

Retinal Features of Family Members With Familial Exudative Vitreoretinopathy Caused By Mutations in *KIF11* Gene

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Purpose: To determine the clinical characteristics of patients and family members with familial exudative vitreoretinopathy (FEVR) caused by mutations in the *KIF11* gene.

Methods: Twenty-one patients from 10 FEVR families with mutations in the *KIF11* gene were studied. The retinal and systemic features were examined. The genetic analyses performed included Sanger sequencing of the *KIF11* gene, whole exome sequencing, as well as array comparative genomic hybridization (CGH) analysis and multiple ligation probe assay (MLPA).

Results: Sequence analysis revealed seven different *KIF11* mutations. Array CGH with MLPA revealed two different exon deletions. All probands had advanced FEVR with retinal detachments (RDs) and microcephaly with or without developmental disabilities. Patients with bilateral RDs were more frequently associated with developmental disabilities ($P = 0.023$). Multimodal imaging of the family members revealed that six of nine patients without RDs (66%) had varying degrees of chorioretinopathy. The retinal folds in FEVR patients were associated with severe retinal avascularization. However, funduscopic changes in the peripheral retina were unremarkable in family members without RDs. A score representing the peripheral vascular anomalies determined from the fluorescein angiograms was lower than that of control eyes of patients with mutations of the Wnt signaling genes ($P = 0.0029$).

Conclusions: The probands with *KIF11* mutations were associated with severe ocular and systemic pathologies, whereas affected family members showed highly variable clinical manifestations. Peripheral vascular anomalies can often be unremarkable in eyes without RDs.

Translational Relevance: These findings highlight more diverse mechanisms that underlie the pathological changes in patients with FEVR.

Introduction

Familial exudative vitreoretinopathy (FEVR, MIM#133780, #305390, #601813, #613310) is a hereditary vitreoretinal disorder first reported by Criswick and Schepens in 1969.¹ This disorder is characterized by abnormalities in the vascular development of the retina, and the secondary abnormalities include retinal neovascularization, vitreous hemor-

rhages, retinal exudates, and various forms of retinal detachments (RDs) such as tractional RDs and falciform retinal folds.^{2,3} Most individuals remain asymptomatic, and the severity is often asymmetric between the two eyes. The consistent signs are abnormal retinal vessels and avascularization in the periphery that are often not detected by fundus examinations and require fluorescein angiographic (FA) examinations.^{2,3}

FEVR is genetically heterogeneous, and several causative genes are known to be causative

including the Wnt signaling genes, that is, *NDP*, *FZD4*, *LRP5*, *TSPAN12*, and *CTNNB1*.^{4–8} The Wnt signaling genes account for 30% to 50% of the patients with FEVR.^{9–11} Although FEVR has been thought to be a nonsyndromic disorder, more severe loss-of-function mutations of the same Wnt signaling genes can cause syndromic disorders associated with more severe vitreoretinopathy, such as osteoporosis-pseudoglioma syndrome (MIM #259770) caused by mutations in the *LRP5* gene and Norrie disease (ND, MIM #310600) caused by mutations in the *NDP* gene.^{12,13} Furthermore, an intellectual disability can be detected in patients with mutations in the *CTNNB1* gene.¹⁴ It has been reported that FEVR can also be caused by enhanced Wnt signaling.¹⁵

The *KIF11* gene that encodes EG5, a homotetramer kinesin motor protein, is crucial for cell division and is associated with physiological and pathological growth of the vascular system.^{16–18} In 2012, Ostergaard et al.¹⁹ identified mutations in the *KIF11* gene that caused an autosomal dominant disorder of microcephaly with or without chorioretinopathy, lymphedema, and mental retardation (MCLMR, MIM#152950). This condition is also known as chorioretinal dysplasia, microcephaly, and mental retardation. Although the ocular features of MCLMR are distinct from FEVR, Robitaille et al.²⁰ found that 6% of FEVR probands had mutations in the *KIF11* gene associated with RDs and microcephaly suggesting a phenotypic overlap between FEVR and MCLMR.²⁰ Later cohort studies showed that mutations in the *KIF11* gene accounted for 5% to 9% of FEVR patients.^{21–23} Most patients with mutations in the *KIF11* gene were found to be sporadic because of de novo mutations,^{11,24} and the expressivity remained unclear whether family members had signs of either FEVR or MCLMR. We report the phenotypes of 21 patients from 10 families associated with mutations in the *KIF11* gene.

Methods

This was a multicenter retrospective case series study. The procedures used conformed to the tenets of the Declaration of Helsinki, and they were approved by the Ethics Committee of the University of Occupational and Environmental Health, Kindai University, and Fukuoka University. A signed informed consent was obtained from all of the patients or their parents. Twenty-one patients from six sporadic and four familial inheritance pattern were studied. An initial diagnosis of FEVR was made for all probands at the age of <1 year because of congenital RDs.

The ocular examinations included measurements of the refractive error, best-corrected visual acuity (BCVA), and intraocular pressure. In addition, slit-lamp, ophthalmoscopy, ultrasonography, and b-mode scanning of swept-source optical coherence tomography (SS-OCT; DRI OCT Triton, Topcon, Tokyo, Japan) were performed. FA was performed by an ultra-widefield fundus camera (Optos 200Tx; Optos PLC, Dunfermline, UK) or the RetCam3 (Clarity, Pleasanton, CA, USA). Fundus autofluorescence (FAF) images were obtained by the Optos 200Tx.

The severity of FEVR was classified as follows: stage 1, avascular peripheral retina; stage 2, retinal neovascularization; stage 3, extramacular RD; stage 4, macula-involving RD; and stage 5, total RD. This classification is based on that of Pendergast et al.²⁵ The MCLMR-associated chorioretinopathy was classified according to Birtel et al.²⁶ as type 1, subclinical retinal changes including retinal thinning or decreased full-field electroretinogram (ERG); type 2, retinal dystrophy characterized by OCT and FAF; type 3, chorioretinopathy with sharply demarcated atrophy outside the vascular arcade; and type 4, FEVR-like retinal folds.

To assess the vascular changes in the peripheral retina by FA, we developed a scoring system for eyes at stage 1 FEVR. Based on the earlier reports on FEVR, the vascular changes in the temporal periphery were categorized as (1) avascular retina, (2) straightening and increased branching of the retinal vessels; (3) vascular looping or arteriovenous anastomoses; (4) supernumerous vascular branching at peripheral vascular ends; (5) V-shaped vascular notch; and (6) staining of retinal vessels in the periphery.^{3,27,28} The presence or absence of each finding was scored by three retinal specialists (I.M., T.N., H.K.) as 0, absent; 1, mild or marginal; and 2, typical or extensive. These scores were summed to be a measure of the vascular anomaly in the periphery (VAP) score. The VAP scores were compared to those of age- and sex-matched control eyes of stage 1 FEVR patients who had mutations of the Wnt signaling genes (Supplementary Table S1).^{29,30} For patients whose VAP scores were available in both eyes, the findings of the eye with the larger VAP score was used for the statistical analyses.

The ERGs were elicited by full-field light stimuli and recorded with a contact lens electrode (LE4000, Tomey, Japan). The stimulus and recording parameters conformed to the standards of the International Society of Clinical Electrophysiology of Vision except for the light intensity at 200 cds/m² for the dark-adapted mixed rod and cone ERGs.³¹ The data were compared with in-house data of 28 unaffected eyes

from 28 healthy subjects or patients with unilateral eye diseases.

Laboratory Studies

Whole Exome and Sanger Sequencing

The reference sequence of the *KIF11* gene (NM_004523.3) was used with a variation number based on its cDNA sequence with +1 corresponding to the first nucleotide of the initiation codon (ATG). DNA samples were extracted from peripheral blood using a DNA extraction kit (QiaAmp; Qiagen, Chatsworth, CA, USA). Nine samples were screened by whole exome sequencing (WES) by clonal clustering of a captured DNA library using the SureSelect human all exon V5 (Agilent, Santa Clara, CA, USA) as described.³² Sanger sequencing was performed in two probands and family members of the probands who had mutations in the *KIF11* gene.

Array-Comparative Genomic Hybridization Analysis

Array-comparative genomic hybridization (CGH) analysis was performed for two probands (patient 5 and 16) whose mutations were not detected by WES or Sanger sequencing. The Agilent Human Genome G3 SurePrint 1 × 1M Microarray (Agilent Technologies, Santa Clara, CA, USA) was used, and labeling and hybridization were carried out according to the manufacturer's instructions (Agilent Technologies). Commercially available genomic DNA was used as a control. The hybridized slide was scanned using the Agilent G4900DA SureScan Microarray Scanner, and image data were extracted and converted to a text file using Agilent Feature Extraction software (v.11.0.1.1). Aberrations were detected using the Aberration Detection Method 2 statistical algorithm with a threshold of 6.0, and the filtering options of Agilent Cytogenomic's software (v5.0.2.5) was used. The detail of the filtering options and mapping data analysis has been described in detail.³³

Multiple Ligation Probe Assay

Multiple ligation probe assay (MLPA) was performed for exons 1 and 21 of the *KIF11* gene in patients 5 and 16, and their family members. Custom oligo DNA probes were designed based on the manufacturer's protocol using a probe designing program suite for MLPA (Supplementary Table S2).³⁴ MLPA was performed using an MLPA kit (Salsa MLPA kit P200-A1 human DNA reference; MRC-Holland, Amsterdam, the Netherlands) following the manufacturer's protocol. In brief, the DNA was hybridized with a mixture of the designed and reference probes, and the probe pairs were ligated, followed

by polymerase chain reaction. Electrophoresis was performed using a DNA sequencer (3730xl DNA Genetic Analyzer; Thermo Fisher Scientific, Waltham, MA, USA) with a size standard mix (GeneScan LIZ600; Thermo Fisher Scientific).

Statistical Analyses

Statistical analyses were performed with the JMP software (version 5.1; SAS Institute, Cary, NC, USA). Wilcoxon tests were used to compare the VAP scores and head circumferences for the two groups, and the Fisher exact test was used to determine the significance of categorized data. The statistical significance was set at $P < 0.05$.

Results

Mutation Analysis

Seven different mutations in the *KIF11* gene were identified by WES and/or Sanger sequencing in eight probands (Table and Fig. 1). Five were novel mutations: c.868C>T (p.Q290*), c.1736_1737insATA (p.D579delinsEY), c.2267+1G>C, c.2541dupA (p.L848Ifs*9), and c.2777delC (p.T926Nfs*14). The other two were known mutations: c.704C>G (p.S235C) and c.1159C>T (p.R387*). Four of five sporadic patients were confirmed to be *de novo* mutations. Sanger sequence revealed that p.Q290*, p.R387*, and c.2267+1G>C were found in affected family members in families 7, 8, and 10 (Fig. 1). Of the five novel mutations, four were null variants affecting the *KIF11* gene, and the other variant was an in-frame 3-bp insertion, p.D579delinsEY, located not in a repeat but a conserved region and found as a *de novo* mutation in patient 6. This variant was not found in the human genome variation databases for the Japanese and other populations (Human Genetic Variation Database, HGVD, <http://www.hgvd.genome.med.kyoto-u.ac.jp/>; the Tohoku Medical Megabank Organization database, <https://www.megabank.tohoku.ac.jp/tommo/>; the 1000 genomes project, <http://www.internationalgenome.org/1000-genomes-browsers>, the genome aggregation database, gnomAD, <https://gnomad.broadinstitute.org>). According to the American College of Medical Genetics and Genomics standard and guidelines,³⁵ this in-frame variant was considered to be "likely pathogenic." The other six variants were also not found in the human genome variation databases.

Two novel deletions in the *KIF11* gene were identified by the array-CGH analysis in

Table. Mutations in the KIF11 Gene and the Associated Clinical Findings

Family	DNA Change (Prediction)	Patient	Age At Last Follow-Up (Yr)	Sex	R/L	Visual Acuity (Refraction, Diopters)	FEVR Stage (Signs, [VAP Score])	MCLMR Type (Signs)	Ocular Remarks	Head Circumference (cm) [SD] (Cognitive Problems)	Other Systemic Remarks
1	c.704C>G	1	25	M	R	LP	4 (FF)	4	LS at 8 yo	48.0 [-5.8] (Mental retardation)	
2	(p.S235C) c.2777delC	Proband 2	1	F	L	NLP	5 (FF, phthisis)	4	PPV at 1 yo	NA (Developmental disability*)	
					R	NA	4 (FF, FA: AR at midperiphery)	4			
3	c.1159C>T	3	9	M	L	NA	4 (FF, FA: AR entire retina)	4		45.0 [-5.4] (Learning disability)	
					R	0.06 (+2.25)	4 (FF, FA: AR at equator)	4 (FAF: hyper at FF, ERG: extinct)			
4	c.2541dupA (p.L848fs*9)	4	6	F	R	NLP	5 (TRD, CO, ACD)	4		36.0 [-5.08]	
					L	NLP	5 (TRD, CO, ACD)	4			
5	[chr10: 94174363-94411713]del*	5	3	F	R	NLP	4 (FF, AR entire retina)	4		42.0 [-5.1] (Mental retardation, epilepsy)	
					L	0.08 (-1.5)	4 (FF, FA: AR at equator)	4 (FAF: hyper at FF, ERG: extinct)			
6	c.1736insATA (p.D579delinsEY)	6	23	F	L	NLP	5 (TRD, CO, ACD)	4		50.0 [-7] (No)	
					R	NLP	5 (FF, phthisis)	4			
7	c.868C>T	7	16	M	R	NLP	4 (FF, phthisis)	4	LS at 0 yo	48.6 [-3.3] (Developmental disability)	
					L	0.4 (-1.5)	3 (FAF: extensive atrophy, ERG: reduced)	4			
8	(p.Q290*) c.868C>T	8	47	F	L	0.15	4 (FF)	4 (FAF: hypo at FF)	LS at 1 yo	52.6 [-1.4] (No)	
					R	1.0 (-0.75)	1 (AR [5])	2 (FAF: hypo at CRA, ERG: reduced, OCT: CRT, ChE)			
8	c.868C>T (p.Q290*)	9	44	F	L	1.0 (-1.0)	1 (AR [8])	2 (FAF: hypo at CRA, ERG: reduced, OCT: CRT, ChE)		49.0 [-4.0] (No)	History of prematurity, a diagnosis of ROP
					R	NLP	5 (TRD, MIC, CO, ACD, phthisis)	4			
8	(p.Q290*)	9	44	F	L	LP	5 (TRD, MIC, CO, ACD, phthisis)	4		49.0 [-4.0] (No)	History of prematurity, a diagnosis of ROP
					R	NLP	5 (TRD, MIC, CO, ACD, phthisis)	4			

Table. Continued

Family	DNA Change (Prediction)	Patient	Age At Last Follow-Up (Yr)	Sex	R/L	Visual Acuity (Refraction, Diopters)	FEVR Stage (Signs, [VAP Score])	MCLMR Type (Sings)	Ocular Remarks	Head Circumference (cm) [SD] (Cognitive Problems)	Other Systemic Remarks
8	c.868C>T	10 Grandmother	71	F	R	FC	1 (AR [1])	unclassified (diffuse CRA, ERG: extinct)		47.0 [-6.5] (No)	Parkinson disease, hypothyroidism, hip dislocation
	(p.Q290*)				L	HM	1 (AR)	unclassified (diffuse CRA, ERG: extinct)			
9	c.1159C>T	11 Proband	14	F	R	0.02 (-3.0)	4 (FF, FA: AR entire retina)	4 (FAF: hyper at FF, ERG: extinct)		46.0 [-4.2] (Developmental disability)	Atrial septal defect
	(p.R387*)				L	0.1 (-10.0)	3 (macula dragged, FA: AR at equator)	4 (FAF: focal hypo-spots, ERG: reduced)			
9	c.1159C>T	12 Sibling	11	M	R	1.5 (nc)	Normal	Normal		46.0 [-3.5] (ADHD)	
	(p.R387*)				L	1.2 (nc)	Normal	Normal			
9	c.1159C>T	13 Sibling	12	M	R	1.5 (nc)	1 (AR [3])	1 (FAF: focal hypo-spots, OCT: ORT)		46.0 [-4.1] (ADHD)	
	(p.R387*)				L	1.2 (nc)	1 (AR [1])	1 (FAF: focal hypo-spots, OCT: ORT)			
9	c.1159C>T	14 Mother	38	F	R	1.5 (-1.25)	1 (AR [3])	3 (FAF: hypo around disc, ERG: reduced, OCT: ORT)		55.0 [+2.4] (No)	
	(p.R387*)				L	1.5 (-2.25)	1 (AR [3])	3 (FAF: hypo around disc, ERG: reduced, OCT: ORT)			
9	c.1159C>T	15	63	M	R	1.2 (-1.125)	1 (AR [3])	3 (FAF: CRA, ERG: reduced, OCT: ORT)	Diabetic retinopathy	55.4 [-0.9] (No)	Diabetes mellitus
	(p.R387*)	Grandfather			L	1 (-0.75)	1 (AR [2])	3 (FAF: CRA, ERG: reduced, OCT: ORT)	Diabetic retinopathy		
10	[chr10:94339429-94357375]del*	16	3	F	R	Follow	4 (FF, MIC, ACD)	4 (ERG: extinct)	LS, PPV at 3 yo	40.5 [-4.7] (No)	

Table. Continued

Family	DNA Change (Prediction)	Patient	Age At Last Follow-Up (Yr)	Sex	R/L	Visual Acuity (Refraction, Diopters)	FEVR Stage (Signs, [VAP Score])	MCLMR Type (Signs)	Ocular Remarks	Head Circumference (cm) [SD] (Cognitive Problems)	Other Systemic Remarks
10	[chr10: 94339429–94357375]del*	Proband	31	F	L	NLP	5 (TRD, MIC, CO, ACD, phthisis)	4 (ERG: extinct)	LS at 2 yo, PPV at 3 yo	49.0 [–2.3] (No)	
					R	0.6 (–6.25)	1 (AR [4])	3 (FAF: CRA, ERG: reduced, OCT: ORT, ChE)			
10	[chr10: 94339429–94357375]del*	Mother	69	M	L	0.8 (–4.75)	1 (AR [4])	3 (FAF: CRA, ERG: reduced, OCT: ORT, ChE)		56.0 [–0.5] (No)	
					R	1.2 (–0.25)	Normal	Normal (FAF: hypo around disc)			
11	c.2267+1G>C	Proband	2	F	L	1 (–0.75)	Normal	Normal (FAF: hypo around disc)		39.0 [–6.1] (No)	
					R	LP	5 (TRD)	4			
11	c.2267+1G>C	Sibling	4	M	L	20/250	4 (FF)	4	LS at 1 yo	44.5 [–4.0] (ADHD)	
					R	20/250	4 (FF)	4			
11	c.2267+1G>C	Father	47	M	L	1.2 (–0.375)	Normal	Normal (FAF: hypo around disc)		49.5 [–4.8] (No)	
					R	1.5 (+0.125)	Normal	Normal (FAF: hypo around disc)			

ACD, anterior chamber disappearance; ADHD, attention deficit/hyperactivity disorder; AR, avascular retina; ChE, choroidal excavation; CO, corneal opacity; CRA, chorioretinal atrophy; F, female; FA, fluorescein angiography; FC, finger count; FAF, fundus autofluorescence; FF, falciform retinal fold; ERG, electroretinogram; HM, hand motion; L, left eye; LP, light perception; LS, lensectomy; M, male; MCLMR: microcephaly with or without chorioretinopathy, lymphedema, or mental retardation; MIC, microphthalmia; NA, not analyzed; NLP, no light perception; OCT, optical coherence tomography; ORT, outer retinal layer thinning; PPV, pars plana vitrectomy; R, right eye; ROP, retinopathy of prematurity; TRD, total retinal detachment; VAP, vascular anomaly in the periphery.

*Pediatric evaluation at age of 3 year-old (after loss of ophthalmologic follow-up).

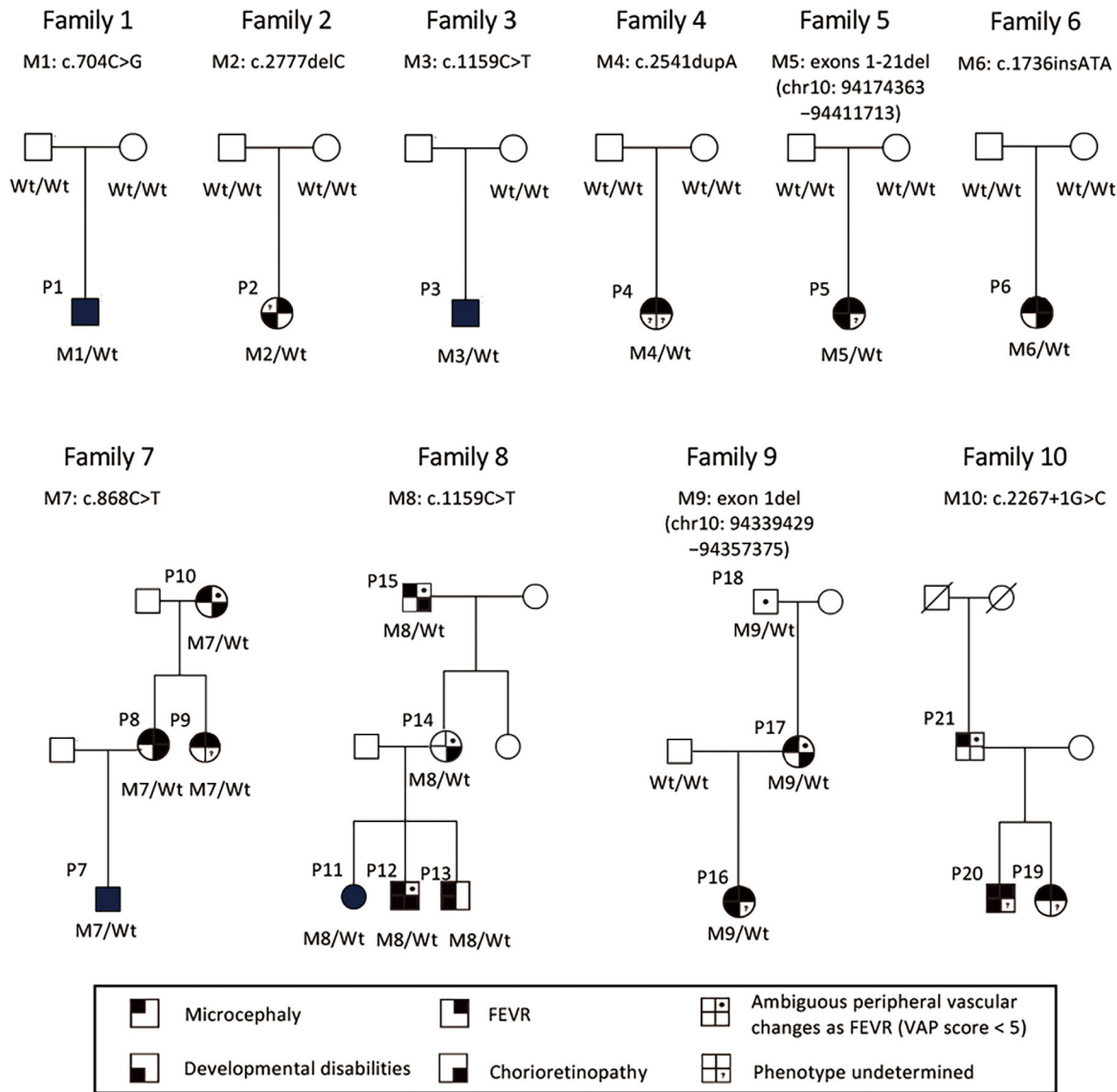


Figure 1. Pedigree charts of the families with mutations in the *KIF11* gene.

patients 5 and 16. A 237-kb deletion: arr[hg19]10q23.33(94174363–94411713)x1 encompassing the entire *IDE* gene and exons 1 through 21 of the *KIF11* gene was found in patient 5, and a 18-kb deletion: arr[hg19]10q23.33(94339429–94357375)x1 encompassing exon 1 of the *KIF11* gene was found in patient 16 (Table, Figs. 1 and 2). MLPA analysis demonstrated that the heights of the peaks of exons 1 and 21 were low for patient 5 but not for the parents indicating a *de novo* mutation. The low heights of the peaks corresponding to exon 1 were detected for patient 16, 17, and 18 (probands, her mother, and asymptomatic maternal grandfather, Figs. 1 and 2).

Clinical Findings

All probands diagnosed with FEVR showed advanced tractional RDs with or without corneal opacities (Fig. 3, Table); bilateral total RD with leukocoria in 2/10, unilateral total RD, and contralateral falciform retinal fold in 3/10, bilateral falciform retinal folds in 3/10, unilateral retinal fold and contralateral dragged macula in 1/10. In addition, patient 6 had severe falciform retinal folds in the right eye and chorioretinal dysplasia consistent with the signs of type 3 MCLMR in the left eye (Fig. 5).

Family members with *KIF11* mutations showed advanced bilateral FEVR (total RD or retinal folds)

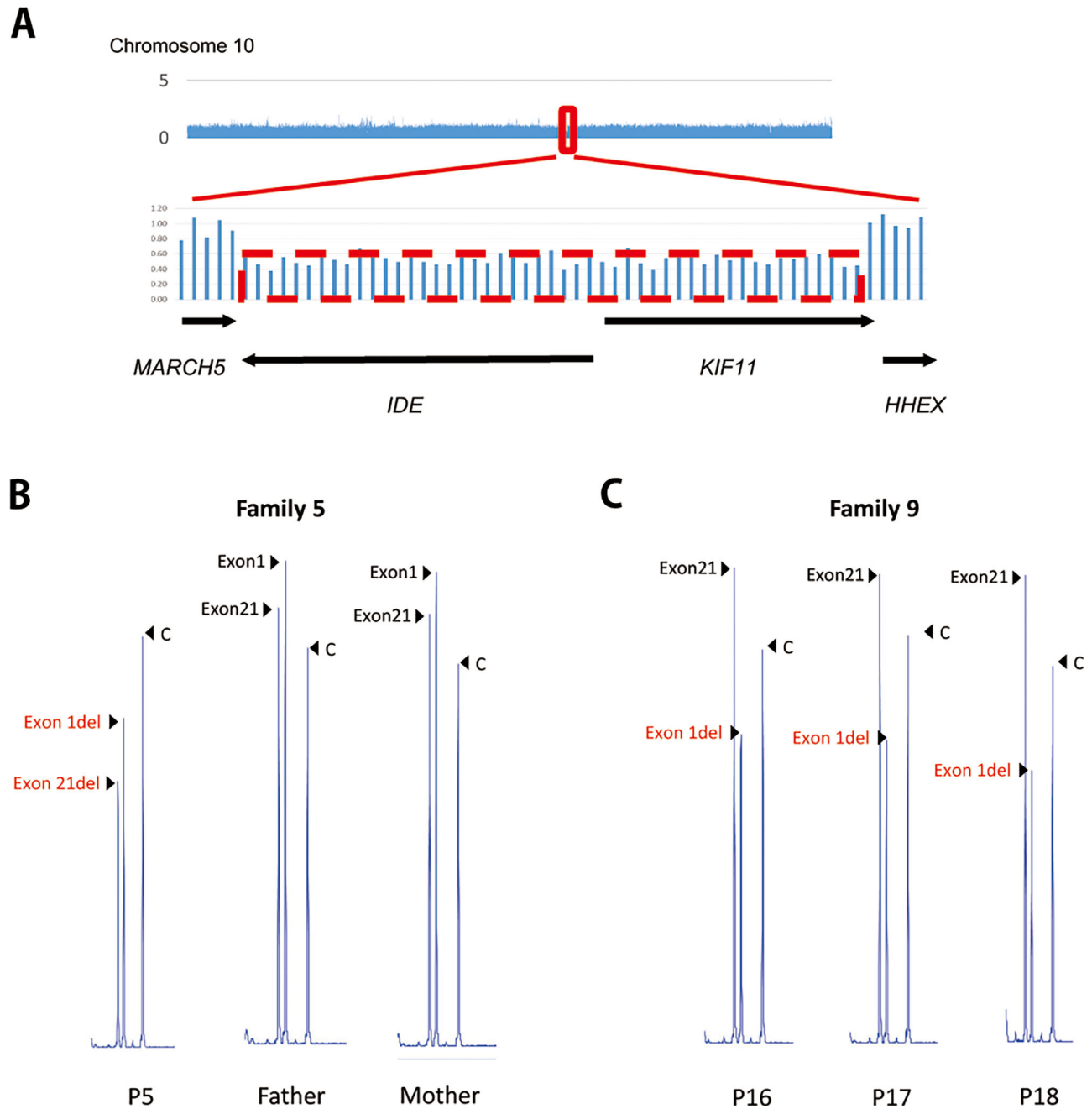


Figure 2. Results of array CGH analysis and MLPA. **(A)** Diagram illustrating a 237-kb deletion: arr[hg19] 10q23.33(94174363–94411713)x1 encompassing the entire *IDE* gene and exons 1 through 21 of the *KIF11* gene in Patient 5. **(B)** MLPA demonstrating the representative chromatogram peaks in exons 1 and 21 of the *KIF11* gene with a length of 100 bp and 95 bp, respectively. *Left*, Family 5: low heights of the peaks for exons 1 and 21 for patient 5 (P5) but not for the parents, indicating a *de novo* mutation. *Right*, Family 9: a low height of the peak of exon 1 was found for patient 16 (P16), her mother (P17), and maternal grandfather (P18). **C** indicates a peak for an internal control probe of exon 1 of the *SOX21* gene with a length of 110 bp.

in 2/11. Of the remaining members without an RD (n = 9), six had signs of chorioretinopathy. Notably, patient 10 had extensive bilateral chorioretinal atrophy resembling retinitis pigmentosa (Supplementary Figure S1). The fundus appearance lacked obvious signs of FEVR in the peripheral retina. Three patients

lacked retinal changes, and two of them had only microcephaly (Fig. 1 and Table).

The decimal BCVA was measured in 19 patients (Table). The exact BCVA depended on the retinal status: light perception or no light perception in eyes with total RDs, light perception to 0.15 in eyes with

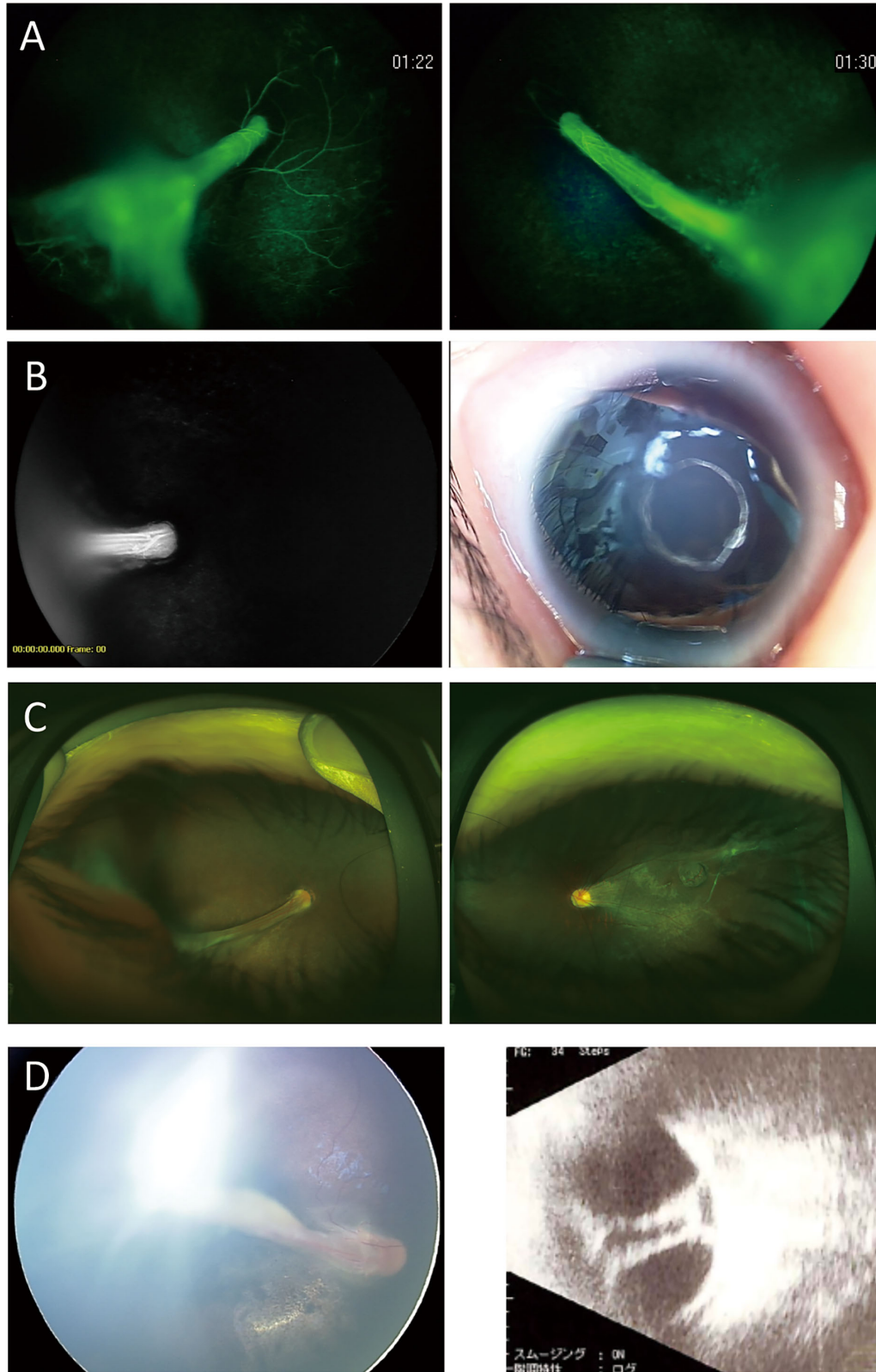


Figure 3. Fundus photographs, fluorescein angiographic images, external photographs, and ultrasonographic images of probands with mutations in the *KIF11* gene. **(A)** Fluorescein angiographic images of patient 2. **(B)** Fluorescein angiographic image OD (left) and external photograph OS (right) of patient 5. **(C)** Ultra-widefield fundus photographs of patient 11. **(D)** Fundus photograph OD (left) and ultrasonography OS (right) of patient 16. Note that retinal vasculature is poorly developed in the fluorescein angiographic images of **A** and **B**.

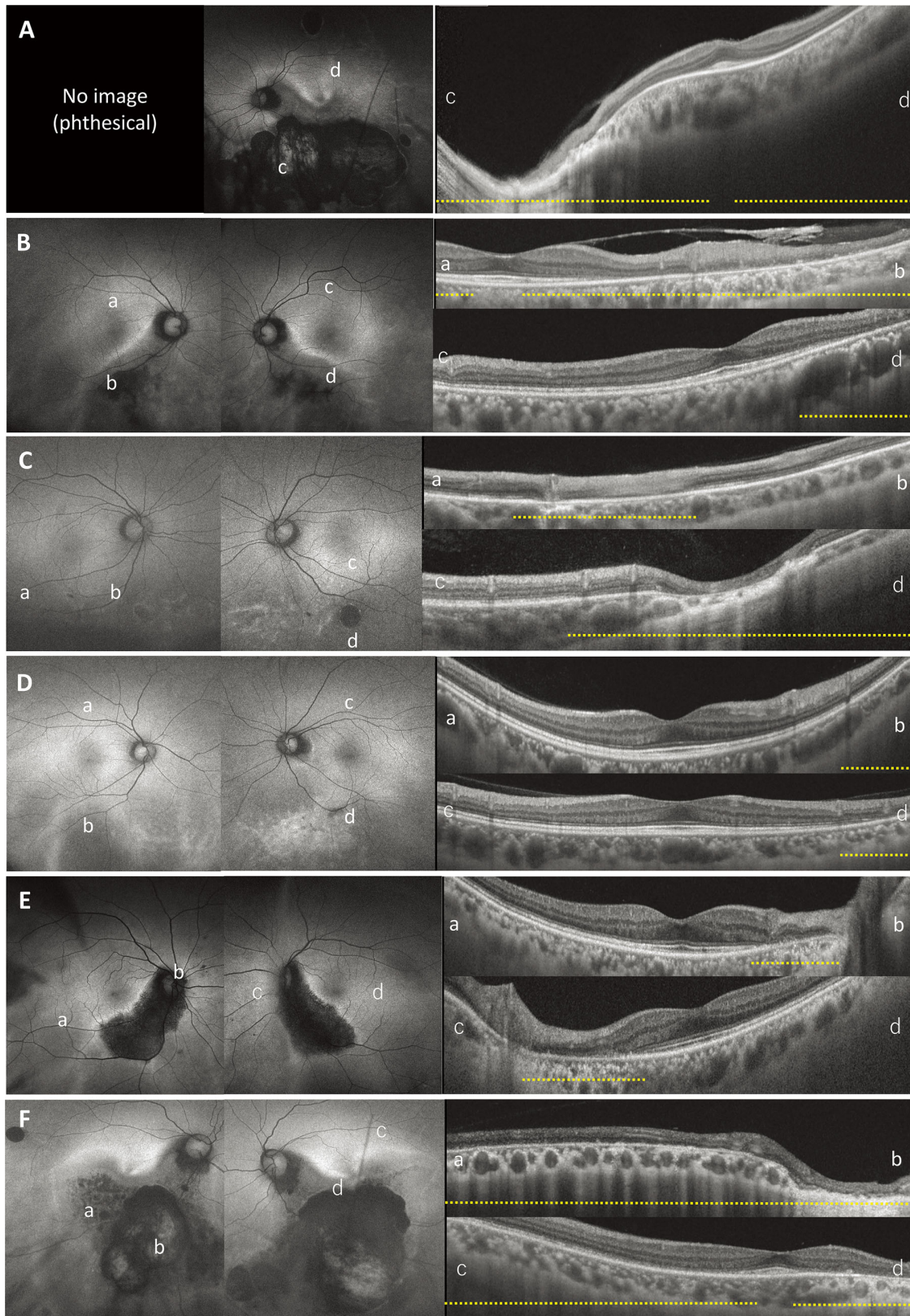


Figure 5. FAF and OCT images of patients with mutations in the *KIF11* gene. **A, B, C, D, E,** and **F** are images from patients 6, 8, 12, 14, 15, and 17, respectively. The right, middle, and left columns indicate FAF images of the right and left eyes and OCT. The OCT images are from line scans of the FAF images designated by *a-b* or *c-d*. *Yellow dashed lines* indicate regions of disrupted outer retinal layers as disappearance of the ellipsoid zone, interdigitation zone, and the outer limiting membrane associated with thinning of the outer nuclear layer.

assessed by Optos 200Tx in 12 eyes without a RD in seven patients. An avascular retina was detected in 9/12, straightening of retinal vessels in 10/12, vascular looping or arteriovenous anastomoses in 8/12, retinal vessels staining in 4/12, and supernumerous vascular branching at the vascular ends in 2/12 (Fig. 4 and Supplementary Figure S1). V-shaped vascular notches were not found. Compared with the FA images of the control FEVR patients with mutations in the Wnt signaling genes, the FA images of patients with the *KIF11* mutations were unremarkable (Fig. 4 and Supplementary Figure S1). The VAP score of the patients with the *KIF11* mutations was significantly lower than that of the control FEVR patients (median VAP score of 3 versus 8, $P = 0.0029$, Fig. 4).

FAF was performed in 14 patients and abnormal FAF signs were found in 10/14 (Fig. 5 and Table). Eyes with retinal folds showed hypo-autofluorescence along the retinal folds. Abnormal hypo-autofluorescence was found preponderantly at the inferior vascular arcade as a sign of chorioretinal atrophy. Small round spots of hypo-autofluorescence were found in eight patients consistent with the signs of chorioretinal atrophy by fundus examination. Moreover, large hypo-autofluorescence was found at the peripapillary area in patients 14 and 21.

Eight eyes of 8 patients were examined by OCT. Signs of various sizes of retinal degeneration, that is, disappearance of the ellipsoid zone, interdigitation zone and the outer limiting membrane associated with thinning of the outer nuclear layer were found posteriorly especially around the inferior vascular arcade in 6/8 patients (Fig. 5 and Table). The area of these atrophy in the outer retina corresponded with the hypo-autofluorescence by FAF.

ERG recordings were performed on nine patients. Seven eyes with RDs or extensive chorioretinopathy in four patients showed extinct ERGs under both scotopic and photopic conditions (Table). The other 10 eyes of six patients who did not have RDs had mild to moderate reduction of the amplitudes of the a- and b-waves under scotopic and photopic conditions (Supplementary Table S3). A reduction of the b-wave amplitude by greater than 2 standard deviations (SDs) from the normal control for the scotopic maximum b-wave amplitudes was found in 4/6 patients.

Systemic symptoms were evaluated in all patients except for one sporadic patient (Table). The head circumferences ranged from -6.1 to $+2.4$ (average -3.6) SD calculated from the age- and sex-adjusted reference in the same population. A head circumference lower than 2 SD was found in 80% (16/20) of the patients. Remarkably, the head circumferences of the probands ($n = 9$) ranged -6.1 to -3.3 (average

-4.8) SD. Smaller head circumferences were found in probands than family members (-4.8 vs -2.7 ; $P = 0.0276$), and in patients with bilateral RDs than the other patients (-4.8 vs -2.5 , $P = 0.0233$). Variable degrees of developmental disabilities such as mental retardation with or without epilepsy, learning disability, and attention deficit/hyperactivity disorder were found in 7/9 (78%) of probands and 3/11 (33%) of family members (Table). The developmental disabilities were found more frequently in patients with than without bilateral RDs (80% vs 20%, $P = 0.0230$). Lymph edema was not detected in all patients evaluated. Patient 12 had atrial septal defect, and patient 15 had diabetes mellitus.

Discussion

We investigated in detail the clinical appearance of patients with FEVR and family members who had mutations in the *KIF11* gene. The probands consistently showed microcephaly and advanced FEVR with RDs associated with poor vision to no light perception and poor visual prognosis. Developmental disabilities were more likely to be associated with patients with bilateral RDs. These findings were as severe as those in ND rather than common FEVR as mentioned.¹¹ The eyes with retinal folds tended to be associated with severe abnormalities of the retinal vasculature. Multimodal imaging showed that 66% of patients without RDs had signs of chorioretinopathy but signs of FEVR were rare at least by fundus examinations. The remaining 33% of the patients had no ocular changes. In addition, there was a finding of extensive retinal degeneration in one patient which has not been reported earlier.

We found nine different mutations in the *KIF11* gene. Based on our cohort of probands with FEVR in which probands were found to have mutations in other FEVR-causing genes or excluded from *KIF11* mutations by WES or Sanger sequence, we estimated that the frequency of *KIF11* mutations to be 4% (10/223). This frequency is comparable to that reported earlier, and the contribution of the *KIF11* gene to FEVR is not small.²⁰⁻²² In fact, a diagnosis of FEVR was first made in seven probands and the mutations were found subsequently. It is important to be aware that the retinal features of the *KIF11*-subset of FEVR are hardly distinguishable in infancy. We found two large deletions that could be identified by array CGH and MLPA analysis and not by DNA sequencing. Although no genetic heterogeneity of MCMLR had been anticipated,³⁶ the reported identification of

KIF11 mutations by sequencing was lower than anticipated. Recently, large deletions of the *KIF11* gene have been identified by methods other than WES or Sanger sequencing that need to be considered for suspicious cases with *KIF11* mutations.^{11,37,38}

According to the Human Gene Mutation Database (professional version 2020.4, <https://portal.biobase-international.com/hgmd/pro/star/php>), 100 different mutations in the *KIF11* gene have been found and about 30 mutations are linked to the phenotypes of FEVR, MCLMR, and simple microcephaly (or developmental disorder). Of these mutations, 18% were missense mutations and the remaining were premature termination mutations. The phenotypes of MCLMR and FEVR have been found in a single family or even in the two eyes of one patient.^{26,37,39} Therefore the phenotypes of MCLMR and FEVR can result from a common mechanism of loss-of-function and a variation in the expressivity of the *KIF11* gene. *KIF11/EG5* plays fundamental physiological roles including neuronal function.⁴⁰ Interfering with this protein inhibits angiogenesis by the vascular endothelial growth factor A and causes vascular defects in zebrafish and chick embryos.¹⁸ Mice model of *KIF11* deficient resembling FEVR was suggested to arise from a distinct mechanism from Wnt signaling.⁴¹

There have been a few studies that reported that eyes with *KIF11* mutations had incomplete vascularization of the peripheral retina whereas the majority of studies on MCLMR did not report signs of FEVR except for retinal folds.^{20,37,42} The severity of FEVR is generally highly variable although peripheral avascularization is believed to be a consistent sign. However, the presence of peripheral avascularization alone is ambiguous for diagnosing FEVR as it is also present in some eyes of normal subjects and other retinal diseases.^{43,44} Eyes with FEVR are characterized by typical vascular changes such as a V-shaped vascular notch and marginal superbranching. Therefore we developed a scoring system to address the severity of the vascular abnormalities of FEVR. The VAP scores in patients with *KIF11* mutations were unremarkable at <5 in all but one patient, whereas the VAP scores of patients who had mutations in the Wnt signaling genes were >5. This suggests that the FEVR-like vascular changes are less penetrant in eyes with *KIF11* mutations although severe RDs can occur and be associated with extensive abnormalities of the retinal vasculature. These findings may reflect a different physiologic property of EG5 from Wnt signaling or a possible genetic redundancy of *KIF11* and *Kif15*.^{41,45} Moreover, the nature of the retinal dystrophy shown by reduced ERG responses in eyes with *KIF11* mutations

is distinct from eyes with common FEVR in which the ERG responses are believed to be normal unless the RDs progresses.^{46,47}

Microcephaly and the associated developmental disabilities were pathological features that were penetrant in patients with *KIF11* mutations although the expressivity of the retinal features was highly variable. The combination of the systemic features is consistent with the autosomal recessive microcephaly and chorioretinopathy that is caused by mutations in the *TUBGCP4*, *TUBGCP6*, and *PLK4* genes.^{42,48,49} These genes share the physiological role in the mitotic spindle with *KIF11*, and microcephaly and chorioretinopathy is indeed indistinguishable from MCLMR except for the mode of inheritance.^{34,39} The term of FEVR tends to be applied to congenital RDs with ischemic vitreoretinal proliferation as a replacement of other terms of congenital retinal folds (falciform retinal folds), posterior hyperplastic primary vitreous, pseudoglioma, and congenital retinal nonattachment.^{50–53} Congenital RDs are highly heterogeneous because they can be found as a part of systemic diseases including not only the Wnt signaling-related diseases such as ND and osteoporosis-pseudoglioma syndrome but also other syndromes associated with mutations in genes unrelated to Wnt signaling, for example, cerebroretinal angiopathy (Coats plus disease), dyskeratosis congenita, and Loeys-Dietz syndrome.^{54–56} These syndromes are clinically and genetically distinct from FEVR; however, their ocular features are often regarded to be FEVR or confused with FEVR as the initial diagnosis.^{57–60} The clinical entity of FEVR is yet to be defined and needs to be discussed whether it should be restricted to an ocular-only disorder with common FEVR findings or included as syndromic disorders as patients with *KIF11* mutations. Nonetheless, a strict determination of the cause of FEVR and the related retinopathy with or without systemic anomalies can be beneficial for offering accurate ocular and extraocular prognosis and proper genetic counseling to the patients and parents.²⁰

The limitations of this study are the small number of cases and its retrospective nature. The strength of this study is providing detailed retinal changes by multimodal imaging and ERGs especially for familial cases that are relatively rare.

In conclusion, mutations in the *KIF11* gene cause severe RDs with ischemic vitreoretinal proliferation, and family members show highly variable expressivity. Peripheral vascular anomalies can often be unremarkable in eyes without RDs. These findings highlight the more variable mechanisms that underlie patients with FEVR and related disorders.

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