

**Title:**

Typical Best vitelliform dystrophy secondary to biallelic variants in BEST1

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***Abstract***

## **Background**

Pathogenic variants in BEST1 can cause autosomal dominant or autosomal recessive dystrophy, typically associated distinct retinal phenotypes. In heterozygous cases the disorder is commonly characterized by yellow submacular lesions in the early stages, known as Best vitelliform macular dystrophy (BVMD). Bi-allelic variants usually cause a more severe phenotype including diffuse retinal pigment epithelial irregularity and widespread generalized progressive retinopathy, known as autosomal recessive bestrophinopathy (ARB). This study describes three cases with clinical changes consistent with BVMD, however, unusually associated with autosomal recessive inheritance.

## **Materials and Methods**

Detailed ophthalmic workup included comprehensive ophthalmologic examination, multimodal retinal imaging, full-field and pattern electroretinography (ERG; PERG) and electrooculogram (EOG).

Genetic analysis of probands and segregation testing, fundus examination of proband relatives was performed where possible.

## **Results:**

Three unrelated cases presented with a clinical phenotype typical for BVMD and were found to have bi-allelic disease-causing variants in BEST1. PERG P50 and ERG were normal in all cases. The EOG was subnormal (probands 1 and 3) or

normal/borderline (proband 2). Probands 1 and 2 were homozygous for the BEST1 missense variant c.139C>T, p.Arg47Cys, while proband 3 was homozygous for a deletion; c.536\_538delACA, p.Asn179del.

The parents of proband 1 were phenotypically normal. Parents of proband 1 and 2 were heterozygous for the same missense variant.

## **Conclusions**

Individuals with bi-allelic variants in BEST1 can present with a phenotype indistinguishable from BVMD. The same clinical phenotype may not be evident in those harboring the same variants in the heterozygous state. This has implications for genetic counselling and prognostication.

## **Main text:**

### **Introduction:**

Heterozygous variants in *BEST1* are usually associated with autosomal dominant Best vitelliform macular dystrophy (BVMD)(OMIM153700), typically characterized by bilateral “egg-yolk” macular lesions. The macular lesions, although not always present, may be further classified into six stages according to severity: previtelliform, vitelliform, vitelliruptive, pseudohypopyon, atrophic, and cicatricial.<sup>1-4</sup> The electrooculogram (EOG) is abnormal in most<sup>5</sup> in the absence of full field electroretinogram (ERG) abnormality, consistent with generalized RPE dysfunction without generalized photoreceptor dysfunction.

Biallelic variants in *BEST1* are predominantly linked with autosomal recessive bestrophinopathy (ARB)(OMIM611809). The presentation of ARB is usually more severe, with widespread fundus changes including RPE irregularity and multiple yellowish subretinal deposits at the posterior pole and in the mid-periphery.<sup>6-8</sup> Additional features can include retinal oedema, serous retinal detachment and subretinal fibrosis in the macula; as well as narrow anterior chamber angles and tendency to angle closure glaucoma.<sup>7,9</sup> Electrophysiologically there is typically severe abnormality of the EOG from a young age with later progressive ERG abnormalities from late childhood or adolescence.<sup>8</sup>

The aforementioned distinctive retinal phenotypes, inheritance pattern, electrophysiology and genetic testing can frequently differentiate between these two disorders. Herein we report three cases with a typical phenotype of BVMD, but due to bi-allelic disease-causing variants in *BEST1*.

### **Material and Methods:**

The protocol of the study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Moorfields Eye Hospital. In all three cases, a medical history was obtained, and a complete ophthalmologic examination performed. Colour fundus photography and fundus autofluorescence (FAF) imaging were performed with wide-field confocal scanning laser imaging (Optos PLC, Dunfermline, UK). Macular scans and FAF imaging of the posterior

pole were performed with spectral-domain optical coherence tomography (SD-OCT, Heidelberg Engineering, Heidelberg, Germany).

The electrophysiological assessment included International-standard full-field ERG and pattern electroretinography (PERG), performed with gold foil corneal (cases 1 and 3) or skin (case 2) electrodes, and EOG was performed.<sup>10-12</sup> The full-field ERG and EOG were used to assess generalized retinal and generalized RPE function respectively; the pattern ERG P50 component was used to assess macular function.<sup>13</sup>

Molecular testing of *BEST1* was by targeted next-generation sequencing and Sanger sequencing (Regional Genetics Laboratory Services, Manchester; Molecular Vision Laboratory, Oregon, United States; NIHR BioResource Rare, London, UK). Segregation analyses was performed in two families and fundus examination of relatives was performed in one family.

## **Results:**

### Case 1

An 11-year-old boy was visually asymptomatic and found to have abnormal fundus changes bilaterally during routine optometry visit. Best-corrected visual acuity (BCVA) was 6/9 in both eyes. Anterior segment examination and intraocular pressures were normal. Retinal examination showed a yellow subfoveal vitelliform lesion bilaterally, less than half a disc diameter in size (Fig:1A,

B). The macular lesions corresponded to areas of high intensity FAF (Fig:1C, D). SD-OCT showed bilateral optically empty spaces with irregular collection of hyperreflective material on the outer retinal surface (Fig:1E, F). The ERG and PERG were normal, and the EOG had a reduced light peak: dark trough (LP:DT) ratio (145% bilaterally; reference range 180-435%).<sup>5,14</sup> Whole genome sequencing identified homozygous c.139C>T, p. Arg47Cys variants in *BEST1*.

Fundus examination of the proband's parents was normal. Segregation analysis showed each parent to be a heterozygous carrier for the same *BEST1* variant. There was no history of consanguinity in the family

Follow up over 10 years showed the BCVA, fundus findings and SD-OCT of the proband to have remained stable.

## Case 2

A 12-year-old girl presented with blurred central vision. BCVA was 6/6 bilaterally, with a hypermetropic correction. Anterior segment examination showed pinpoint posterior subcapsular cataract in the right eye; the left eye was unremarkable. Intraocular pressures were normal. Her younger sister was diagnosed as having bilateral congenital cataract. The wide-field fundus images showed bilateral vitelliform lesions in the pseudohypopyon stage, with accumulation of yellowish material in the sub-macular space (Fig:2A, B). FAF of the lesion showed increased autofluorescence inferiorly, while a low to absent FAF signal was seen in the central area bilaterally (Fig:2C, D). SD-OCT scans

through the lesion showed bilateral thickening of the RPE and the photoreceptor layer. An optically empty space was evident on the right and there was evidence of deposition of hyperreflective material sub-foveally bilaterally (Fig:2E,F). The ERG, PERG and EOG (LP:DT ratio right eye 190%; left eye 185%) were normal. Her parents were asymptomatic and there was no history of consanguinity in the family, however they were not available for clinical examination.

Genetic testing identified homozygous, c.139C>T, p. Arg47Cys variant in *BEST1* in the proband, while her mother was a heterozygous carrier of the same missense.

### Case 3

A twenty-nine-year-old male had a history of reduced vision from the age of 16. The left eye had been treated elsewhere with laser photocoagulation for choroidal neovascular membrane. BCVA was 6/9 on the right and 6/12 on the left. The anterior segment examination and intraocular pressure were normal. Fundus examination revealed a yellow vitelliruptive macular lesion in the right eye and a cicatricial stage lesion in the left (Fig:3A, B). FAF in the right showed an increased signal on the inferior edge of the lesion and decreased FAF centrally and superiorly; on the left there was an area of absent FAF surrounded by a border of hyperautofluorescence associated with scar tissue (Fig:3C,D). SD-OCT scans showed an optically empty space with thinning of the outer retina on the right. There was patchy thickening of the outer retina with a hyporeflective sub-foveal mass in the left (Fig:E,F). The ERG and PERG were normal. Eye movement accuracy during the light phase of EOG testing became variable after 9-10 minutes, but no light peak was detectable (LP:DT ratio 100% bilaterally).

His parents were first cousins from Bangladesh and visually asymptomatic (not available for examination). The proband was found to have homozygous c.536\_538delACA, p.Asn179del variant in *BEST1*. Clinical findings of the proband have been stable over 14 years of follow-up

### **Discussion:**

This study details the findings in three unrelated individuals with clinical and electrophysiological features typical of BVMD, but inherited in an autosomal recessive manner, usually associated with a distinctly more severe and progressive retinal and functional phenotype (ARB). Our case study provides further evidence that rare sequence variants in *BEST1* behave in a recessive manner but are phenotypically identical to BVMD.

In two of the three cases, stability of the BVMD phenotype was demonstrated, including one case of a child monitored into adulthood. Two different homozygous disease-causing variants in *BEST1* were identified in each of the 3 families. (c.139C>T, p.Arg47Cys in two families and c.536\_538delACA, p.Asn179del. in one family)

Several studies have reported that the majority of disease-causing variants in *BEST1* associated with BVMD are heterozygous missense variants, which differ from those causing ARB.<sup>3,7,15,16</sup> However, there are rare reports of bi-allelic *BEST1* variants causing BVMD.<sup>15,17-20</sup> Heterozygous family members can present with the same retinal phenotype showing variable expressivity, recognized as a cause of inter and intra- familial differences in other *BEST1* pedigrees.<sup>17,21-23</sup>

Rarely BVMD has also been associated with novel variants, which can be pathogenic, associated with manifest disease, only when in bi-allelic form and are unlikely to cause retinal dystrophy in the heterozygous state.<sup>24-26</sup>



The clinical presentation and disease course in our 3 patients was indistinguishable from BVMD, in keeping with limited previous reports. Sodi et al <sup>26</sup> described four cases of BVMD with compound heterozygous or homozygous variants in *BEST1*. These included two of the variants seen in our cases in homozygous state (p. Arg47Cys, p. Asn179del). The family members who were heterozygous carriers had a normal clinical phenotype, although some showed a reduced EOG LP:DT ratio. The bi-allelic variants causing BVMD were noted to be often deletion rather than missense and were located outside the BVMD *BEST1* mutational hot spot.<sup>26</sup> Some were associated with ARB. It was concluded that these sequence variants were pathogenic only when inherited in recessive form.

Variable BVMD phenotypes associated with autosomal recessive inheritance have also been described, ranging from typical BVMD lesions to atypical extramacular deposits and parents with normal fundi.<sup>25</sup> The authors proposed that, in contrast to autosomal dominant BVMD cases, variants may have caused minimal protein changes i.e., maintaining function in the heterozygous state but resulting in a BVMD phenotype when combined with another mild disease-causing variant.

Bitner et al <sup>24</sup> further reported a family with c.1415delT, p.Leu472ProfsX10, homozygous frameshift in *BEST1* presenting as BVMD. Interestingly, although the probands parents had normal fundus findings, both grandmothers harboring heterozygous variants and a deceased great grandmother, showed a recessive inheritance pattern with dry age-related macular degeneration (AMD). All except the great-grandmother in whom EOG was not tested, had a normal EOG and ERG. However, in previous studies *no* statistically significant difference between *BEST1* mutations in the AMD group and the control group was found.<sup>27,28</sup>

Collectively, these case reports and our case series indicate that homozygous or compound heterozygous *BEST1* variants can be associated not only with ARB, but also with autosomal

recessive BVMD.<sup>24-26</sup> Unless a functional analysis is performed, it will remain inconclusive why certain *BEST1* variants cause BVMD, only when coupled with a disease-causing variant on the second allele. Nevertheless, it is of utmost importance to have clarity about the *BEST1* variants and their inheritance pattern, as this will ensure accurate genetic counselling and family planning, but also in providing potential treatment in the future.

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