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Novel *BEST1* mutation in autosomal recessive bestrophinopathy in Japanese siblings

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Abstract:

PURPOSE: Autosomal recessive bestrophinopathy (ARB) is a disease that results from the mutations in the *BEST1* gene. It is characterized by multifocal yellowish lipofuscin deposits, cystoid macular edema, and subretinal fluid. Among approximately 270 *BEST1* mutations, only 40 that include both heterozygous and homozygous mutations are associated with ARB. However, very few ARB-related mutations have been reported in the Japanese population. Therefore, in this study, we aimed to identify *BEST1* mutations and describe the genotype–phenotype relationship in Japanese dizygotic twins presenting with ARB.

MATERIALS AND METHODS: We performed clinical examinations in Japanese dizygotic twin patients (male: 29 years) with ARB as well as whole-exome sequencing in seven family members of these twins.

RESULTS: In this study, we have reported on a novel *BEST1* mutation, the p. Phe151Cys mutation, associated with ARB in Japanese dizygotic twins who had bi-allelic p. Ala160Pro mutations in *BEST1*. The clinical features observed were binocular abnormalities of the fundus, such as multifocal yellowish subretinal deposits, cystoid macular edema, and subretinal fluid. The full-field electroretinography results were subnormal.

CONCLUSION: It was indicated that the novel *BEST1* mutations identified may be strongly correlated with binocular ARB. This study provides significant information of the genotype–phenotype association in Japanese ARB patients. Further, the genetic analysis that we performed was very useful for the differential diagnosis and might have implications in the development of future treatment modalities.

Keywords:

Best vitelliform macular dystrophy, bestrophin-1, dizygotic twins, genotype–phenotype correlation, macular edema

Introduction

The retinal pigment epithelium (RPE) plays an essential role in retinal function and visual acuity.[1] Several retinal diseases, such as retinal dystrophy and age-related macular dystrophy (AMD), are caused by RPE dysfunction.[2] Mutations in the *BEST1* gene lead to RPE dysfunction, and thus far, approximately 270 mutations have been reported.[3] The *BEST1* gene is located on chromosome 11 (*11q12.3*), and it encodes the transmembrane protein, bestrophin-1, which is localized in RPE cells.[3] Bestrophin-1 functions as a calcium-activated chloride channel and affects RPE metabolism, the transportation of subretinal fluid to the choroids, and the phagocytosis of the outer photoreceptor segment. Thus, a dysfunction in the synthesis or function of bestrophin-1 may lead to an increase in subretinal fluid, macular edema, and the accumulation of lipofuscin within and beneath the RPE.[1,2] Best vitelliform macular dystrophy (BVMD) and autosomal

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recessive bestrophinopathy (ARB) are known as *BEST1* mutation diseases. BVMD is an autosomal dominant retinopathy with the following clinical characteristics: the accumulation of lipofuscin within and beneath the RPE, which presents as yellowish, vitelliform, or egg-yolk-like lesions in the macula.[4] In contrast, ARB is characterized by multifocal yellowish lipofuscin deposits, cystoid macular edema, and subretinal fluid.[5] BVMD and ARB are associated with central visual loss.[6] Identifying the genotype–phenotype correlations that are associated with *BEST1* mutations may be significant to clarify their pathophysiological mechanism.

Among approximately 270 *BEST1* mutations, only 40 that include both heterozygous and homozygous mutations are associated with ARB. Very few ARB-related mutations have been reported in the Japanese population. Therefore, in this study, we aimed to report on *BEST1* mutations and to identify the genotype–phenotype correlations that are associated with *BEST1* mutations in dizygotic twin patients with ARB from Japan.

Materials and Methods

Clinical examination

The family and medical histories of seven family members of the two siblings with ARB were recorded, and clinical examinations, including best-corrected visual acuity, intraocular pressure, a slit-lamp examination, a dilated fundus examination, imaging fundus photography, Swept Source OCT (SS-OCT) and fundus autofluorescence (DRI OCT Triton Plus; TOPCON, Tokyo, Japan), fundus fluorescein angiography (VX-10i; KOWA, Tokyo, Japan)), and full-field electroretinography (ERG) (ERG and VEP LE-3000; TOMEY, Nagoya, Japan) were performed to examine the dizygotic twins.

Deoxyribose nucleic acid isolation, exome library preparation, and sequencing

Deoxyribose nucleic acid (DNA) was isolated from whole blood using a Gentra Puregene Blood Kit (Qiagen, Valencia, CA, USA; cat no. 158467). The exome library was enriched using a Nextera Exome Kit (Illumina, San Diego, CA, USA). The library was sequenced on the Illumina NextSeq 500 platform (Illumina, San Diego, CA, USA) to obtain 56.6 ± 0.2 M 75-bp paired-end reads per sample. A mean region coverage of 127.4 ± 4.1 with 90.2 ± 0.8% of target coverage at ×20 was obtained.

Variant annotation and filtering

Whole-exome sequencing data were processed using GATK's best practices workflow for DNA-Seq.[7] Briefly, raw-sequencing reads were aligned to the human reference genome version, genome reference consortium Human genome build 37, using the BWA Enrichment software program (version 2.1.0, (Illumina, San Diego, CA, USA)). The resulting variant call format files from all the samples were analyzed to annotate and extract potentially pathogenic variants using VariantStudio 3.0 (Illumina, San Diego, CA, USA). Simultaneously, the binary alignment map files that were outputted by the BWA Enrichment program were visualized to estimate the confidence of called variants using Integrative Genomics Viewer.[8] Two filtering methods were applied to identify candidate pathogenic nucleotide variants. The variants that were present at >1% in either the 1000 Genome Project 31 database or Exome Aggregation Consortium database 33 were filtered out, and the variants that followed autosomal or X-linked recessive modes of inheritance were selected for the further analysis. Nonsynonymous nucleotide variants were evaluated using the polymorphism phenotyping (PolyPhen)and the sorting intolerant from tolerant (SIFT) to determine whether alteration of the amino residue impaired protein function. Potentially pathogenic nucleotide variants were confirmed through direct Sanger sequencing using the primer set, 5′-*GGCTTCTACGTGACGCTGGT*-3′ and 3′-*TCCACCCATCTTCCATTCCT*-5′ to amplify and sequence the *BEST1* exon4.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee at Jichi Medical University (No.18-018), and written informed consent was obtained from all the participants. These processes were carried out in accordance with the tenets of the Helsinki Declaration.

Results

Patient clinical characteristics

The proband [Figure 1, III-1] and the affected sibling [Figure 1, III-2], both aged 29 years, were male dizygotic twins. They presented with reduced vision in both eyes as they simultaneously developed cystoid macular edema and subretinal fluid accumulation [Figures 2d and 3b]. At the first visit, the best-corrected visual acuities of the proband were 20/29 (oculus dexter [OD], right eye) and 20/33 (oculus sinister [OS], left eye), and those of the affected sibling were 20/25 (OD) and 20/66 (OS), whereas approximately 5 and 7 months later, the best-corrected visual acuities of the proband decreased to 20/40 (OD) and 20/40 (OS) while those of the affected sibling remained unchanged [Table 1]. Before the onset of the visual impairment, the best-corrected visual acuities of the proband and affected sibling were above 20/20. The diagnosed intraocular pressure of both the siblings was normal.

The proband and affected sibling did not exhibit hyper hyperopia (the refractive-errors ranged from −0.5 to +0.5 diopter), and their anterior chamber depth

Table 1: Summary of first clinical characteristics and genetics findings

	Age	Sex	BCVA	OCT	ERG	Mutation
Proband	29	Male	20/29, 20/33 (OD, OS)	Cystoid macular edema Subretinal fluid	Subnormal	p.Phe151Cys (hetero) p.Ala160Pro (hetero)
Sibling	29	Male	20/25, 20/66 (OD, OS)	Cystoid macular edema Subretinal fluid	Subnormal	p.Phe151Cys (hetero) p.Ala160Pro (hetero)

*hetero; heterozygous, BCVA; best-corrected visual acuity

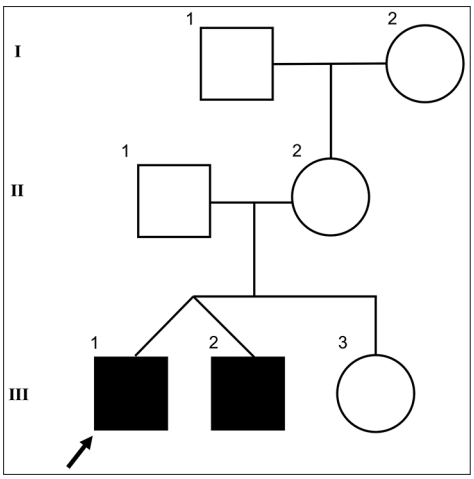

Figure 1: Pedigree of the family with two affected members. The proband is marked with an arrow (III-1), and the affected sibling is indicated by a black box (III-2)

and anterior chamber angles were normal. They did not have microcornea, cataract, or staphyloma. Apart from these dizygotic twin patients, the other family members did not have visual disturbances [Figure 1]. The fundus photographic images of the proband and affected sibling revealed sparse yellowish macular lesions [Figures 2a and 3a]. The fundus autofluorescence results of the proband indicated hyper signals corresponding to the yellowish lesions [Figure 2b]. The fundus fluorescein angiography of the proband indicated no vessel leaks [Figure 2c]. These dizygotic twin patients had very similar cystoid macular edema and subretinal fluid as indicated by OCT imaging [Figures 2d and 3b], and the full-field ERG test results indicated reduced amplification (subnormal) corresponding to rod and cone dysfunction in flash, flicker, cone, rod, and op waves [Figures 2e and 3c]. The clinical treatment of cystoid macular edema and subretinal fluid with subtenon triamcinolone acetonide (STA) was ineffective.

Exome sequencing and causal variant identification

The whole-exome sequencing of the seven family members resulted in an output of a total of 292,238 variants (mean per sample = 41,748 ± 230). Among these, 668 variants in 43 autosomal genes and one rare variant in an X-linked gene (minor allele frequency <1%) were segregated in the affected and unaffected family members in the recessive mode. These 668 rare variants were predicted to have an effect on protein function. Two variants in the *BEST1* gene (NM_004183.4 (NP_004174.1)), c.452T>G (p. F151C) and c. 478G > C (p. A160P)) [Figure 4a], were predicted to be solely deleterious to the function of the protein, bestrophin1. The grandmother (I-2) and mother (II-2) were found to have a p. F151C mutation, the father (II-1) was found to have a p. A160P mutation, and the dizygotic twins (III-1,2) exhibited double mutations of p. F151C and p. A160P; however, the younger sister (III-3) did not have any of the abovementioned mutations. As reported on RetNet, *BEST1* is associated with an ocular disease phenotype (https://sph.uth.edu/retnet/), which supports the pathogenicity of these variants. These mutation areas are highly conserved among species [Figure 4b], and it is highly probable that an alteration in the amino residues lead to an impairment in bestrophin1 function [Figure 4c]. According to the American College of Medical Genetics and Genomics guideline, p. F151C is likely to be pathogenic (PM2, PM3, PP1, PP3, and PP4), and p. A160P is pathogenic (PS1, PM2, PP1, PP3, PP4, and PP5).[9]

Discussion

BEST1 mutations are associated with a wide range of ocular phenotypes.[10,11] BVMD and ARB are quite different in both their genotypes and clinical characteristics. BVMD is an autosomal dominant disease with a phenotype consisting of five stages: previtelliform, vitelliform (egg-yolk), pseudohypopyon, vitelleruptive, and atrophic.[12-14] Unlike BVMD, ARB is an autosomal recessive disease, and its phenotype consists of cystoid macular edema, subretinal fluid, and multifocal yellowish retinal deposits.[5,15] The *BEST1* mutation loci affect bestrophin1 protein modeling and its function.[16-18] Among approximately 270 *BEST1* mutations, only about 40 have been reported to be associated with ARB, and reports pertaining to Japanese ARB are especially scarce.

In this study, Japanese male dizygotic twins, aged 29, suffered from the simultaneous onset of visual loss with cystoid macular edema and subretinal fluid accumulation. At first, the patients were suspected to have X-linked juvenile retinoschisis (XLRS),[19] uveitis,[20] or acute posterior multifocal placoid pigment epitheliopathy[21] based on the observed clinical features, such as macular edema. However, other family members

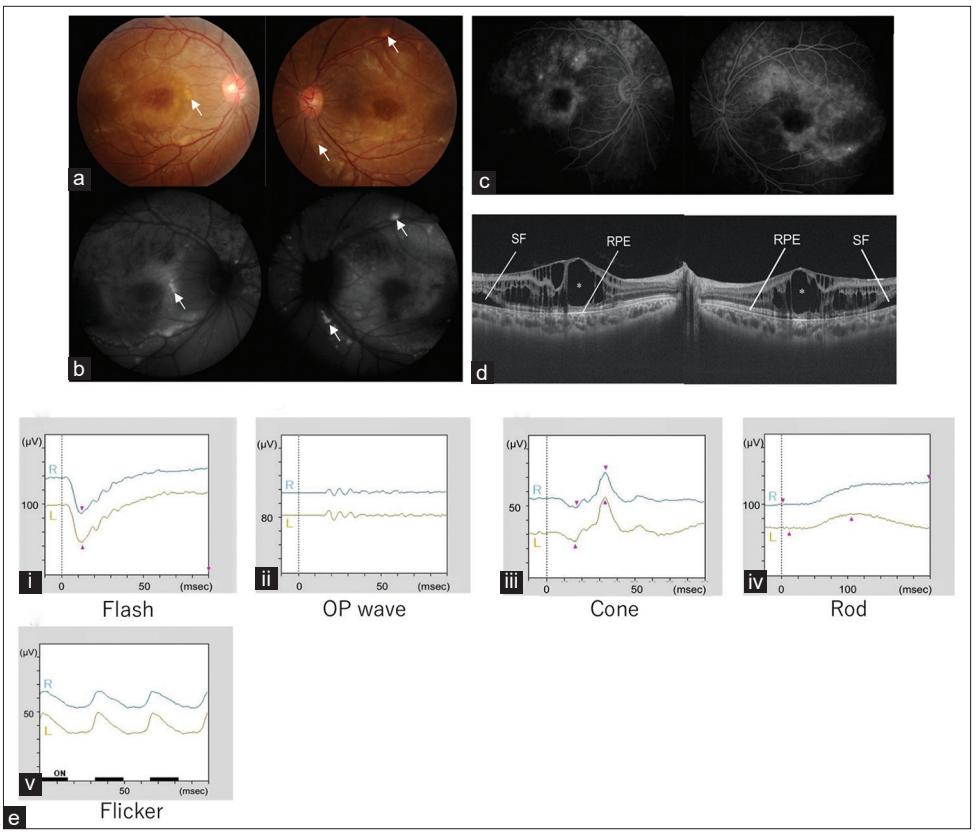

Figure 2: Clinical features of the proband. Fundus photographs (a), fundus autofluorescence image (b), fluorescein fundus angiography (c), spectral-domain optical coherence tomography (d), full-field electroretinography (first visit) (e). White arrows indicate yellowish deposit lesions (a and b) and white asterisk, cystoid macular edema (d). RPE = Retinal pigment epithelium, SF = Subretinal fluid

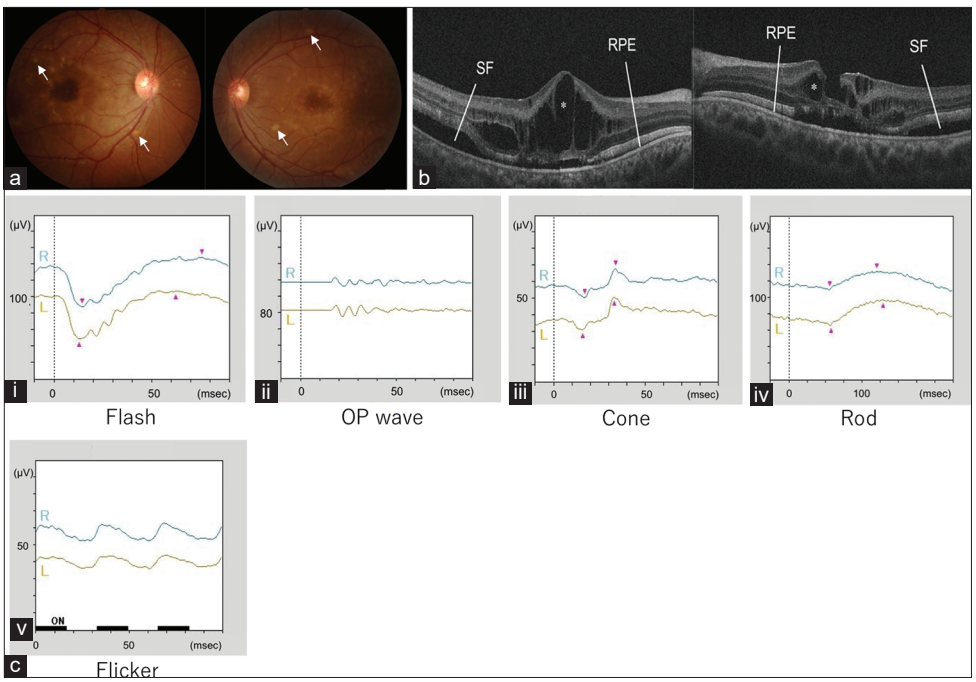

Figure 3: Clinical features of the affected sibling. Fundus photographs (a), spectral-domain optical coherence tomography (b), full-field electroretinography during the first visit (c). White arrows show yellowish deposit lesions (a). The white asterisk depicts cystoid macular edema (b). RPE = Retinal pigment epithelium, SF = Subretinal fluid

of these patients did not have visual problems. In addition, these patients did not exhibit severe myopia. Furthermore, the tests for uveitis, including those for specific antibodies, were all negative. In addition, the STA treatment was ineffective. Although we were unable to confirm a clinical diagnosis based on the clinical findings, a hereditary disease was suspected, and a whole-exome sequence analysis was performed. The results indicated that these Japanese dizygotic twins were carrying the same combined-heterozygous *BEST1* mutations at c. 425T>G (p. F151C) and c. 478G>C (p. A160 P). The p. F151C mutation was derived from the mother, and the p. A160 P one, from the father. The PolyPhen software predicted that p. A160 P and p. F151C amino acid substitutions might affect and possibly impair bestrophin1 function [Figure 4c]. Gene mutations associated with other hereditary diseases, such as XLRS, RP, or Stargardt disease, were not found. The confirmed clinical diagnosis of ARB was substantiated by matching clinical features. Unfortunately, at the time of this study, our hospital did not own electro-oculogram (EOG) equipment and was not able to perform EOG measurements.

Gene analysis is a powerful approach that can be utilized not only for the diagnosis and proper treatment of an eye disease but also for the clarification of its pathology.[16] In clinical practice, it may be challenging to appropriately diagnose a disease owing to other diseases that have similar clinical features and to those that have atypical clinical features.[16] There are several retinal degenerative diseases, such as BVMD, ARB, Stargardt's disease, and AMD. Most of them are caused by RPE dysfunction, but the relationships among these diseases have not been studied in detail.[2] In addition, genotype-phenotype relationships among retinal diseases suspected to be caused by the same gene have not been effectively analyzed. Several eye diseases, such as ARB (MIM 611809),[16,17] BVMD (MIM 153700),[13,14] autosomal dominant vitreoretinochoroidopathy (MIM 193220),[22,23] autosomal dominant microcornea, rod-cone dystrophy, early-onset cataract posterior staphyloma syndrome, and retinitis pigmentosa (MIM 613194),[10,11] appear to be associated with *BEST1* mutations. However, their pathological relationships are not yet known. Although the p. A160P mutation has already been reported as an ARB-associated mutation in Japanese patients,[9] the ARB characteristics that have been reported are very different from those corresponding to our new bi-allelic mutations (p. F151C and p. A160P) and those of p. A160P and p. A195V.[9] As for the characteristics of ARB resulting from the new bi-allelic mutations (p. F151C and p. A160P), the main phenotype is cystoid macular edema, and a significant reduced ERG amplification is observed (subnormal). On the other hand, the main phenotype of ARB resulting from bi-allelic mutations of p. A160P and p. A195V is subretinal fluid, and the ERG amplification is normal.[9] It is very interesting that the phenotype and physiological function differ greatly depending on the combination of mutations.

Accurate diagnoses and analyses of genotype–phenotype relationships may contribute toward determining optimal treatment strategies for patients as well as toward the development of therapeutic alternatives. Genetic analysis has become more affordable and quicker because of next-generation sequencing technologies.[24] Therefore, the genetic analysis of patients is expected to be a useful strategy in the future gene therapy.

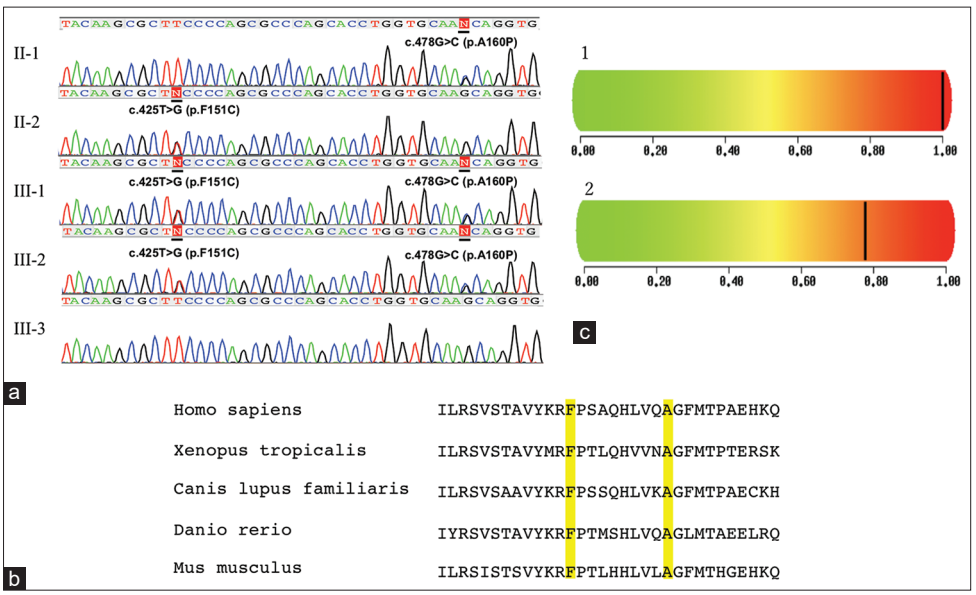

Figure 4: Identification of mutations of patients (a) heterozygous mutations of c. 452T>G (p. F151C) and c. 478G>C (p. A160P) in *Best1* gene. (b) Multiple amino acid sequence alignments of *Best1* protein (bestrophin1) in different species. The yellow color areas indicate conserved positions across species. (c) PoplyPhen predicted bestrophin1 protein damage derived from amino acid substitution of p. F151C and p. A160P. It indicates p. F151C substitution is probably damaging (1) and p. A160P substitution is possibly damaging (2)

Recently, gene therapies for Leber congenital amaurosis, a type of retinal dystrophy, have been approved as a clinical treatment modality.[25] Several clinical trials involving gene therapies for Stargardt disease, choroideremia, autosomal dominant optic atrophy, and AMD have been conducted, in which the therapies were proven to be effective.[26] Moreover, with the development of new genome-editing technologies, including the CRISPR–Cas 9 system, the number of target diseases that are treatable by gene therapy is increasing.[27] On a final note, early and accurate clinical diagnoses using gene panels might ensure the administration of appropriate and timely treatment, facilitate accurate prognoses, and lower medical expenses.[28]

Conclusion

Identification of disease-related gene mutations is very important for clinical diagnosis and for elucidating the pathology. As a result of our study, a novel *BEST1* gene mutation was found in Japanese twins. This finding can contribute to a better understanding of the possible mechanisms and genotype–phenotype relationships for *BEST1* gene-related retinal diseases.

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Conflicts of interest

The authors declare that there are no conflicts of interests of this article.

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