REPORT

Mutations in *KIF11* Cause Autosomal-Dominant Microcephaly Variably Associated with Congenital Lymphedema and Chorioretinopathy

Pia Ostergaard,[1] Michael A. Simpson,[2] Antonella Mendola,[3] Pradeep Vasudevan,[4] Fiona C. Connell,[5] Andreas van Impel,[6] Anthony T. Moore,[7] Bart L. Loeys,[8] Arash Ghalamkarpour,[3] Alexandros Onoufriadis,[2] Ines Martinez-Corral,[9] Sophie Devery,[10] Jules G. Leroy,[11] Lut van Laer,[8] Amihood Singer,[12] Martin G. Bialer,[13] Meriel McEntagart,[14] Oliver Quarrell,[15] Glen Brice,[14] Richard C. Trembath,[2] Stefan Schulte-Merker,[6] Taija Makinen,[9] Miikka Vikkula,[3,16] Peter S. Mortimer,[17] Sahar Mansour,[14] and Steve Jeffery[1,*]

We have identified *KIF11* mutations in individuals with syndromic autosomal-dominant microcephaly associated with lymphedema and/or chorioretinopathy. Initial whole-exome sequencing revealed heterozygous *KIF11* mutations in three individuals with a combination of microcephaly and lymphedema from a microcephaly-lymphedema-chorioretinal-dysplasia cohort. Subsequent Sanger sequencing of *KIF11* in a further 15 unrelated microcephalic probands with lymphedema and/or chorioretinopathy identified additional heterozygous mutations in 12 of them. *KIF11* encodes EG5, a homotetramer kinesin motor. The variety of mutations we have found (two nonsense, two splice site, four missense, and six indels causing frameshifts) are all predicted to have an impact on protein function. EG5 has previously been shown to play a role in spindle assembly and function, and these findings highlight the critical role of proteins necessary for spindle formation in CNS development. Moreover, identification of *KIF11* mutations in patients with chorioretinopathy and lymphedema suggests that EG5 is involved in the development and maintenance of retinal and lymphatic structures.

There is substantial phenotypic overlap between MLCRD (microcephaly, primary lymphedema, and chorioretinal dysplasia) syndrome (MIM 152950) and CDMMR (chorioretinal dysplasia, microcephaly, and mental retardation) syndrome (MIM 156590).[1] Both have been observed to segregate with autosomal-dominant inheritance and present with an overlapping, yet variable, spectrum of CNS and ocular developmental anomalies.[2,3] Microcephaly, ranging from mild to severe, is the critical component of both syndromes and is often associated with mild to moderate developmental delay and a characteristic facial phenotype[4] (Figures 1A and 1B). Chorioretinopathy constitutes the most common eye abnormality (Figure 1C). However, retinal folds, microphthalmia, and myopic and hypermetropic astigmatism have also been reported, and some individuals have no overt ocular phenotype.[5] The presence of lymphedema has historically been seen as the critical differentiating feature between the two syndromes. The lymphedema in MLCRD is congenital and typically confined to the dorsa of the feet (Figure 1D), and lymphoscintigraphy reveals the absence of radioactive isotope (technetium 99) uptake from the webspaces between the toes (Figure 1E). We have identified causative variants for this complex disease, and the fact that we show that *KIF11* mutations can cause both MLCRD and CDMMR suggests that together, these syndromes should be considered as a single entity that has variable clinical features but a unified molecular basis.

Whole-exome sequencing has proved to be a successful approach to identifying causative mutations in primary lymphoedema.[6–8] We therefore sought to establish the molecular genetic basis of MLCRD by whole-exome sequencing the DNA of five unrelated probands (individuals MLCRD01:II-2, MLCRD02:II-2, MLCRD03:III-2, MLCRD04:I-1, and MLCRD05:I-1 in Table 1 and Table S1, available online) with microcephaly and lymphedema. Subjects for this study were recruited from genetic, lymphovascular, and ophthalmic clinics in Europe. Informed consent was obtained from all subjects. All affected individuals and family members underwent a detailed physical

[1]Medical Genetics Unit, Biomedical Sciences, St. George's University of London, London SW17 0RE, UK; [2]Division of Genetics and Molecular Medicine, King's College London School of Medicine, Guy's Hospital, London SE1 9RT, UK; [3]Laboratory of Human Molecular Genetics, de Duve Institute, Université Catholique de Louvain, B-1200 Brussels, Belgium; [4]Clinical Genetics, University Hospitals of Leicester National Health Services Trust, Leicester Royal Infirmary, Leicester LE1 5WW, UK; [5]Clinical Genetics, Guy's and St. Thomas' National Health Services Foundation Trust, Guy's Hospital, London SE1 9RT, UK; [6]Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences, in collaboration with the Universitair Medisch Centrum Utrecht, 3584 CT Utrecht, Netherlands; [7]University College London Institute of Ophthalmology, Moorfields Eye Hospital and Hospital for Children, London EC1V 2PD, UK; [8]Center for Medical Genetics, Antwerp University Hospital and University of Antwerp, B-2650 Antwerp, Belgium; [9]Lymphatic Development Laboratory, Cancer Research UK London Research Institute, London WC2A 3PX, U; [10]Moorfields Eye Hospital NHS Foundation Trust, London EC1V 2PD, UK; [11]C. Hooft Children's Hospital, Ghent University Hospital, B-9000 Ghent, Belgium; [12]Pediatrics and Medical Genetics, Barzilai Medical Center, 78306 Ashkelon, Israel; [13]Division of Medical Genetics, North Shore-Long Island Jewish Health System, Manhasset, NY 11030, USA; [14]South West Thames Regional Genetics Service, St. George's University of London, London SW17 0RE, UK; [15]Sheffield Clinical Genetics Service, Sheffield Children's Hospital, Sheffield S10 2TH, UK; [16]Walloon Excellence in Lifesciences and Biotechnology, Université catholique de Louvain, B-1200 Brussels, Belgium; [17]Department of Cardiac and Vascular Sciences, St. George's University of London, London SW17 0RE, UK
*Correspondence: sggt100@sgul.ac.uk
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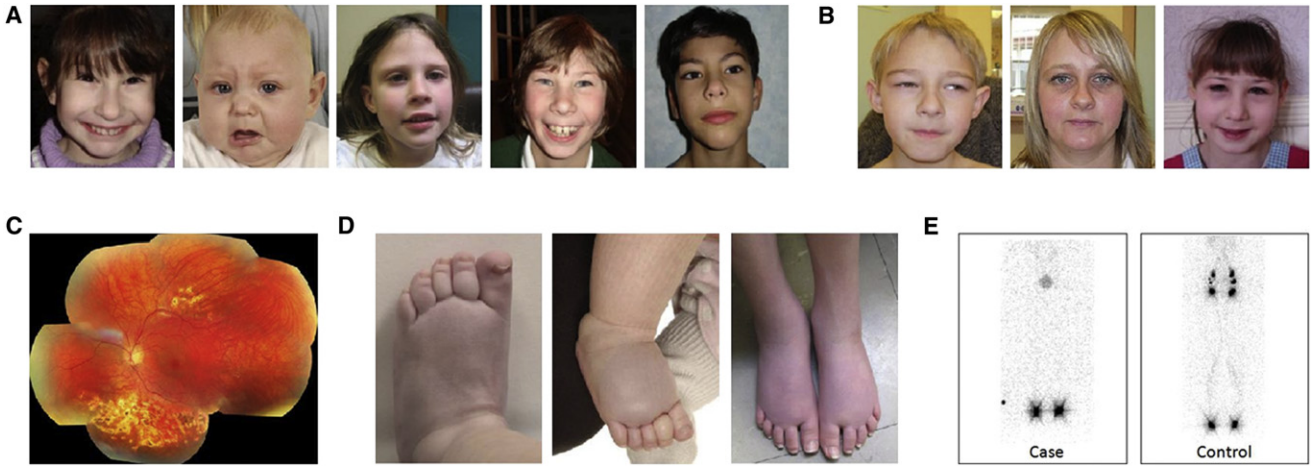

Figure 1. Clinical Features of *KIF11* Mutation-Positive Patients with MLCRD and CDMMR
(A) Facial features of individuals diagnosed with MLCRD.
(B) Facial features of individuals diagnosed with CDMMR. The faces in (A) and (B) are characteristic of the syndrome and have upslanting palpebral fissures, a broad nose with a rounded tip, a long philtrum with a thin upper lip, a prominent chin, and prominent ears.
(C) A composite color photograph of the left fundus in patient CDMMR05 II-1 shows focal areas of peripheral chorioretinal atrophy.
(D) Bilateral, congenital lower-limb primary lymphedema in subjects with MLCRD involves the dorsa of the feet, which show pitting edema, deep interphalangeal creases, small dysplastic nails, and wide-caliber veins.
(E) Comparison of lower-limb lymphoscintigraphy (imaging taken 2 hr after injection of radioactive isotope [technetium 99] into the webspaces between the toes) in patient MLCRD01 II-2 and an unaffected control. The patient's lymphoscintigraphy shows no significant main-tract filling and therefore suggests initial lymphatic-vessel dysfunction.

examination. Ethical approval for this study was obtained from the South West London Research Ethics Committee (REC Ref: 05/Q0803/257).

Whole-exome capture was performed by in-solution hybridization, followed by massively parallel sequencing with the SureSelect All Exon CCDS (Consensus Coding Sequences) Target Enrichment System (Agilent)[9] and sequencing on a Genome AnalyserIIx (Illumina) with 76 bp paired-end reads. Sequence reads were aligned to the reference genome (hg18) with Novoalign (Novocraft Technologies SdnBhd). Duplicate reads, resulting from PCR clonality or optical duplicates, and reads mapping to multiple locations were excluded from downstream analysis. Depth and breadth of sequence coverage were calculated with custom scripts and the BedTools package.[10] More than 5.1 Gb of sequence was generated for each subject, such that >75% of the coding bases of the CCDS-defined exome were represented by at least 20 reads (Table S2). Single-nucleotide substitutions and small indel variants were identified and quality filtered within the SamTools software package[11] and in-house software tools (Table S3).[12] Variants were annotated with respect to genes and transcripts with the Annovar tool.[13] We filtered variants for novelty by comparing them to dbSNP132 and 1000 Genomes SNP calls (December 2010) and to variants identified in 250 control exomes (primarily of European origin), which we sequenced and analyzed by the same method described above.

Analysis of the exome-variant profiles was performed under a model of a rare autosomal-dominant disorder; this model required at least one previously unobserved, heterozygous nonsynonymous or splice-site substitution or an insertion or deletion in the same gene in all five individuals. This analysis failed to identify a single gene matching these criteria (Table S4). Further evaluation of the data with an expectation of genetic heterogeneity among these five cases revealed *KIF11* to be the only gene that harbored previously unobserved variants in three of the five individuals (Table S4). All three allelic mutations (a nonsense variant [p.Arg387*], a single-nucleotide deletion [p.Ile1006Leufs*62], and a dinucleotide deletion [p.Leu347Glufs*8]) are predicted to cause changes to the *KIF11* protein product (Table 1). All were confirmed by Sanger sequencing (primer sequences are listed in Table S5). Sequencing in the two subjects, in whom exome sequencing did not reveal novel *KIF11* variants, confirmed the wild-type coding sequence. Our findings indicate that heterozygous mutations of *KIF11* underlie a significant proportion of MLCRD, and we therefore assessed *KIF11* for mutations in other MLCRD cases by Sanger sequencing in probands from nine additional families, including a large multigenerational pedigree (MLCRD11, Figure S1). In these probands, sequencing revealed seven more independent heterozygous *KIF11* variants: three frameshift insertions and deletions, two missense substitutions, an acceptor splice-site substitution, and a donor splice-site change that was observed in the proband from the multigenerational pedigree (Table 1). Each of the ten identified *KIF11* alleles was assessed in all available relatives; two were shown to have arisen de novo, and eight demonstrated cosegregation with microcephaly, variable in its severity, and with a spectrum of eye and lymphatic abnormalities (Table 1). In total, we identified *KIF11* mutations in 10 of the 14 MLCRD-affected families examined.

Table 1. Clinical and Genetic Findings in *KIF11*-Mutation-Positive Patients

Pedigree	Individual	Gender	Head Circumference[b]	Lymphedema	Eye Abnormalities	Additional Clinical Features	Nucleotide Variant	Exon	Protein Alteration
MLCRD01	I-1	male	−3.0	minimal edema	none	mild LD	c.1159C>T	10	p.Arg387*
	II-2[a]	female	−7.2	congenital, bilateral, and lower limb	none	mild LD and dysmorphic features	c.1159C>T	10	p.Arg387*
MLCRD02	I-1	female	−1.6	none	diabetic retinopathy	mild LD	c.3016delA	21	p.Ile1006Leufs*62
	II-2[a]	female	−4.0	congenital, bilateral, and lower limb	none	mild dysmorphic features	c.3016delA	21	p.Ile1006Leufs*62
MLCRD03	II-1	male	−2.0	none	none	mild LD	c.1039_1040delCT	9	p.Leu347Glufs*8
	III-2[a]	female	−7.5	congenital, bilateral, and lower limb plus pleural effusions	hypermetropic astigmatism and chorioretinopathy	mild LD	c.1039_1040delCT	9	p.Leu347Glufs*8
MLCRD06	I-1	male	−4.3	congenital, bilateral, and lower limb	none	low birth weight, failure to thrive,	c.432T>G	5	p.Phe144Leu
MLCRD07	I-1[c]	male	−2.3	congenital, bilateral, and lower limb	bilateral chorioretinopathy	mild LD, ASD, and myoclonic epilepsy	c.2830C>T (de novo)	20	p.Arg944Cys
MLCRD08	I-1	male	−3.5	none	none	none	c.1425_1426delinsAAA	12	p.Val476Asnfs*2
	II-1	male	−5.5	congenital, mild, bilateral, and lower limb	myopia	moderate LD	c.1425_1426delinsAAA	12	p.Val476Asnfs*2
MLCRD09	I-1	female	−3.0	none	none	none	c.1592delA	13	p.Gln531Argfs*8
	II-1[d]	male	−4.0	congenital, bilateral, and lower limb	bilateral chorioretinopathy	ASD and dysmorphic features	c.1592delA	13	p.Gln531Argfs*8
MLCRD10	I-1	male	−5.5	congenital, mild, bilateral, and mild lower limb (resolved)	bilateral chorioretinopathy	mild LD	c.699-2A>G (de novo)	6/7	acceptor splice site
MLCRD11	II-1	male	low by history	congenital, bilateral, and lower limb plus mild in hands	none	none	c.2547+2T>C	18/19	donor splice site
	III-2	female	−4.7	none	none	mild LD	c.2547+2T>C	18/19	donor splice site
	IV-1	male	−4.0	congenital, bilateral, and lower limb plus mild in hands	none	moderate LD and hypospadias	c.2547+2T>C	18/19	donor splice site
	IV-3	male	−3.7	congenital, mild, bilateral, and lower limb	none	moderate LD	c.2547+2T>C	18/19	donor splice site
	IV-5	female	−4.1	none	none	mild LD	c.2547+2T>C	18/19	donor splice site

Table 1. *Continued*

Pedigree	Individual	Gender	Head Circumference[b]	Lymphedema	Eye Abnormalities	Additional Clinical Features	Nucleotide Variant	Exon	Protein Alteration
MLCRD12	I-1	male	−6.4	congenital, mild, bilateral, and lower limb	bilateral chorioretinopathy	moderate LD	c.1963_1964dupAA	15	p.His656Serfs*8
CDMMR01	II-1	female	−5.0	adult onset and post-traumatic mild edema	chorioretinopathy	mild LD	c.1159C>T (de novo)	10	p.Arg387*
	III-1	male	−5.5	none	hypermetropic astigmatism and chorioretinopathy	moderate LD	c.1159C>T	10	p.Arg387*
CDMMR02	I-1	female	low by history	none	bilateral chorioretinopathy	none	c.704C>G	7	p.Ser235Cys
	II-1	female	low by history	none	bilateral chorioretinopathy	moderate LD	c.704C>G	7	p.Ser235Cys
CDMMR03	I-1	male	−3.4	none	no vision in right eye (retinal detachment) and peripheral retinal atrophy in left eye	mild LD	c.2304_2305delCA (de novo)	18	p.His768Glnfs*7
CDMMR04	I-1	male	−5.1	none	chorioretinopathy, nystagmus, and exotropia	moderate LD	c.700C>T	7	p.Arg234Cys
CDMMR05	II-1	female	−3.9	none	chorioretinopathy	mild LD	c.1804C>T	14	p.Gln602*
	II-2	female	−6.1	none	hypermetropic astigmatism	none	c.1804C>T	14	p.Gln602*

Abbreviations are as follows: LD, learning difficulties; and ASD, atrial septal defect. Please see main text for additional abbreviations.
[a] Individuals exome sequenced in primary analysis.
[b] Head circumference measured as occipitofrontal head circumference in cm and corrected for age and sex.
[c] Case description in Vasudevan et al. (2005).[4]
[d] Case description in Eventon-Friedman et al. (2009).[24]

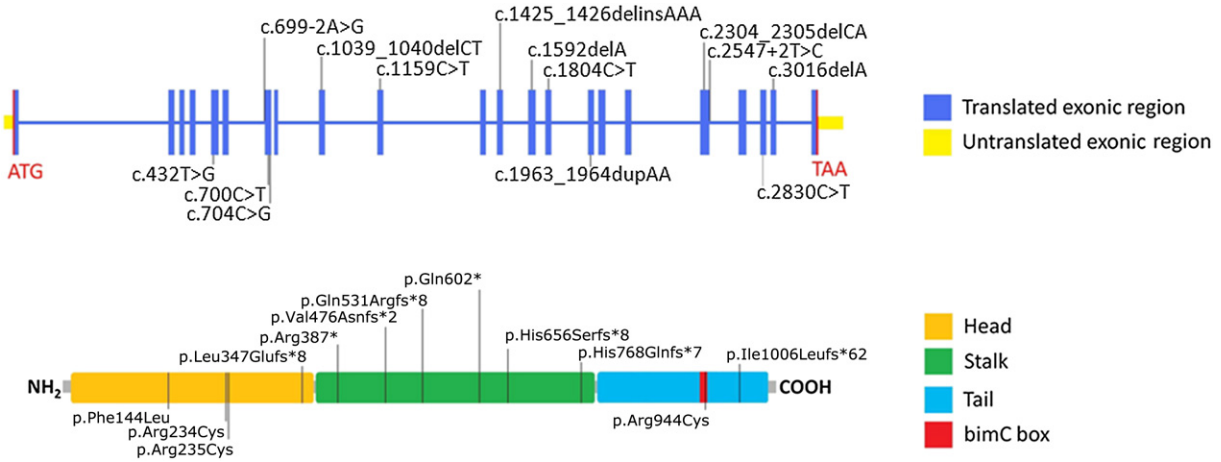

Figure 2. Location of the Identified Mutations in *KIF11*
Upper panel: Mutations are indicated with respect to the genomic organization of *KIF11*.
Lower panel: KIF11 domain structure excludes the two identified splice-site mutations.

Within the extended MLCRD-affected families, we observed individuals who had heterozygous mutations in *KIF11* and in whom there was no evidence of lymphatic involvement (Table 1). The considerable and recognizable overlap between the two conditions led us to address the hypothesis that MLCRD and CDMMR are allelic disorders. Sequencing of *KIF11* in a cohort of six independent families in which microcephaly and eye abnormalities had been observed in the absence of lymphedema revealed five independent variants: Two missense substitutions and three variants, including one nonsense mutation (p.Arg387*) that we had previously identified in pedigree MLCRD01, were predicted to lead to premature termination of the protein product (Table 1). In total, we identified 27 carriers of 14 mutant *KIF11* alleles in 15 independent families. None of the 14 variants is present in dbSNP, has been identified by the 1000 Genomes Project, or was observed in a cohort of 250 control samples.

We failed to identify coding variation in *KIF11* in five families from which four probands had previously been diagnosed with MLCRD and one with CDMMR (Table S1). These cases might represent *KIF11* alleles comprising noncoding or undetected coding variants. They might represent phenocopies of MLCRD and CDMMR or be explained by locus heterogeneity. We were unable to investigate the latter because none of the *KIF11*-mutation-negative families were of sufficient size to allow exclusion of the locus by linkage analysis.

KIF11 encodes EG5, a bipolar, homotetrameric, slow-processive, plus-end-directed spindle motor protein of the kinesin-5 family.[14] Each monomer contains a conserved N-terminal motor, a central coiled-coil domain, and a C-terminal tail that contains a bimC box, a conserved sequence of positively charged amino acid residues (Figure 2).[15,16] The homotetramer consists of four monomers that are arranged in an antiparallel fashion so that the resulting molecule possesses two motor domains and two nonmotor tails at each end of a central stalk. The contact of EG5 with microtubules is established through the C-terminal tails, whereas the subsequent sliding of the antiparallel microtubules is driven by the motor domains.[17] Seven of the identified allelic mutations, two nonsense and five frameshift, including insertions and deletions, are predicted to lead to premature termination of the protein. A sixth frameshift variant, a single-base deletion in the second-to-last exon, is predicted to result in substitution of the terminal 50 residues of the 1,056 amino acid wild-type protein and extension of the reading frame by a further 12 residues. The two splice-site mutations are both predicted in HSF (Human Splicing Finder v.2.4.1)[18] to have significant impact on the splicing of the 5 kb transcript (Table S6). The four missense mutations all alter evolutionarily conserved amino acid residues and are predicted to have a damaging effect on protein function according to SIFT and PolyPhen; three (p.Phe144Leu, p.Arg234Cys, and p.Ser235Cys) are located within the motor domain,[15] and the fourth (p.Arg944Cys) is located within the bimC box in the C-terminal tail of the molecule (Figure S2).[16]

Kif11 has been shown to be widely expressed during murine embryonic development and is elevated in proliferating tissues.[19,20] During zebrafish development, *kif11* is dynamically expressed in tissues that are associated with rapid proliferation (Figure S3). Interestingly, homozygous disruption of *Kif11* leads to early embryonic lethality, and signs of a proliferation defect are shown at embryonic day 2.5.[19,20] It has been previously demonstrated that EG5 localizes to spindle microtubules during mitosis and also contributes to the assembly of the bipolar spindle,[21] as well as the regulation of axonal outgrowth[22] and CNS development.[23] Several genes (*CENPJ, MCPH1, ASPM, CDK5RAP2, STIL, CEP152,* and *WDR62* [MIM 608393, 251200, 608716, 604804, 612703, 604321, and 604317, respectively]) mutated in recessively inherited microcephaly have products with roles in centrosome formation and spindle development. Our identification

of heterozygous *KIF11* mutations in dominant forms of microcephaly provides further evidence of the critical role of molecules involved in mitotic spindle function in CNS development. The observation of lymphedema and chorioretinopathy provides evidence of a role of EG5 in the development and maintenance of the lymphatic and retinal structures. It is currently unclear whether MLCRD and CDMMR result from disruption of the mitotic function of EG5 or from other roles of EG5 in the cell. The recently defined function of EG5 as a brake on microtubule activity as part of axonal turning[22] provides the basis for speculating that the dominant mutations observed in this study might disrupt the control of lymphatic development in a similar manner.

Our findings demonstrate a pleiotropic phenotypic expression of mutant *KIF11* alleles and show that MLCRD and CDMMR are allelic disorders. Beyond the observed variability of lymphatic and eye involvement, there is also a range in the severity of microcephaly. Microcephaly was primary (congenital) in all subjects, and there was some correlation between the degree of microcephaly and the severity of the learning disorder. Although all probands were chosen because they had microcephaly, there was marked intrafamilial variation (Table 1); one parent had a normal head circumference, mild learning difficulties, no lymphedema, and only a retinopathy associated with her diabetes (MLCRD02 I-1).

Chorioretinopathy was a highly specific finding in patients with *KIF11* mutations, but none of the patients had retinal folds or microphthalmia to suggest that the condition originally described by Jarmas et al.[5] might be nonallelic. A number of patients with additional abnormalities (e.g., thrombocytopenia and craniosynostosis) were not found to have *KIF11* mutations. It could be relevant for clinical differentiation that chorioretinopathy was not observed in any of the *KIF11*-mutation-negative subjects (Table S1), although numbers are too small for this to be a definitive observation.

The lymphedema in the mutation-positive subjects was present at birth and was restricted to both lower limbs and rarely extended above the knees. The dorsa of the feet were particularly affected and had small, dysplastic nails and deep interphalangeal creases. There were often large-caliber veins in the lower limbs. Clinically, the findings resemble those seen in Milroy disease (Figure 1D). The lymphedema was usually persistent but responded well to compression garments. Interestingly, there appeared to be some intrafamilial consistency among the clinical features regardless of the type of mutation, but such consistency is difficult to verify with such small numbers.

In conclusion, we have identified *KIF11* mutations that cause autosomal-dominant forms of microcephaly that are variably associated with congenital lymphedema and/or chorioretinopathy, demonstrating that MLCRD and CDMMR are allelic disorders. The extreme variability of the phenotype, even between individuals with the same *KIF11* mutation, suggests that there might be additional genetic or environmental factors that contribute to the extent of disruption. In addition, our findings also provide a substantial foundation for the existence of a link between EG5 and the development and maintenance of retinal and lymphatic structures. It remains to be elucidated how far the established mitotic function of EG5 can account for the different phenotypical aspects of MLCRD and CDMMR and whether at least some defects are consequences of a different role of EG5 during development.

Supplemental Data

Supplemental Data include three figures and six tables and can be found with this article online at http://www.cell.com/AJHG.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

1000 Genomes Project, http://www.1000genomes.org
Consensus Coding Sequence Project (CCDS) project, http://www.ncbi.nlm.nih.gov/projects/CCDS/
dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/

Human Splicing Finder, http://www.umd.be/HSF/
Online Mendelian Inheritance in man (OMIM), http://www.omim.org/
PolyPhen, http://genetics.bwh.harvard.edu/pph2/
SIFT, http://sift.jcvi.org/

Accession Numbers

The accession number for the *KIF11* sequence reported in this paper is NM_004523.

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