in a severely reduced Arden light peak-to-dark trough ratio in the presence of normal or mildly reduced full-field electroretinographic (FFERG) responses. Progression of the disease leads to destruction of the RPE and overlying photoreceptors in the macular area, often culminating in loss of central vision in late adolescence or adulthood. Best disease shows an autosomal dominant (AD) inheritance in the vast majority of cases and is mostly caused by heterozygous mutations in the *BEST1* (VMD2) gene.5,6 Reduced penetrance was reported in some families in which individuals who carry a disease-causing mutation heterozygously had abnormal EOG Arden ratios but normal visual acuity and normal fundus appearance.7,8

*BEST1* encodes a 585-amino acid protein called Best (1)-, whose function remains controversial. It is hypothesized to act as a Ca2+-activated Cl− channel, regulator of ion transport, or both (reviewed in9). One hundred and twenty-two *BEST1* mutations causing AD Best disease have thus far been reported, more than 90% of which are missense point mutations within the first 310 amino acid residues, often within or close to transmembrane domains. Some of the exons (mainly exons 4 and 8) are relatively enriched with mutations (an updated list of *BEST1* mutations can found at http://www-huge.uni-regensburg.de/BEST1_database/home.php). In addition, four in-frame deletions and three truncating mutations have thus far been reported (reviewed in Ref. 10 and listed in the VMD2 mutation database).

Heterozygous mutations in *BEST1* have also been reported as the cause of disease in adult-onset vitelliform macular dystrophy (AVMD)11 and AD vitreo-retino-choroidopathy (ADVIRC).12 The clinical features of AVMD partially overlap those of Best disease, but patients usually become symptomatic at a more advanced age (fourth or fifth decade of life), and the EOG is in the normal to subnormal range. *BEST1* was reported to cause approximately 25% of AVMD cases,11 and the RDS (retinal degeneration, slow) gene is responsible for approximately 36%.13 The clinical features of ADVIRC are different from those of Best disease and include retinal and vitreal involvement as well as ocular developmental abnormalities such as nanophthalmos, microcornea, closed-angle glaucoma, and congenital cataract.12 The mutations causing ADVIRC disrupt *BEST1* pre-mRNA splicing and are predicted to cause in-frame deletions.12 Yet another phenotype was reported to be associated with biallelic mutations of *BEST1*, which cause a
distinct retinopathy termed autosomal recessive (AR) bestrophinopathy (ARB).\textsuperscript{14} ARB is characterized by abnormal and reduced FFERG responses and severe reduction or absence of the EOG light rise, but without the characteristic funduscopic findings of Best disease.\textsuperscript{14} Missense mutations in \textit{BEST1} have also been reported to cause retinitis pigmentosa (RP) inherited in an AD (in four families) or AR (in a single family) fashion.\textsuperscript{15} Recently the association of biallelic \textit{BEST1} mutations and the Best vitelliform phenotype has been reported in four families.\textsuperscript{16, 17}

We present here a family of Ashkenazi Jewish descent with autosomal recessive Best disease. The two affected siblings are homozygous for a novel frameshift mutation in \textit{BEST1} while the middle-aged heterozygous (carrier) parents have normal visual acuities, EOG Arden ratios, and fundus appearance. Interestingly, one of the two carrier grandmothers manifests moderate to severe non-neovascular age-related macular degeneration (AMD) changes at the relatively young age of 61 years.

\textbf{METHODS}

The tenets of the Declaration of Helsinki were followed, and before donation of a blood sample for DNA analysis, informed consent was obtained from all patients and family members who participated in this study. Clinical evaluation included a detailed family history, a full ophthalmologic examination, EOG, FFERG, color vision testing using the Panel-D-15 test, OCT, color and infrared fundus photos, autofluorescence (AF) imaging, and fluorescein angiography (FA), performed as previously described.\textsuperscript{18} Briefly, FFERGs were recorded using monopolar corneal electrodes (Henkes-type, Medical Workshop B.V., Groningen, the Netherlands) and a computerized system (UTAS 3000; LKC, Gaithersburg, MD). In the dark-adapted state, a rod response to a dim blue flash and a mixed cone-rod response to a white flash were acquired. Cone responses to 50-Hz flashes of white light were acquired under a background light of 21 cd/m². Between two and four sets of responses were recorded in each condition to verify repeatability. All ERG responses were filtered at 0.3 to 500 Hz, and signal averaging was used. EOG was performed according to the International Society for Clinical Electrophysiology of Vision standard using bilateral skin electrodes on both canthi, and the Arden ratio (light peak to dark trough) was derived. OCT imaging was performed (Zeiss Stratus OCT3 system or Heidelberg Spectralis OCT), and presented as horizontal scans through the center of the fovea. Autofluorescence and infrared images were obtained using an SLO-based system (Heidelberg Retina Angiograph II; Heidelberg Engineering, Heidelberg, Germany).

Genomic DNA was extracted from peripheral blood of all family members (FlexiGene DNA kit; Qiagen, Valencia, CA). Primers for exons 1 to 3 and 5 to 11 of \textit{BEST1} were previously reported.\textsuperscript{3} Primers for exon 4 (F 5′AGAAAGCTGTGAGGACCGGA3′, R 5′-TCCACCCATCTTCCATTCGTC3′) were designed (Primer3 software; http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). PCR reactions were performed, and the mutations were analyzed by direct sequencing of the PCR products. A set of 112 ethnicity-matched normal controls was used to estimate the frequency of the c.1415delT mutation using the \textit{DdeI} restriction enzyme.

Genotyping of the following six AMD-associated polymorphisms were performed in individuals II:1 and II:3 (MOL0632): C2 p.E318D (rs95327359), ILOC3877715 p.A695 (rs10490924), CX3CR1 p.T280M (rs37525783), CFH p.Y402H (rs1061170), CFH p.I62V (rs800292), and CFB p.R32Q (rs641153). Primers for PCR (available by request) were designed using the Primer3 software. PCR products were restricted using the restriction enzymes BsmBI, PvuII, Alu26I, and TaqI, respectively. The SNPs rs800292 and rs641153 were genotyped by direct sequencing.

\textbf{RESULTS}

\textbf{Clinical Evaluation of MOL0632}

A nonconsanguineous family (MOL0632) of Ashkenazi-Jewish ancestry (Fig. 1A) was recruited for this study. The 9-year-old asymptomatic index case (IV:1) was found to have foveal changes on a routine eye examination (Figs. 2A, B, E, F), and subsequently further evaluation and testing was performed on her and on other family members. Visual acuity of the index case was normal (Table 1), and her fundus showed signs compatible with subclinical, early (previtelliform) stages of Best disease, including yellow foveal spots (Figs. 2A, E) that are more readily seen on infrared imaging (Figs. 2B, F) and show very minimal hyperautofluorescence (Figs. 2C, G). Horizontal spectral OCT scans through the fovea show the physiologic hyporeflective cleft that characterizes the outer segment/RPE complex in the subfoveal region and are normal (Figs. 2D, H).

Full-field ERG testing revealed normal light-adapted cone ERG responses and supernormal dark-adapted rod and mixed cone-rod ERG amplitudes with normal implicit times (the cause and significance of the elevated dark-adapted ERG responses are not clear; the hypermetropia that is present may partially account for this). The EOG Arden ratio, however, was severely reduced, with practically no light rise (Table 1). Panel-D15 color vision testing did not reveal any deficiencies. Based on these findings, she was clinically diagnosed with Best disease.

After her diagnosis, her younger brother (IV:4) was referred for examination. At the age of 2.5 years, visual acuity (using a picture cube) was 0.8 in each eye, but maculopathy was already clearly evident (Figs. 2I–N). Interestingly, foveal changes were more prominent than in his older sister, and spectral OCT shows significant subfoveal deposits located between the RPE and Bruchs membrane (Figs. 2K, N). ERG testing under anesthesia (using a short protocol, without full dark adaptation) was within normal limits (Table 1). Because of the young age of the patient, EOG testing as well as more detailed retinal imaging could not be performed. Clinical evaluation of the father at 40 years of age (III:4) revealed normal retinal appearance and function including a normal fundus examination (Figs. 3A, B), OCT (Figs. 3C, D), color vision, and EOG. Fundus findings, color vision, and EOG were also normal in the 34 year-old mother (III:3) of the affected children. After the molecular genetic work-up (detailed below), we also performed clinical evaluation of the two grandmothers (II:1 and II:3). In both, EOG, FFERG, and color vision were within normal limits (Table 1). Individual II:1 (Fig. 1A), at age 62, had a practically normal fundus examination, with mild extramacular RPE changes manifesting as deep, pale yellow spots that showed some staining on late-phase FA images (Figs. 3E, F). In contrast, individual II:3 (Fig. 1A), at age 61, had marked macular changes compatible with moderate to severe dry (nonneovascular) AMD, including large soft drusen (some of them confluent) that are clearly evident on fundus color photos (Figs. 3H, I) and spectral OCT imaging (Fig. 3J). Interestingly, the deceased mother of this patient (individual I:1) was also referred to manifest severe AMD, causing legal blindness in her early sixties.

\textbf{Molecular Analysis of MOL0632}

Screening the index case (IV:1) for mutations in the \textit{BEST1} gene revealed a novel homozygous deletion of thymine in exon 10 (c.1415delT, p.Leu472ProfsX10) leading to a frameshift followed by a premature stop at position 481 (Figs. 1B, C). In addition, the patient was homozygous for four silent changes (c.1097>C, c.219>C>A, c.1410>A>G, c.1608T>G) previously described as nonpathogenic.\textsuperscript{19} Segregation analysis (Fig. 1A) revealed a recessive inheritance pattern in which the affected
brother (IV:4) was also homozygous for the c.1415delT mutation, the three unaffected siblings were either heterozygous or carried normal alleles, and the unaffected parents (III:3 and III:4) were heterozygous. In addition, the two grandmothers (II:1 and II:3) and three uncles (III:1, III:6 and III:7) were heterozygous for the mutation (Fig. 1A). We screened a set of 112 ethnicity-matched normal controls and identified one carrier of the c.1415delT mutation.

Aiming to exclude the possibility that other BEST1 sequence variants protect from the disease in unaffected heterozygous individuals, we sequenced the BEST1 gene in the two heterozygous parents. Both were found to carry the four above-mentioned polymorphisms that are located on the chromosome harboring the disease-causing mutation. No sequence changes were identified on the counter allele.

To evaluate the possible contribution of polymorphisms previously reported to be associated with AMD, we genotyped six SNPs (three high-risk and three protective alleles) in the two grandmothers who are heterozygous for the BEST1 frameshift mutation. The individual who is affected by AMD, II:3,
carried heterozygously two high-risk alleles (\textit{LOC387715}\textendash rs10490924 and \textit{CFH}\textendash rs1061170) as well as two protective alleles (\textit{CFB}\textendash rs641153 and \textit{CFH}\textendash rs800292). Individual II:1 was also heterozygous for the two risk alleles, did not carry the \textit{CFB}\textendash rs641153 protective allele, and was homozygous for the common protective allele of \textit{CFH}\textendash rs800292.

**DISCUSSION**

The data we present here add to the complexity of phenotypes and inheritance patterns linked to mutations in the \textit{BEST1} gene. We provide conclusive evidence of transmission of the vitelliform phenotype of Best disease in an autosomal recessive inheritance (AR) pattern. In addition, unlike the majority of mutations causing Best disease thus far reported (which are missense mutations), the patients reported herein harbored a homozygous frameshift mutation that can either be null and degraded by the nonsense mediated mRNA decay system (NMD),\textsuperscript{19} or produce a truncated protein, similar to \textit{BEST1} splice variants.\textsuperscript{20} Approximately 90% of AD Best disease mutations thus far reported in the \textit{BEST1} gene are missense, predicted to result in production of an abnormal protein product that interferes with the normal protein, thus causing disease via a dominant negative mechanism (reviewed in Ref. 21). Frameshift and nonsense mutations in the \textit{BEST1} gene have been previously reported, few of which were associated with Best disease. Two of the mutations, \textit{p.His490fsX514} and \textit{p.Pro260fsX288}, were identified heterozygously in isolate Best cases with no information available on any of the family members and no mutation identified on the counter allele.\textsuperscript{8,22} These cases might represent either de novo AD mutations or AR cases with compound heterozygous mutations, one of which could not be identified by regular mutation screening of the exons and splice sites. An intriguing family was reported to include individuals who are either heterozygous for the \textit{p.R141H} missense mutation, a nonsense mutation (\textit{p.Y29X}), or compound heterozygous for both.\textsuperscript{23} Interestingly, none of the individuals (ages 6, 11, and 67) who are heterozygous for only the nonsense mutation were reported to have any signs of Best disease, whereas the two compound heterozygous sisters had a relatively severe form of Best disease.\textsuperscript{23} An additional family in which a similar combination of a null (\textit{p.Tyr5X}) and a missense (\textit{p.Ser144Gly}) mutation occurred has been recently reported, leading to multifocal Best disease.\textsuperscript{24} In another study a nonsense \textit{BEST1} mutation, \textit{p.Arg200X}, was found to cause ARB in a single family with affected homozygous individuals and unaffected heterozygous parents.\textsuperscript{14} The phenotype we report here is clinically different from ARB in two main aspects: First, the funduscopic findings in the two patients we describe here are

![Figure 2: Fundus imaging of individuals from family MOL0632 affected by Best disease.](image_url)

- **(A–H)** Imaging of the index patient (IV:1). (A) Right and (E) left color fundus photos show early macular changes, manifest as yellow foveal spots. These changes can be better seen in infrared photos (B, F) and exhibit only very mild hyper-autofluorescence (C, G). (D) Right and (H) left horizontal spectral OCT scans through the fovea are normal at this stage, with no obvious deposits. (I) Right and (L) left color fundus photos of her younger brother (IV:4) show more prominent foveal changes, which are also seen on infrared imaging (J, M), and correlate with significant subfoveal deposits shown to be located between the RPE and Bruchs membrane on spectral OCT scans (K, N).
The hypothesis that BEST1 mutations contribute to the risk of developing AMD was examined in two previous studies. In one of these studies, two AMD patients (with no available EOG data) were found to carry the nonsense p.Leu472ProfsX10 mutation heterozygously. The authors did not find a statistically significant difference in the presence of multifocal disease. In addition to being carriers of the c.1415delT mutation, these two individuals carried both risk and protective AMD-associated polymorphisms in other genes. Although the data are inconclusive, our findings, taken together with the aforementioned reports, suggest that recessive heterozygous BEST1 mutations, perhaps in conjunction with established AMD risk genetic variants (as those seen in MOL0632 II:3 with AMD), may confer further increase in the risk of developing AMD over time. This needs to be further explored in larger patient cohorts.

The AR inheritance pattern of Best disease in humans might be similar to the one identified in a canine model of Best disease. Homozygous mutations (p.Arg25X and p.Gly161Asp) in canine BEST1 were reported to cause canine multifocal retinopathy (cmr), a recessive disorder that shares clinical and pathologic similarities with BVMD and in which heterozygous dogs were asymptomatic. The haplosufficiency theory can explain the recessive inheritance of the p.Arg25X nonsense mutation, but the authors noted that variable expression and/or incomplete penetrance may also occur in dogs, and therefore a larger number of animals heterozygous for the p.Gly161Asp mutation need to be examined to determine the inheritance pattern of this mutation. In our patients, at least at the relatively young ages examined, there is as yet no evidence for multifocal disease.

There are two main possible mechanisms by which the mutation we identified could cause Best disease. One possibility is that the mutant transcript is recognized and degraded by the NMD mechanism, leading to a null phenotype, as reported to be the case for a few null mutations reported to cause an ARB phenotype in the homozygous state. A second possibility is that a truncated protein lacking the C-terminus but including the transmembrane domains is expressed, resulting in impaired, partial, or no protein function. Recent studies in the Bestrophin W93C knock-in mouse, which shares many similarities with BVMD, suggest that defects in Ca2+ channels may underlie Best disease rather than a null or insufficiency phenotype. Indeed, it is the C-terminal domain, which would be truncated by the mutation we report here, that was shown to interact with voltage-dependent Ca2+ channels.

Genes that can cause the same disease with different inheritance patterns have been well known for many years. Among the retinal disease genes, missense mutations in RHOD and RP1 mainly act in a dominant negative manner, causing adRP, and null mutations in these genes cause arRP with unaffected heterozygotes.

The variety of ocular phenotypes and inheritance patterns caused by BEST1 mutations is exceptional (Fig. 4). BEST1 is now known to cause Best disease, ADVIRC, and RP in a dominant inheritance pattern and, on the other hand, RP and ARB in an autosomal recessive pattern (reviewed in Ref. 21). This complex pattern can be only partially explained by the type of mutations in the BEST1 gene, and it is likely that interactions
FIGURE 3. Fundus imaging of the heterozygous (carrier) father and grandmothers of the affected children of family MOL0652. (A–D) Imaging of the unaffected carrier father (III:4) at the age of 40 years. (A) Right and (B) left fundus photos show normal appearance of the maculas and foveas. (C) Right and (D) left time domain OCT scans through the foveas are likewise normal. (E–G) The maternal carrier grandmother (II:1) showed mild extramacular RPE changes at the age of 62, manifesting as faint deep pale yellow spots compatible with drusen-like deposits that are better seen in late-phase fluorescein angiography images (F). Spectral OCT image of the fovea in this eye (G) is within normal limits. Images shown are of the right eye; the left eye had similar findings. (H–I) The paternal carrier grandmother (II:5), at age 61, had fundus findings compatible with moderate-to-severe dry AMD. Note large soft drusen on color photos of the right (H) and left (I) maculas. A representative spectral OCT image (J) of the left eye shows drusen under the RPE layer in the foveal area (arrows).
with other genes modulate disease type and severity. The present study, along with two additional recent reports,16,17 confirms that BEST1 can also cause AR Best disease.

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**References**


