Mutation analysis of *BEST1* in Japanese patients with Best vitelliform macular dystrophy

Subtitle: *BEST1* mutations in Japanese patients

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Abstract

Purpose: To describe the clinical and genetic features of Japanese patients with Best vitelliform macular dystrophy (BVMD).

Patients and Methods: This study examined 22 patients, including 16 probands from 16 families with BVMD. Comprehensive ophthalmic examinations were performed, including dilated funduscopy, full-field electroretinography (ERG), and electrooculography (EOG). *BEST1* mutation analysis was performed by Sanger sequencing.

Results: All 16 probands exhibited characteristic BVMD fundus appearances, abnormal EOG, and normal ERG responses with the exception of one diabetic retinopathy proband. Genetic analysis identified 12 *BEST1* mutations in 13 probands (81%). Of these, 10 mutations (p.T2A, p.R25W, p.F80L, p.V81M, p.A195V, p.R218H, p.G222E, p.V242M, p.D304del, and p.E306D) have been previously reported, while two mutations (p.S7N and p.P346H) were novel. Single *BEST1* mutations were found in 12 probands. The one proband with compound heterozygous mutations (p.S7N and p.R218H) exhibited typical BVMD phenotypes (pseudohypopyon stage and vitelliruptive stage in the right and left eyes, respectively).

Conclusions: Twelve different mutations, two of which (p.S7N and p.P346H) were novel, were identified in the 13 Japanese families with BVMD. Compound heterozygous mutations were found in one proband exhibiting a typical BVMD phenotype. Our results suggest that *BEST1* mutations do play a large role in Japanese BVMD patients.

INTRODUCTION

The *BEST1* gene (OMIM *607854; also known as *VMD2*) encodes a 585-amino acid transmembrane protein (bestrophin-1) that has been mapped to the long arm of chromosome 11 (11q12.3).[1, 2] The *BEST1* gene was first described in 1998 as the causative gene of Best vitelliform macular dystrophy (OMIM #153700; BVMD).[1, 2] To date, *BEST1* mutations have been reported to underlie at least four major ocular phenotypes; BVMD, autosomal dominant vitreoretinochoroidopathy, retinitis pigmentosa, and autosomal recessive bestrophinopathy (ARB).[3-6] In addition to *BEST1*, it is reported that *PRPH2* mutations are also associated with vitelliform macular dystrophy and adult-onset vitelliform macular dystrophy.[7, 8]

The clinical features of BVMD caused by the *BEST1* mutations were first described by Best in 1905.[9] Although the majority of BVMD is inherited in an autosomal dominant fashion,[4] on occasion, some BVMD shows autosomal recessive inheritance.[10] BVMD is ophthalmoscopically characterized by an elevated macular lesion filled with large deposits of lipofuscin-like material, which creates a yellowish lesion resembling an egg yolk (vitelliform stage). Before the vitelliform stage, discrete alterlations of the retinal pigment epithelium (RPE) are often observed (previtelliform stage). Over time, the vitelliform lesion breaks up, resembling scrambled egg-like appearance (vitelliruptive stage), and becomes a niveau formation of yellowish materials in the inferior part of lesion (pseudohypopyon stage). Subsequently, chorioretinal atrophy ensues (atrophic stage).[4, 11] Choroidal neovascularization (CNV) occurs in 2-9% of BVMD and is sometimes associated with the fibrotic (or cicatricial) stage, which is the final stage of BVMD.[4, 12] Thus, the six stages of BVMD are: previtelliform, vitelliform, vitelliruptive, pseudohypopyon, atrophic, and fibrotic.[4]

The *BEST1* mutation analysis in BVMD patients is routinely performed in several ethnic groups.[13-15] However, since only a few cases of BVMD with the *BEST1* mutations have been reported in the Japanese population,[16, 17] there have yet to be any Japanese population studies performed. Thus, the current study investigated the *BEST1* gene in 16 Japanese families with BVMD and evaluated the genotype-phenotype correlations.

PATIENTS AND METHODS

Subjects were recruited from the patient population seen at Jikei University School of Medicine with 16 families with BVMD enrolled. The protocol of this study was approved by the Institutional Review Board at the two participating institutions (Jikei University School of Medicine and National Hospital Organization Tokyo Medical Center). The protocol adhered to the tenets of the Declaration of Helsinki, and informed consent was obtained from all participants.

Clinical studies

This study examined 16 BVMD probands (from 16 unrelated Japanese families, A to P) and their affected or unaffected family members. All participants underwent comprehensive ophthalmic examinations, which included decimal best-corrected visual acuity (BCVA), slit-lamp biomicroscopy, dilated funduscopy, short-wavelength fundus autofluorescence (FAF) (Spectralis HRA; Heidelberg Engineering, Heidelberg, Germany) and optical coherence tomography (OCT) (Cirrus HD-OCT; Carl Zeiss Meditec AG, Dublin, CA, USA). In addition, in almost all probands, full-field electroretinography (ERG) and electrooculography (EOG) were performed according to the protocols of the International Society for Clinical Electrophysiology of Vision. The procedure and conditions have been previously reported in detail.[18]

The diagnosis of BVMD was determined based on the characteristic findings for BVMD that included the fundus appearance, normal or slightly subnormal ERG, and abnormal EOG.[4, 9]

Molecular genetic studies

Blood samples were obtained from all probands and their family members. Genomic DNA that was extracted from peripheral blood leukocytes by a Gentra Puregene Blood Kit (Qiagen, Hilden, Germany) was used as the template for the polymerase chain reaction (PCR) to amplify each of the coding exons (exons 2ï 11) of the *BEST1* gene and the coding exons (exons 1-3) of the *PRPH2* gene. Supplemental Tables S1 and S2 list the primer pairs used for the amplification. The PCR products were purified by a QIAquick PCR Purification Kit (Qiagen) or an Agencourt AMPure XP (Beckman Coulter, Brea, CA, USA), and used as the template for sequencing. Both strands were sequenced on an automated sequencer (3730*xl* DNA Analyzer; Applied Biosystems, Foster City, CA, USA) by using a BigDye Terminator Kit V3.1 (Applied Biosystems). We used accession number (NM_004183.3) of the BEST1 mRNA reference sequence from the National Center for Biotechnology Information.

RESULTS

Identification of the *BEST1* mutations

Among the 16 Japanese probands with BVMD, 12 different *BEST1* variants were identified in 13 BVMD probands from 13 families, A to N, whereas no pathological *PRPH* variants were found in all probands. The *BEST1* variants are summarized in Figure 1 and Table 1. Of the variants, 10 [c.4A>G (p.T2A), c.73C>T (p.R25W), c.240C>A(p.F80L), c.241G>A (p.V81M), c.584C>T (p.A195V), c.653G>A

(p.R218H), c.665G>A (p.G222E), c.724G>A (p.V242M), c.910_912delGAT (p.D304del), and c.918G>C (p.E306D)] have been previously reported as the disease-causing mutations, while two [c.20G>A (p.S7N) and c.1037C>A (p.P346H)] variants had never been reported to be the cause of BVMD. The p.T2A and p.R218H mutations were each found in two families. With the exception of proband II-1 (family A) who had two (compound heterozygous) mutations (p.S7N and p.R218H), each proband carried one mutation.

The two new variants (p.S7N and p.P346H) had not been previously registered at the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/) or at the Mutation Database of Retina International (http://www.retina-international.org/sci-news/databases/mutation-database/). The amino acid alignment of p.S7N was mildly conserved, whereas that of p.346H was completely conserved in different vertebrates. We used the *in silico* bioinformatic programs, PolyPhen-2, SIFT and PROVEAN, to predict the pathogenicity. For the new variants (p.S7N and p.P346H), all programs generated non-pathogenic scores and pathogenic scores, respectively (Table 2). Regarding allele frequency, we found that not only p.P346H but also the p.S7N were extremely rare variants in five different SNPs database (Table 2). In addition, two patients from two generations, who had the p.S7N variant, exhibited BVMD phenotypes in family A. Taken together, we concluded that both new variants are likely to be disease-causing mutations.

Clinical phenotypes

Table 1 summarizes the clinical phenotypes of the 13 families with the *BEST1* mutations. All the probands showed macular lesions of the typical BVMD stages. Figure 2 presents fundus photographs, FAF images and OCTs for representative cases

of each stage. Figure 3 presents fundus photographs, FAF images and OCTs in families A and H with the new mutations (p.S7N and p.P346H). In FAF, the vitelliform lesions indicated hyperautofluorescence during the vitelliform stage, whereas the disrupted vitelliform lesions became mottled with hypoautofluoresent areas during the vitelliruptive and pseudohypopyon stages (Figures 2 and 3). The OCT images revealed marked abnormalities with hyperreflective deposits above the RPE layer at the vitelliform stage, and subretinal detachment accompanied with hyperreflective materials during the vitelliruptive and pseudohypopyon stages (Figures 2 and 3).

Proband II-1 in family A had compound heterozygous mutations (p.S7N and p.R218H) and exhibited typical BVMD phenotypes (Figure 3A). His parents had either the p.S7N or p.R218H mutation. The father (I-1 in family A) with the p.S7N mutation showed typical phenotypes of BVMD [atrophic stage in his right eye (Figure 3B) and vitelliruptive stage in his left eye] and decreased EOG. However, his mother (I-2) who had the p.R218H mutation exhibited normal fundus findings (Figure 3C), but had decreased EOG. One patient with diabetic retinopathy (proband II-2 in family G), exhibited decreased ERG responses. In the two patients who developed CNV unilaterally (patient I-1 in family D and proband II-2 in family M), both received intravitreal injections of anti-vascular endothelial growth factor agents.

DISCUSSION

This study performed a mutational analysis of the *BEST1* and *PRPH2* genes in 16 Japanese families with BVMD. Twelve different *BEST1* mutations were found in 13 Japanese families. Among all of the enrolled participants, 19 BVMD patients from the 13 families had heterozygous or compound heterozygous *BEST1* mutations.

While two mutations (p.A195V and p.I295T) have been previously reported in Japanese BVMD patients,[16, 17] to date, there have never been any population studies of Japanese BVMD patients. In the current study, we identified 12 *BEST1* mutations, 10 of which have been previously reported in non-Japanese populations. These findings suggest that the *BEST1* mutations in Japanese patients overlap those found for other ethnic groups.

Previous reports have shown that the amino acid residues (R25 and R218) are likely to be the hot spot residues among the *BEST1* mutations.[1, 2, 7, 15] The two BVMD mutations (p.T2A and p.R218H) observed in our current study were found in four Japanese families (families A, B, C, D). The p.A195V mutation (family G) was a recurrent mutation that was previously reported in an earlier Japanese study.[17] The other three mutations (p.T2A, p.A195V, and p.R218H) were observed in more than the two BVMD families and thus, might be frequent mutations found within the Japanese population. A larger cohort study will be necessary to clarify the *BEST1* mutation spectra in the Japanese population.

Proband II-1 in family A had compound heterozygous mutations (p.S7N and p.R218H), in addition to showing typical BVMD phenotypes (pseudohypopyon stage and vitelliruptive stage in the right and left eyes, respectively) (Figure 3A and Table 1). Biallelic *BEST1* mutations are reported to primarily manifest in two distinct phenotypes, BVMD and ARB.[3, 6, 19, 20] ARB is a unique retinal dystrophy characterized by RPE abnormalities throughout the posterior fundus that is often accompanied by scattered punctate flecks, exhibits a severe reduction in the EOG light rise, and shows reduced ERG amplitudes.[3, 4, 6] Although the differences in the *BEST1* mutations between BVMD and ARB remain unclear, it has been reported that

ARB might be caused by particular *BEST1* mutations that lead to a markedly decreased or absent bestrophin-1 function.[6]

Although the *in silico* analysis of the new p.S7N mutation (found in family A) predicted a non-pathogenic mutation, in the probandôs father (I-1) who had the heterozygous p.S7N mutation, he exhibited decreased EOG and the atrophic stage in his right eye (Figure 3B) and the vitelliruptive stage in his left eye, convincing that the p.S7N co-segregated with the BVMD phenotype. The p.S7N is located on the evolutionarily conserved N-terminal part (amino acid positions 6 to 30), one of three main mutation hotspots.[4] The *in silico* analysis for the other new p.P346H mutation showed there was severe protein damage (family H). The proband II-2 had the heterozygous p.P346H mutation and exhibited the vitelliruptive stage in both eyes (Figure 3D and Table 1).

Among the four different mutations (p.R218C, p.R218G, p.R218H, and p.R218S) in the R218 residue, the p.R218H mutation is a reported hot spot mutation among the *BEST1* mutations[1, 2, 7, 13, 20-26] that shows various phenotypes of BVMD.[13, 25] Indeed, the members from families A and D who had the p.R218H mutation, all showed broad clinical BVMD phenotypes that ranged from a normal fundus appearance to the fibrotic stage with CNV (Table 1). Previous studies have also showed that the age of onset and disease severity/progression are highly variable among BVMD patients,[4, 15] even within family members with the same *BEST1* mutations.[27] Also, there is a report that showed a phenotypic variability between two BVMD siblings with biallelic/homozygous *BEST1* mutations, whose parents exhibited normal fundus appearance.[28]

Recently, two new causal genes (*IMPG1* and *IMPG2*) have been identified in patients with adult-onset vitelliform dystrophy, whose EOG results were normal or

borderline.[29, 30] The two proteins (IMPG1 and IMPG2) are localized in the interphotoreceptor matrix from the external limiting membrane to the apical surface of the RPE.[30] The reason EOG is normal or borderline can be considered that the two proteins are not present in the extracellular space surrounding the basal part of RPE.[30] In our study, both *BEST1* and *PRPH2* mutations were not identified in three probands (families N to P), who showed decreased EOG but normal ERG (Table 1). Although we could not exclude the possibility of intronic mutations or genomic rearrangements such as large deletions/duplications in *BEST1*, any other gene including *IMPG1* or *IMPG2* might be involved in the three probands.

In conclusion, we identified 12 *BEST1* mutations in 13 Japanese families, with two mutations (p.S7N and p.P346H) being novel. The typical phenotype of BVMD was observed in the one patient who had compound heterozygous *BEST1* mutations (p.S7N and p.R218H). Since a variety of *BEST1* mutations were identified as causes of BVMD in the current study, this suggests that *BEST1* mutations do play a large role in Japanese BVMD patients.

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REFERENCES

1 Petrukhin K, Koisti MJ, Bakall B*, et al.* Identification of the gene responsible for Best macular dystrophy. *Nat Genet* 1998;19:241-7.

2 Marquardt A, Stohr H, Passmore LA*, et al.* Mutations in a novel gene, *VMD2*, encoding a protein of unknown properties cause juvenile-onset vitelliform macular dystrophy (Best's disease). *Hum Mol Genet* 1998;7:1517-25.

3 Burgess R, Millar ID, Leroy BP*, et al.* Biallelic mutation of *BEST1* causes a distinct retinopathy in humans. *Am J Hum Genet* 2008;82:19-31.

4 Boon CJ, Klevering BJ, Leroy BP*, et al.* The spectrum of ocular phenotypes caused by mutations in the *BEST1* gene. *Prog Retin Eye Res* 2009;28:187-205.

5 Davidson AE, Millar ID, Urquhart JE*, et al.* Missense mutations in a retinal pigment epithelium protein, bestrophin-1, cause retinitis pigmentosa. *Am J Hum Genet* 2009;85:581-92.

6 Boon CJ, van den Born LI, Visser L*, et al.* Autosomal recessive bestrophinopathy: differential diagnosis and treatment options. *Ophthalmology* 2013;120:809-20.

7 Krämer F, White K, Pauleikhoff D*, et al.* Mutations in the *VMD2* gene are associated with juvenile-onset vitelliform macular dystrophy (Best disease) and adult vitelliform macular dystrophy but not age-related macular degeneration. *Eur J Hum Genet* 2000;8:286-92.

8 Meunier I, Senechal A, Dhaenens CM*, et al.* Systematic screening of *BEST1* and *PRPH2* in juvenile and adult vitelliform macular dystrophies: a rationale for molecular analysis. *Ophthalmology* 2011;118:1130-6.

9 Best F. Ueber eine hereditaere Maculaaffektion. *Z Augenheilk* 1905;13:199-212.

10 Iannaccone A, Kerr NC, Kinnick TR*, et al.* Autosomal recessive best vitelliform macular dystrophy: report of a family and management of early-onset neovascular complications. *Arch Ophthalmol* 2011;129:211-7.

11 Bitner H, Mizrahi-Meissonnier L, Griefner G*, et al.* A homozygous frameshift mutation in BEST1 causes the classical form of Best disease in an autosomal recessive mode. *Invest Ophthalmol Vis Sci* 2011;52:5332-8.

12 Noble KG, Scher BM, Carr RE. Polymorphous presentations in vitelliform macular dystrophy: subretinal neovascularisation and central choroidal atrophy. *Br J Ophthalmol* 1978;62:561-70.

13 Lotery AJ, Munier FL, Fishman GA*, et al.* Allelic variation in the *VMD2* gene in best disease and age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2000;41:1291-6.

14 Bitner H, Schatz P, Mizrahi-Meissonnier L*, et al.* Frequency, genotype, and clinical spectrum of best vitelliform macular dystrophy: data from a national center in Denmark. *Am J Ophthalmol* 2012;154:403-12 e4.

15 Sodi A, Passerini I, Murro V*, et al. BEST1* sequence variants in Italian patients with vitelliform macular dystrophy. *Mol Vis* 2012;18:2736-48.

16 Yanagi Y, Sekine H, Mori M. Identification of a novel VMD2 mutation in Japanese patients with Best disease. *Ophthalmic Genet* 2002;23:129-33.

17 Shiose S, Yoshida S, Ishikawa K*, et al. VMD2* mutational analysis in a Japanese family with Best macular dystrophy. *Oman J Ophthalmol* 2009;2:143-4.

18 Takeuchi T, Hayashi T, Bedell M*, et al.* A novel haplotype with the R345W mutation in the *EFEMP1* gene associated with autosomal dominant drusen in a Japanese family. *Invest Ophthalmol Vis Sci* 2010;51:1643-50.

19 Schatz P, Klar J, Andreasson S*, et al.* Variant phenotype of Best vitelliform macular dystrophy associated with compound heterozygous mutations in *VMD2*. *Ophthalmic Genet* 2006;27:51-6.

20 Kinnick TR, Mullins RF, Dev S*, et al.* Autosomal recessive vitelliform macular dystrophy in a large cohort of vitelliform macular dystrophy patients. *Retina* 2011;31:581-95.

21 Bakall B, Marknell T, Ingvast S*, et al.* The mutation spectrum of the bestrophin protein--functional implications. *Hum Genet* 1999;104:383-9.

22 Caldwell GM, Kakuk LE, Griesinger IB*, et al.* Bestrophin gene mutations in patients with Best vitelliform macular dystrophy. *Genomics* 1999;58:98-101.

23 Sodi A, Passerini I, Simonelli F*, et al.* A novel mutation in the *VMD2* gene in an Italian family with Best maculopathy. *J Fr Ophtalmol* 2007;30:616-20.

24 Atchaneeyasakul LO, Jinda W, Sakolsatayadorn N*, et al.* Mutation analysis of the VMD2 gene in thai families with best macular dystrophy. *Ophthalmic Genet* 2008;29:139-44.

25 Marchant D, Gogat K, Boutboul S*, et al.* Identification of novel VMD2 gene mutations in patients with best vitelliform macular dystrophy. *Hum Mutat* 2001;17:235.

26 Marchant D, Yu K, Bigot K*, et al.* New *VMD2* gene mutations identified in patients affected by Best vitelliform macular dystrophy. *J Med Genet* 2007;44:e70.

27 Lacassagne E, Dhuez A, Rigaudiere F*, et al.* Phenotypic variability in a French family with a novel mutation in the *BEST1* gene causing multifocal best vitelliform macular dystrophy. *Mol Vis* 2011;17:309-22.

28 Pineiro-Gallego T, Alvarez M, Pereiro I*, et al.* Clinical evaluation of two consanguineous families with homozygous mutations in *BEST1*. *Mol Vis* 2011;17:1607-17.

29 Manes G, Meunier I, Avila-Fernandez A*, et al.* Mutations in *IMPG1* cause vitelliform macular dystrophies. *Am J Hum Genet* 2013;93:571-8.

30 Meunier I, Manes G, Bocquet B*, et al.* Frequency and clinical pattern of vitelliform macular dystrophy caused by mutations of interphotoreceptor matrix *IMPG1* and *IMPG2* genes. *Ophthalmology* 2014;121:2406-14.

Figure legends

Figure 1. Pedigrees of the families with Best vitelliform macular dystrophy. Black arrows show the probands of the families.

Asterisks indicate family members who were not clinically examined but elucidated whether the members have macular abnormalities or decreased visual acuity.

Daggers indicate family members who were genetically examined.

Figure 2. Fundus photographs, short-wavelength fundus autofluorescence images and optical coherence tomography of the different stages of Best vitelliform macular dystrophy.

A: Previtelliform stage (right eye of proband I-1 in family L).

B: Vitelliform stage (left eye of proband II-1 in family D).

C: Vitelliruptive stage (right eye of proband I-2 in family J).

D: Pseudohypopyon stage (right eye of proband II-1 in family E).

E: Atrophic stage (right eye of proband II-1 in family K).

F: Fibrotic (or cicatricial) stage (right eye of patient I-1 in family D).

Figure 3. Fundus photographs, short-wavelength fundus autofluorescence images (FAF) and optical coherence tomography (OCT) of the patients from families A and H with the novel mutations, p.S7N and p.P346H.

A: Fundus photographs, FAF and OCT of proband II-1 in family A, who exhibited the pseudohypopyon stage in the right and the vitelliruptive stage in the left eye.

B: Fundus photograph of the right eye in the probandôs father (I-1, family A) with p.S7N.

C: Fundus photograph of the right eye in the probandôs mother (I-2, family A) with p.R218H.

D: Fundus photographs, FAF and OCT of proband II-2 in family H, who exhibited the vitelliruptive stage in both eyes.

Table 1. Phenotypes and genotypes of Best vitelliform macular dystrophy examined in this study

FN,Pt,Gender	Examined Age	BCVA RE, LE	Fundus RE, LE	Notes	EOG A/D ratio RE, LE	ERG	Nucleotide Change		Amino Acid Change	
							Allele 1	Allele 2	Allele 1	Allele 2
A, I-1, M	60	0.5, 1.2	AT, VR		decreased	ND	c.20g>a	WT	p.S7N	WT
A, I-2, F	59	1.2, 1.2	Normal, Normal		decreased	ND	c.653G>A	WT	p.R218H	WT
A, II-1, M	37	1.0, 1.2	PH, VR		1.16, ND	normal	c.20g>a	c.653G>A	p.S7N	p.R218H
B, I-1, M	36	0.8, 1,5	VR, PV		decreased	normal	c.4A>G	WT	p.T2A	WT
C, II-3, M	26	0.2, 1.0	VR, PH		1.08, ND	normal	c.4A>G	WT	p.T2A	WT
D, I-1, M	71	0.06, 0.4	FI, VR	RE-CNV	1.15, 1.30	normal	c.653G>A	WT	p.R218H	WT
D, II-1, M	43	0.5, 1.2	AT, VL		1.11, 1.04	normal	c.653G>A	WT	p.R218H	WT
E, I-1, M	76	0.3, 0.04	AT, AT	LE-OA	1.24, 1.71	normal	c.241G>A	WT	p.V81M	WT
E, II-1, F	47	1.0, 1.2	PH, PV		1.09, 1.10	normal	c.241G>A	WT	p.V81M	WT
F, I-1, M	66	0.5, 0.5	VL, VL		1.31, 1.27	normal	c.724G>A	WT	p.V242M	WT
G, II-2, M	56	1.2, 1.0	VR, VR	BE-DR	1.76, 1.70	decreased	c.584C>T	WT	p.A195V	WT
H, II-2, F	38	0.6, 1.0	VR, VR		1.31, 1.50	normal	c.1037C>A	WT	p.P346H	WT
I, II-3, M	58	1.0, 0.7	Normal, VL		1.18, 1.12	normal	c.665G>A	WT	p.G222E	WT
J, I-2, F	74	1.0, 0.9	VR, VR		1.23, ND	ND	c.918G>C	WT	p.E306D	WT
K, II-1, M	73	0.15, 1.2	AT, VL		1.38, 1.47	ND	c.910_912delGAT	WT	p.D304del	WT
L, I-1, M	50	0.6, 1.0	PV, VL		1.11, 1.17	normal	c.73C>T	WT	p.R25W	WT
L, II-2, F	11	1.5, 2.0	Normal, Normal		ND, ND	ND	c.73C>T	WT	p.R25W	WT
M, I-2, F	85	0.5, 0.5	PV, PV	BE-Cat	ND, ND	ND	c.240C>A	WT	p.F80L	WT
M, II-2, F	60	0.5, 0.4	VR, VR/FI	LE-CNV	ND, 1.20	ND	c.240C>A	WT	p.F80L	WT
N, pronad, M	39	0.3, 0.2	AT, AT		1.25, 1.26	Normal	WT	WT	WT	WT
O, proband, M	40	1.0, 1.0	VL, VL		1.39, 1.66	Normal	WT	WT	WT	WT
P, proband, F	47	0.9, 1.0	VR, PH		1.49, 1.38	Normal	WT	WT	WT	WT

FN = family number, Pt = patient, BCVA = best corrected visual acuity, EOG = electrooculogram, ERG = electroretinogram, M = male, F = female, WT = wild type, ND = not done or not described, RE = right eye, LE = left eye, BE = both eyes, CNV = choroidal neovasucularization, OA = optic atrophy, DR = diabetic retinopathy, Cat = cataract, PV = previtelliform stage, VL = vitelliform stage, VR = vitelliruptive stage, PH = pseudohypopyon stage, AT = atrophic stage, FI = fibrotic (or cicatricial) stage

Table 2. Characterization of two new *BEST1* variants found in this study

Amino acid substitution	Results of *in silico* bioinformatics tools			Allele frequency in various SNPs database				
	PolyPhen-2 (score)	SIFT (score)	PROVEAN (score)	dbSNP	ESP	ExAC	HTD	HGVD
p.S7N	Benign (0.003)	Tolerated (0.18)	Neutral (-0.351)	rs199508634 1 in 5,000 (0.0002)	0 in 8598	2 in 121,398 (0.00001647)	0 in 2,178	0 in 2,416
p.P346H	Probably damaging (0.999)	Damaging (0.01)	Deleterious (-8.347)	Not registered	0 in 8598	0 in 121,398	0 in 2,178	0 in 2,416

SNPs: single nucleotide polymorphisms, dbSNP: Single Nucleotide Polymorphism Database (URL: http://www.ncbi.nlm.nih.gov/projects/SNP/), ESP: Exome Sequencing Project (URL: http://evs.gs.washington.edu/EVS/), ExAC: Exome Aggregation Consortium (URL: http://exac.broadinstitute.org/), HTD: Human Transporter Database (URL: http://htd.cbi.pku.edu.cn/), HGVD: Human Genetic Variation Database (URL: http://www.genome.med.kyoto-u.ac.jp/SnpDB)

Supplemental Table 1. *BEST1* primers and PCR conditions

Region to amplify	Primer name	Sequence (5´ to 3´)	Annealing temperature
Exon 2 (376 bp)	VMD2-2F	CTTGGAGACCACTTCATC	58º
	VMD2-2R	CCCAGCTTAGTGTTGTAGTG	
Exon 3 (349 bp)	VMD2-3F	CATCGAGGCAGTCCCACTC	64º
	VMD2-3R	ACCTTTCCCTACAGAGAGGC	
Exon 4 (317 bp)	VMD2-4F	GGCTTCTACGTGACGCTGGT	64º
	VMD2-4R	TCCACCCATCTTCCATTC	
Exon 5 (274 bp)	VMD2-5F	ATCCCTTCTGCAGGTTCTCC	60º
	VMD2-5R	AAACCTTGTTTCCTGTGGACC	
Exon 6 (183 bp)	VMD2-6F	GGGCAGGTGGTGTTCAGA	60º
	VMD2-6R	CCTTGGTCCTTCTAGCCTCAG	
Exon 7 (301 bp)	VMD2-7F	CATCCTGATTTCAGGGTTCC	60º
	VMD2-7R	GCATCCTCGTCTCAGGCAG	
Exon 8 (215 bp)	VMD2-8F	AGCTGAGGTTTAAAGGGGGA	56º
	VMD2-8R	TCTCTTTGGGTCCACTTTGG	
Exon 9 (298 bp)	VMD2-9F	ACATACAAGGTCCTGCCTGG	60º
	VMD2-9R	GCATTAACTAGTGCTATTCTAAGTTCC	
Exons 10a (591 bp)	VMD2-10aF	GGTGTTGGTCCTTTGTCCAC	58º
	VMD2-10aR	CTCTGGCATATCCGTCAGGT	
Exons 10b (457 bp)	VMD2-10bF	CTTCAAGTCTGCCCCACTGT	62º
	VMD2-10bR	TAGGCTCAGAGCAAGGGAAG	
Exon 11 (216 bp)	VMD2-11F	CATTTTGGTATTTGAAATGAAGG	54º
	VMD2-11R	CCATTTGATTCAGGCTGTTG	

Direct sequencing is also performed using VMD2-3F and VMD2-4R at the annealing temperature 64º.

Supplemental Table 2.

PRPH2 primers and PCR conditions

Region to amplify	Primer name	Sequence (5´ to 3´)	Annealing temperature
Exon 1 (717 bp)	RDS1-aF	cgcctctgggctcgttaagg	66º
	RDS1-aR	aggcctgagcctcagtgtcc	
Exon 2 (372 bp)	RDS2-aF	tggcccctgttgagaagccc	66º
	RDS2-aR	cccccattagacccaaatggg	
Exon 3 (331 bp)	RDS3-aF	ggtccagctcccagcgattc	66º
	RDS3-aR	atccacgtttcttggagtgcac	

Figure1

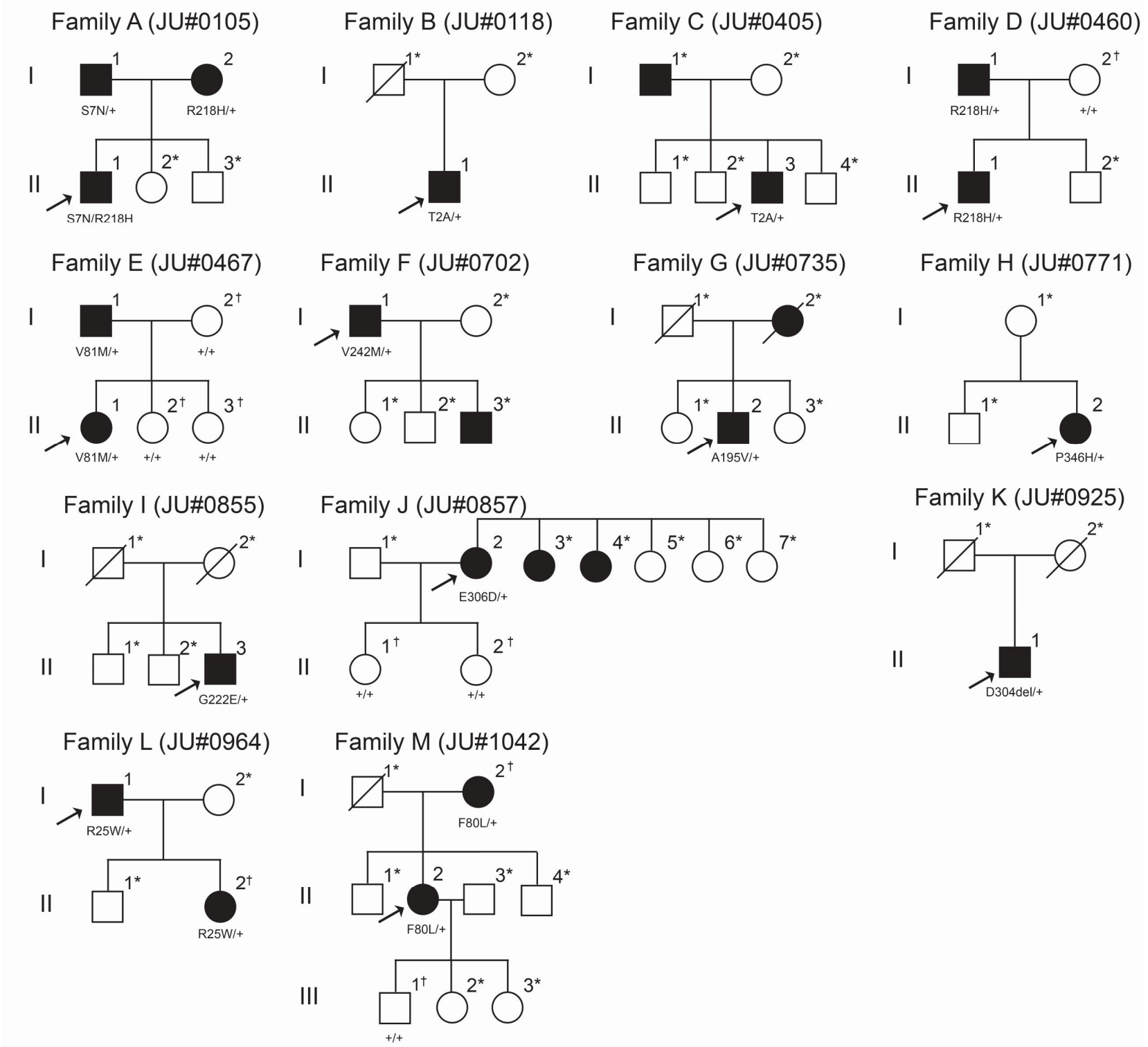
Family A (JU#0105)
I
1
2
S7N/+
R218H/+
II
1
2*
3*
S7N/R218H
Family B (JU#0118)
I
1*
2*
II
1
T2A/+
Family C (JU#0405)
I
1*
2*
II
1*
2*
3
4*
T2A/+
Family D (JU#0460)
I
1
2†
R218H/+
+/+
II
1
2*
R218H/+
Family E (JU#0467)
I
1
2†
V81M/+
+/+
II
1
2†
3†
V81M/+
+/+
+/+
Family F (JU#0702)
I
1
2*
V242M/+
II
1*
2*
3*
Family G (JU#0735)
I
1*
2*
II
1*
2
3*
A195V/+
Family H (JU#0771)
I
1*
II
1*
2
P346H/+
Family I (JU#0855)
I
1*
2*
II
1*
2*
3
G222E/+
Family J (JU#0857)
I
1*
2
3*
4*
5*
6*
7*
E306D/+
II
1†
2†
+/+
+/+
Family K (JU#0925)
I
1*
2*
II
1
D304del/+
Family L (JU#0964)
I
1
2*
R25W/+
II
1*
2†
R25W/+
Family M (JU#1042)
I
1*
2†
F80L/+
II
1*
2
3*
4*
F80L/+
III
1†
2*
3*
+/+

Figure2

A
Previtelliform stage
B
Vitelliform stage
C
Vitelliruptive stage
D
Pseudohypopyon stage
E
Atrophic stage
F
Fibrotic/cicatricial stage

Figure3