Original Investigation

Phenotypic Overlap Between Familial Exudative Vitreoretinopathy and Microcephaly, Lymphedema, and Chorioretinal Dysplasia Caused by *KIF11* Mutations

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IMPORTANCE Retinal detachment with avascularity of the peripheral retina, typically associated with familial exudative vitreoretinopathy (FEVR), can result from mutations in *KIF11*, a gene recently identified to cause microcephaly, lymphedema, and chorioretinal dysplasia (MLCRD) as well as chorioretinal dysplasia, microcephaly, and mental retardation (CDMMR). Ophthalmologists should be aware of the range of presentations for mutations in *KIF11* because the phenotypic distinction between FEVR and MLCRD/CDMMR portends management implications in patients with these conditions.

OBJECTIVE To identify gene mutations in patients who present with a FEVR phenotype and explore the spectrum of ocular and systemic abnormalities caused by *KIF11* mutations in a cohort of patients with FEVR or microcephaly in conjunction with chorioretinopathy or FEVR.

DESIGN, SETTING, AND PARTICIPANTS Clinical data and DNA were collected from each participant between 1998 and 2013 from the clinical practices of ophthalmologists and clinical geneticists internationally. Twenty-eight FEVR probands with diagnoses made by the referring physician and without a known FEVR gene mutation, and 3 with microcephaly and chorioretinopathy, were included. At least 1 patient in each pedigree manifested 1 or more of the following: macular dragging, partial retinal detachment, falciform folds, or total retinal detachment.

EXPOSURES Whole-exome sequencing was conducted on affected members in multiplex pedigrees, and Sanger sequencing of the 22 exons of the *KIF11* gene was performed on singletons. Clinical data and history were collected and reviewed.

MAIN OUTCOMES AND MEASURES Identification of mutations in *KIF11*.

RESULTS Four novel heterozygous *KIF11* mutations and 1 previously published mutation were identified in probands with FEVR: p.A218Gfs*15, p.E470X, p.R221G, c.790-1G>T, and the previously described heterozygous p.R47X. Documentation of peripheral avascular areas on intravenous fluorescein angiography was possible in 2 probands with fibrovascular proliferation demonstrating phenotypic overlap with FEVR.

CONCLUSIONS AND RELEVANCE Mutations in *KIF11* cause a broader spectrum of ocular disease than previously reported, including retinal detachment. The *KIF11* gene likely plays a role in retinal vascular development and mutations in this gene can lead to clinical overlap with FEVR. Cases of FEVR should be carefully inspected for the presence of microcephaly as a marker for *KIF11*-related disease to enhance the accuracy of the prognosis and genetic counseling.

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Microcephaly, lymphedema, and chorioretinal dysplasia (MLCRD), as well as chorioretinal dysplasia, microcephaly, and mental retardation (CDMMR), were recently reported by Ostergaard et al[1] to result from mutations in the *KIF11* gene (NCBI NM_004523), demonstrating that these conditions are allelic (OMIM 152950) with highly variable phenotypic expression. None of the patients with *KIF11* mutations in that study had retinal folds or microphthalmia. Of the 27 mutation carriers identified, unilateral total retinal detachment was present in only 1 individual.

Familial exudative vitreoretinopathy (FEVR) is a hereditary developmental disorder characterized by the failure of peripheral retinal vascularization at birth. Complications include partial and total retinal detachment, and the disease may present with retinal folds that mimic persistent fetal vasculature.[2] To date, 5 genes have been identified that account for approximately 50% of cases of FEVR: *NDP* (OMIM 300658),[3] *FZD4* (OMIM 604579),[4] *LRP5* (OMIM 603506),[5,6] *TSPAN12* (OMIM 613138),[7,8] and, more recently, *ZNF408* (NCBI 79797).[9]

We performed whole-exome sequencing on DNA from affected members of multiplex FEVR pedigrees with no known causative FEVR gene mutations and identified 1 pedigree with a heterozygous KIF11 mutation (p.E470X). To test the hypothesis that more cases of FEVR are caused by *KIF11* mutations, we screened the *KIF11* gene in a cohort of FEVR probands who did not have a mutation in any of the known FEVR genes. We also screened the *KIF11* gene in typical and atypical cases of microcephaly with chorioretinopathy and/or retinal detachment. In addition, 2 of the 5 pedigrees with *KIF11* mutations were investigated further to identify a mechanism for the phenotypic overlap and differences between MLCRD and FEVR.

Methods

Participants and Clinical Data Collection

The study was approved by the research ethics board of the IWK Health Centre and written informed consent was obtained according to Canadian Tri-Council guidelines. No stipend was offered.

Participants were identified from a database of individuals in a study aiming to identify novel FEVR genes and describe the phenotypic spectrum of the disease. For recruitment in this database, the laboratory was listed on the GeneTests website[10] and potential participants were recruited at the treating physician's request. Patients were included if they manifested signs compatible with one of the following diagnoses: FEVR, atypical/typical persistent fetal vasculature, Coats disease, and congenital retinal folds or detachment. Pedigrees with microcephaly and bilateral or unilateral chorioretinopathy were recruited separately for specific *KIF11* screening.

Clinical data were collected prospectively and retrospectively by the referring physician or the study investigator (J.M.R.). These data included results from eye examinations and, whenever possible, ultrasonography, electrophysiologic testing, intravenous fluorescein angiography (IVFA), and fundus photography. Relatives at risk were also invited to participate by the referring physician or study investigator, and an eye examination was performed including best-corrected visual acuity, ocular alignment, slitlamp examination, dilated fundus examination, and in some cases, IVFA. Ethnicity was recorded for each participant by the referring physician who completed the study history questionnaire. This information was used in the analysis of novel mutation screening in a random population and in available databases.

Genetic Testing

Exome Capture and Sequencing, Read Mapping, and Variant Annotation

Blood and/or saliva samples were obtained from each participant for genomic DNA extraction using standard protocols. Whole-exome capture and sequencing were performed at the McGill University and Genome Quebec Innovation Centre as previously described.[11] In brief, a total of 3 µg of DNA was used for exome capture (SureSelect Human All Exon Kit, version 3; Agilent Technologies Inc). The captured DNA was sequenced with 100 base pair paired-end reads (HiSeq 2000 sequencer; Illumina). High-quality reads were mapped against the human genomic reference sequence (NCBI37/*hg19*) using the Burrows-Wheeler Aligner software.[12] Genomic variants were called using the Genome Analysis Toolkit pipeline[13] and annotated with ANNOVAR.[14]

All variants were compared against the Cardiff University Human Gene Mutation Database,[15] dbSNP,[16] 1000 Genomes Project,[17] Exome Variant Server,[18] and a pool of more than 300 in-house exomes from unrelated projects. Variants considered potentially damaging included nonsynonymous mutations (missense and nonsense), splice-site variants, and frameshift changes due to short insertions and/or deletions (indels). Depending on the type of mutation discovered, novel mutations were evaluated for functional significance using various software programs including SIFT,[19-23] PolyPhen-2,[24] PROVEAN (version 1.13),[25,26] CRYP-SKIP,[27-30] and Spliceman.[31,32]

Sanger Sequencing and Mutation Identification

Direct automated Sanger sequencing was performed (ABI PRISM 3100 Genetic Analyzer; Applied Biosystems). Regions sequenced included the 2 coding exons of the *FZD4* gene, the 23 coding exons of the *LRP5* gene, the 3 exons of the *NDP* gene (including the 5′ untranslated and promoter regions), the 8 coding exons of the *TSPAN12* gene, and the 22 coding exons of the *KIF11* gene (primer pairs available upon request). Because all *KIF11* mutations were detected before discovery of the *ZNF408* gene, *ZNF408* was not screened for variants in this study.

Mutation calling was performed (Mutation Surveyor, version 3.97; SoftGenetics LLC). Sequence changes were confirmed either in the forward and reverse direction or twice in the same direction. The variants were compared as stated above using the University of California, Santa Cruz, Genome Browser with the Human February 2009 (GRCh37/hg19) Assembly (http://genome.ucsc.edu/).[33] Novel missense mutations were screened using Sanger sequencing in 95 random population samples (180 chromosomes) of white race origin.

Results

Cohort Description and Mutation Detection Rate

Participants were recruited between April 1998 and September 2013. Seventy-two probands were enrolled to screen for a *FEVR* gene mutation because of ocular features compatible with a diagnosis of FEVR. Of these, 28 probands were found to not have a mutation in any of the following genes: *FZD4*, *LRP5*, *TSPAN12*, and *NDP*. A mutation in the *KIF11* gene was identified in 4 of 72 probands (5.6% mutation detection rate) with a phenotype mimicking FEVR. Two of these 4 probands were not reported to have microcephaly at the time of recruitment. Three of 6 probands with microcephaly associated with unilateral or bilateral chorioretinopathy and/or retinal folds or retinal detachment had a *KIF11* mutation. Clinical details for each participant are summarized in the eTable in the Supplement.

Genetic Analysis

Whole-exome sequencing was initially performed on an affected male and his affected sister (pedigree I) to identify a shared disease-causing variant. There were 267 shared missense or nonsense variants after filtering for suspected sequencing artifacts and with a multiple allele frequency greater than 5%. The siblings shared 3 truncating mutations and 1 frame shift deletion: the frame shift deletion was seen in 12 other sequenced exomes; 1 truncating mutation (*CORO7*) had been identified in an additional in-house exome and another was in the olfactory receptor gene *OR52N4*. The remaining heterozygous truncating mutation, KIF11 p.E470X (c.1408 G>T), was selected as the candidate, because heterozygous truncating mutations in *KIF11* had previously been reported [1] as causative for MLCRD. There were no other siblings and no family history of a condition compatible with a diagnosis of FEVR or MLCRD. The mutation was absent in both parents, suggesting mosaicism in one of the parents.

Sanger sequencing identified additional novel heterozygous mutations in 3 probands (**Figure 1**): p.A218Gfs*15 (c.652DupG), p.R221G (c.661 A>G), and *KIF11* c.790-1G>T that alters an acceptor splice site. A previously reported[34] heterozygous mutation, KIF11 p.R47X (c.139C>T), was found in a fifth proband. No participant harbored compound heterozygous mutations.

Clinical Description

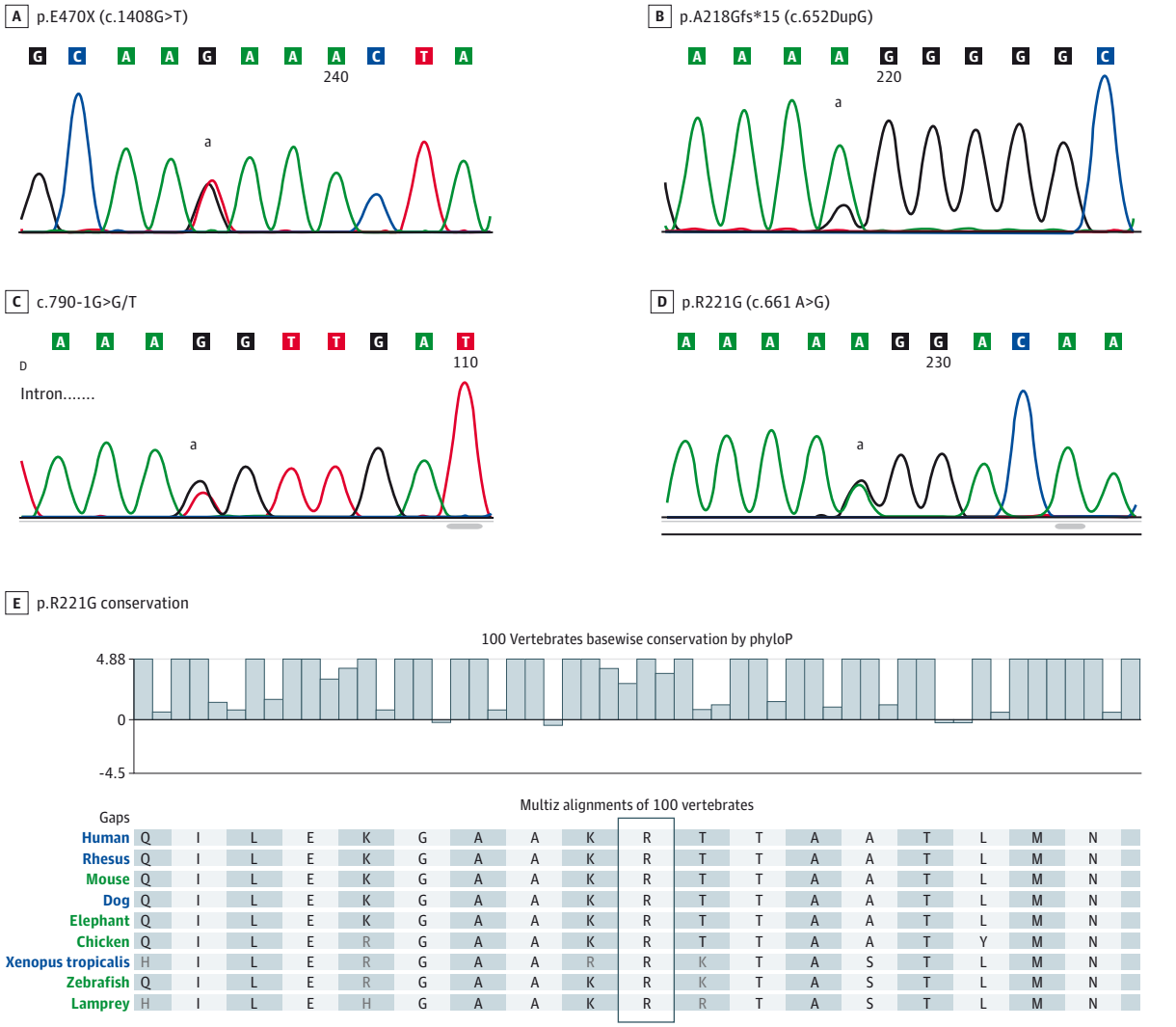
The first mutation, KIF11 p. E470X, identified in 2 siblings (1 male and 1 female) was associated with a FEVR phenotype and with microcephaly that was noted prior to enrollment. The family was recruited in the FEVR project specifically because of the fundus appearance mimicking FEVR. The proband was an 8-year-old boy at the time of recruitment. He had been noted to have intermittent esotropia since age 1½ years and had amblyopia that did not respond to patching or atropine treatment. The retinal abnormalities were first noted at age 3 years. He was born full-term with a birth weight of 3.0 kg. His past medical history was significant for microcephaly (head circumference measures not available). His best-corrected visual acuity was 20/50 OD and 20/210 OS, with a correction of +1.50 + 1.50X85 in the right eye and −1.25 + 1.75X100 in the left eye. Chorioretinal atrophy without retinal detachment was present in both eyes (**Figure 2**A). The younger sister was examined at age 5½ years. She had a history of eyes drifting in or out intermittently and had received patching and atropine treatment that had been discontinued at age 2 years. She also was noted to have microcephaly. On eye examination, her best-corrected visual acuity was 20/170 OD (−2.25 + 2.25X70) and 20/120 OS (−4.25 + 5.25X110). She had a unilateral retinal fold in addition to bilateral areas of chorioretinopathy (Figure 2B).

The KIF11 p.A218Gfs*15 mutation was discovered in a boy who was born full-term and had a history of vision problems since age 6 months. There was no family history of FEVR or congenital retina problems. In addition, there was no family history of microcephaly or lymphedema, and the family noted large head sizes on the paternal side. The boy was not noted to have an anomalous head size on review of his medical records. At age 4 years, he had noncentral, unsteady, and nonmaintained fixation in either eye (cycloplegic refraction +1.00 in the right eye and +0.50 in the left eye). He had an alternating esotropia of 40 prism diopters. On fundus examination, retinal folds were present in both eyes without evidence of chorioretinopathy typically seen in MLCRD (Figure 2C). Intravenous fluorescein angiography revealed peripheral avascular areas of retina typical of FEVR (Figure 2D). Results of a head computed tomography scan were reported as normal. Following the discovery of the *KIF11* mutation, the head circumference was measured and found to be below the second percentile. The parents and a sibling did not share the *KIF11* mutation, suggesting that the mutation occurred de novo in the proband.

The KIF11 p.R47X mutation was identified in a 6-year-old boy who was born full-term. The visual acuity was light perception with both eyes open. The retina was completely detached, forming a fibrous mass behind the lens on the right side. The left eye manifested a falciform fold. The anterior segment was normal. The child was lost to follow-up, precluding further investigation of head circumference measurements and formal systemic evaluation. The parents' fundus examination including IVFA showed peripheral vascular tortuosity in both. Tissue samples were not collected from the parents.

The intronic sequence mutation *KIF11* c.790-1G>T was identified in a singleton with recognized microcephaly and diagnosed with FEVR by the ophthalmologist. There was no family history of health problems compatible with a diagnosis of FEVR or MLCRD, and the mutation was not found in either parent or sister. The boy was born full-term. Vision problems were noted soon after birth at which time he received a diagnosis of FEVR. At age 2, his development appeared normal, but his head circumference was 5 SDs below the mean. On eye examination, he had a large-angle esotropia with leukocoria on the right side. At age 15 months, he was able to fix and follow objects with his left eye (+3.25-2.00X180) but had no light perception on the right side. The right eye had a complete retinal detachment with a cloudy cornea. The left fundus showed peripheral vitreous condensation with subretinal fibrosis inferiorly after prior treatment with cryotherapy and scleral buckle.

Figure 1. Novel *KIF11* Mutations

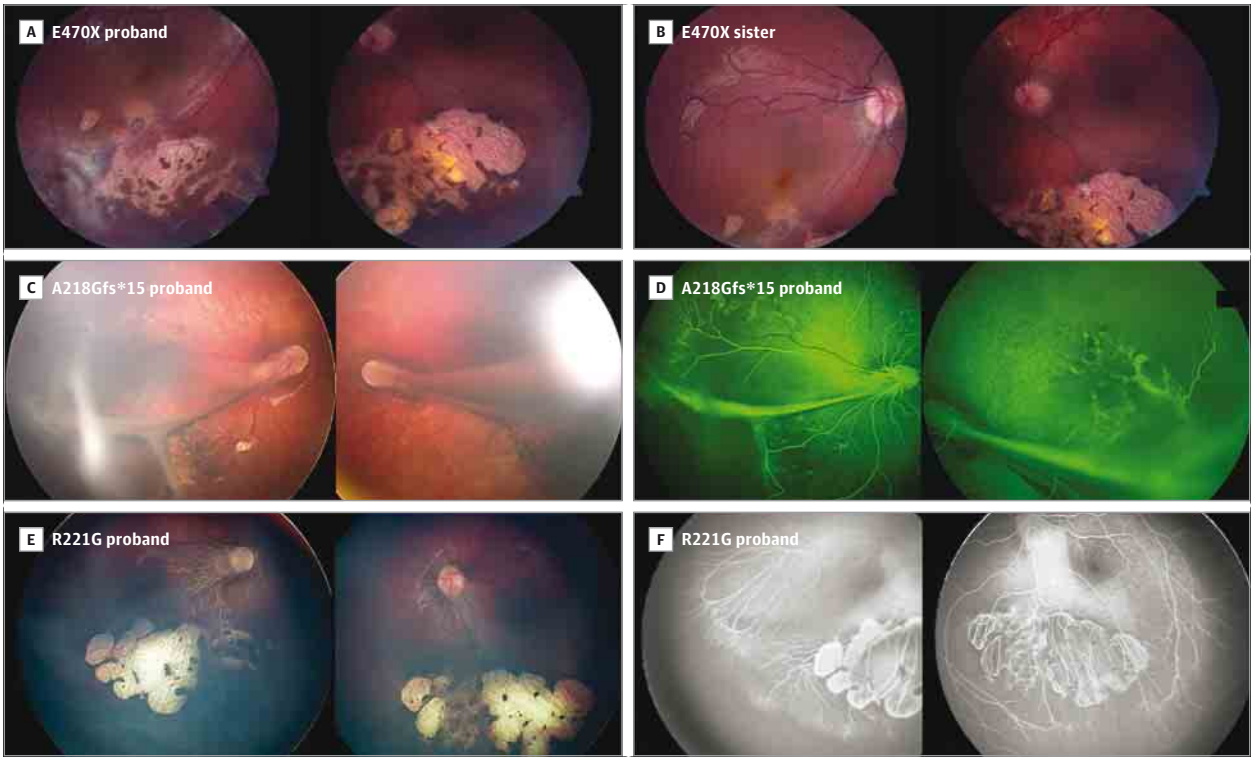


A-D, Location of novel *KIF11* mutations with respect to the genomic organization of *KIF11*. Three of the novel mutations, KIF11 p.E470X (c.1408G>T), p.A218Gfs*15 (c.652DupG), and *KIF11* c.790-1G>T, lead to a truncation of the KIF11 protein coding sequence. *KIF11* c.790-1G>T alters an acceptor splice site. The mutation occurs in a nucleotide that is highly conserved across a variety of taxa. The mutation was evaluated for functional significance using the software CRYP-SKIP, and Spliceman. Both programs predicted that the mutation has a high probability of affecting splicing. The nonsynonymous KIF11 p.R221G (c.661 A>G) mutation segregated with the disease in a pedigree with 4 affected individuals. The mutation, located in the kinesin motor domain, was evaluated for functional significance using the software programs PolyPhen-2, PROVEAN, and SIFT. All programs predicted that the mutation is damaging. The mutation was absent in the 300 in-house exomes and the Exome Variant Server, as well as in 180 chromosomes from a random white population. E, Sequence comparison of the KIF11 protein demonstrating the evolutionary conservation of the mutated KIF11 arginine 221 residue across a variety of taxa.

Three probands were recruited specifically to investigate a genetic origin for likely or possible MLCRD. None had retinal detachment. Of the 3 pedigrees, one family of Acadian descent with 4 affected members was identified with KIF11 p.R221G. The proband was born full-term. She was noted to have microcephaly in infancy, but according to the family, this was deemed to represent uncomplicated hereditary microcephaly before discovering the eye problems, because she was meeting her developmental milestones and her mother was also microcephalic but otherwise healthy. There were no siblings, and a maternal uncle was known to have significant developmental delays, having lived most of his life in a special care facility, and he had a long-standing history of blindness. The family was further investigated by medical genetics (eTable in the Supplement). Although not examined, the maternal grandmother's head size was described as normal but the maternal uncle with significant developmental delays was known to have a small head size. Medical ocular records of the maternal uncle described retinal lesions similar to those found in the proband.

At age 20 months, the proband had a right esotropia and subnormal visual behavior. The corneal diameter was reduced at 10 mm with mild sclerocornea over 360° in both eyes. The axial length was normal at 20.4 mm in the right eye and

Figure 2. Fundus Photographs and Intravenous Fluorescein Angiography (IVFA)



A, E470X proband: the right fundus shows peripheral atrophic changes inferotemporally and an area of mild elevation. The left fundus manifests an inferior peripheral area of chorioretinal atrophy with retinal pigment epithelium clumping. B, E470X sister: a peripheral retinal fold courses inferotemporally with areas of atrophy in the right eye and peripheral retinal pigmentary changes only on the left. C, A218Gfs*15 proband: fibrovascular tissue with retinal folds involving the macula was present in both eyes without evidence of chorioretinopathy typically seen in microcephaly,lymphedema, and chorioretinal dysplasia. Retinal pigment epithelial changes were evident in keeping with retinal detachment and ischemic stress. D, IVFA revealed peripheral areas of an avascular retina. E, R221G proband: multiple inferior chorioretinal atrophic lesions are present with moderate, diffuse atrophy of the optic nerve. The macula is featureless and the retinal vessels are fine and straightened, in keeping with severe retinal dysplasia. The vascular arcade is dragged temporally toward an atrophic scar with a temporal fibrous mass. F, IVFA revealed an area of avascular retina peripheral to the chorioretinal scars and the fibrous mass as well as retinal vascular anomalous formation in the areas without chorioretinal atrophy, resembling what is typically seen in familial exudative vitreoretinopathy with the presence of a fibrovascular mass at the junction of the avascular area.

20.16 mm in the left eye, and the cycloplegic refraction was +1.50 spherical equivalent hyperopic astigmatism in both eyes. Multiple bilateral inferior chorioretinal atrophic lesions were present. The macula was dragged peripherally and there was retinal detachment in both eyes (Figure 2E). Intravenous fluorescein angiography revealed bilateral areas of peripheral avascular retina that was otherwise difficult to detect with indirect ophthalmoscopy alone (Figure 2F). The proband's mother underwent a full eye examination including IVFA that failed to show vascular abnormalities or chorioretinopathy.

Discussion

In this study, we have extended the description of the spectrum of ocular manifestation resulting from mutations in *KIF11* to include retinal detachment that can mimic FEVR. Ostergaard et al[1] recently reported on cases of MLCRD/CDMMR associated with *KIF11* mutations (OMIM 152950). The major ocular phenotype described in 27 patients consisted of chorioretinopathy characterized by peripheral areas of atrophic patches involving the choroid and retina. Other ocular characteristics included refractive errors, nystagmus, strabismus, and only a single eye with a total retinal detachment. Because previous reports[35-37] of MLCRD and CDMMR have described the presence of retinal folds, persistent fetal vasculature, and microphthalmia, none of which were present in their patients with *KIF11* mutations, the authors[1] suggested that microcephaly with retinal folds and microphthalmia could be caused by mutations in a different gene. Consistent with this hypothesis, the only other patient with MLCRD who had a *KIF11* mutation also did not have a retinal fold or detachment.[34]

The present study suggests that retinal folds and detachments are more common in the *KIF11*-related MLCRD spectrum of conditions than was previously reported, with the identification of *KIF11* mutations in 5.6% of all FEVR probands and 14.3% (4 of 28) of probands without a known FEVR gene mutation. The difference in prevalence could be attributed to ascertainment bias. Children with FEVR presenting with partial or complete retinal detachments at a very young age should be examined for mild to moderate microcephaly that could

point to a more precise diagnosis. Microcephaly that ranges from mild to severe,[1] especially in the absence of developmental delays, can be easily overlooked as unrelated to the FEVR phenotype. Patients with FEVR should be carefully examined for the presence of microcephaly as a marker for *KIF11*-related disease, because differentiating FEVR from MLCRD/CDMMR is important for the accuracy of genetic counseling. Of particular significance is the possibility of variable degrees of mental retardation, as is the case for our pedigree carrying the KIF11 p.R221G mutation.

An ocular feature that can help distinguish FEVR from MLCRD/CDMMR is the presence of chorioretinopathy typically affecting the inferior retina. However, this feature may be subtle or absent, as was the case for the patient with KIF11 p.A218Gfs*15. We have shown that the identification of a peripheral avascular retina on examination and/or IVFA does not exclude *KIF11*-related disease. Our data do not enable us to determine the exact frequency of *KIF11* mutations in cases with such a presentation at a young age, but we hypothesize that the frequency is likely to be higher in those patients than the 5.6% of all FEVR cases in our cohort.

In FEVR, the primary abnormality is variable incomplete vascularization of the peripheral retina, a process that should be largely completed by term birth. Secondary fibrovascular proliferation is responsible for retinal detachment that can range from partial to complete. More severe cases usually present in infancy or early childhood. Until now, the cause of retinal detachment in some cases of MLCRD and CDMMR had been unresolved. To our knowledge, we have demonstrated for the first time that *KIF11* mutations can result in abnormal peripheral retinal vascular development that can present with or without retinal detachment, which is identical to what occurs in patients with FEVR. This feature is different from retinal vascular attenuation or paucity associated with retinal dysplasia. The identification of peripheral avascular retina is important to minimize vision loss from secondary complications. Our findings suggest that newly diagnosed cases and newborns at risk for MLCRD should receive IVFA as part of their workup with consideration to treat the avascular zones with laser in selected cases to avoid tractional detachment and prevent vision loss. At a minimum, such patients will require closer follow-up, especially at a young age, to detect potential complications early and improve the visual outcome.

A kinesin family member motor protein, KIF11 localizes to spindle microtubules during mitosis and is required for mitotic progression. Several genes with roles in spindle development, including *KIF11*, result in microcephaly when mutated.[1] An insertional mutagenesis screen in zebrafish identified the *kif11* gene as being required for normal development of secondary motor neurons and oligodendrocytes, as well as for glial cell viability. Glial cells from the *kif11* mutants arrested in mitosis and subsequently underwent apoptotic cell death.[38] However, the role of KIF11 in retinal vascular development is less clear. Of interest is the report of a case of congenital retinal folds, microcephaly, and mild mental retardation associated with a chromosomal abnormality disrupting *CDK19*,[39] suggesting that genes other than *KIF11* that can cause microcephaly and that retinal folds likely play a role in the development of retinal vasculature. Further research will clarify the role of these genes in the development of retinal vessels.

Conclusions

Mutations of *KIF11* are associated with a broad spectrum of phenotypes ranging from apparently classical FEVR to MLCRD and CDMMR. Careful clinical examination is necessary to identify associated microcephaly. Genotyping patients with newly diagnosed FEVR should include *KIF11* in addition to classical FEVR genes.

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Author Contributions: Drs Robitaille and Bedard had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Robitaille, LeBlanc, Ells, Shalev, Fernandez, McMaster, Bedard.
Acquisition, analysis, or interpretation of data: Robitaille, Gillett, LeBlanc, Gaston, Nightingale, Mackley, Parkash, Hathaway, Thomas, Ells, Traboulsi, Héon, Roy, MacGillivray, Wallace, Fahiminiya, Majewski, McMaster, Bedard.
Drafting of the manuscript: Robitaille, Gillett, LeBlanc, Gaston, Nightingale, Mackley, Shalev, McMaster.
Critical revision of the manuscript for important intellectual content: Robitaille, Gillett, LeBlanc, Gaston, Parkash, Hathaway, Thomas, Ells, Traboulsi, Héon, Roy, Fernandez, MacGillivray, Wallace, Fahiminiya, Majewski, McMaster, Bedard.
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