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Supplemental Data

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

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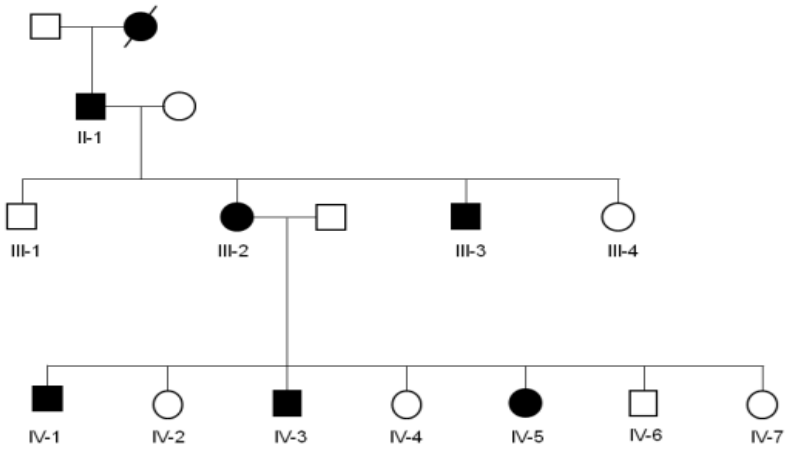

Figure S1. Pedigree Structure and Mutation Status for Family MLCRD11

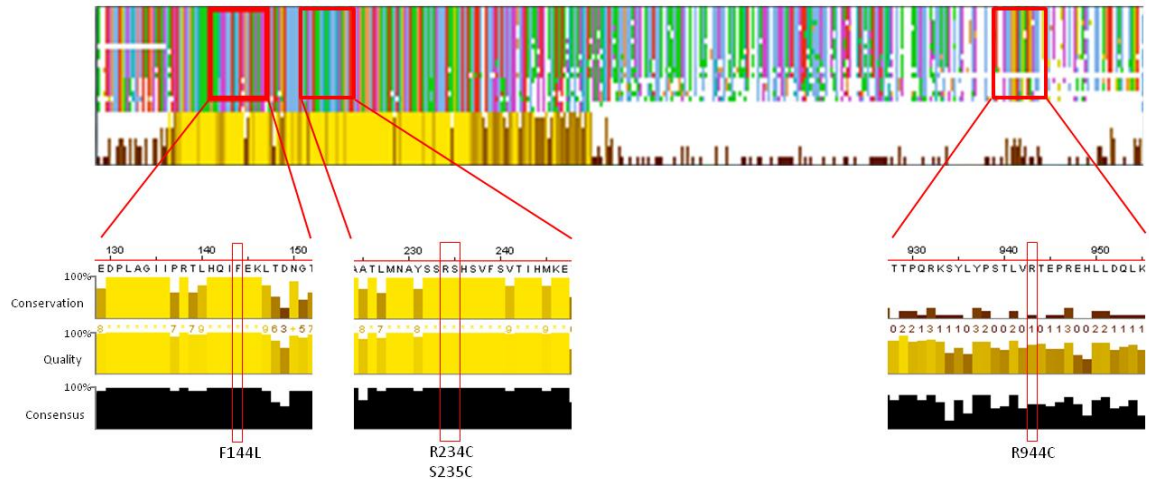

Figure S2. Conservation of Residues for the Four Missense Mutations

Top panel illustrates regions of conservation in *KIF11* across 18 species. Conserved residues are indicated by color. Lower panel zooms in on the regions of each of the missense mutations. Residues F144, R234C and S235C are highly conserved across all compared species and have PSIC scores of 2.551, 3.065 and 2.321 respectively, which indicate that the amino acid changes are likely to be deleterious to protein function. The R944C is conserved in fourteen out of the eighteen species included in the comparison and has a PSIC score of 2.42.

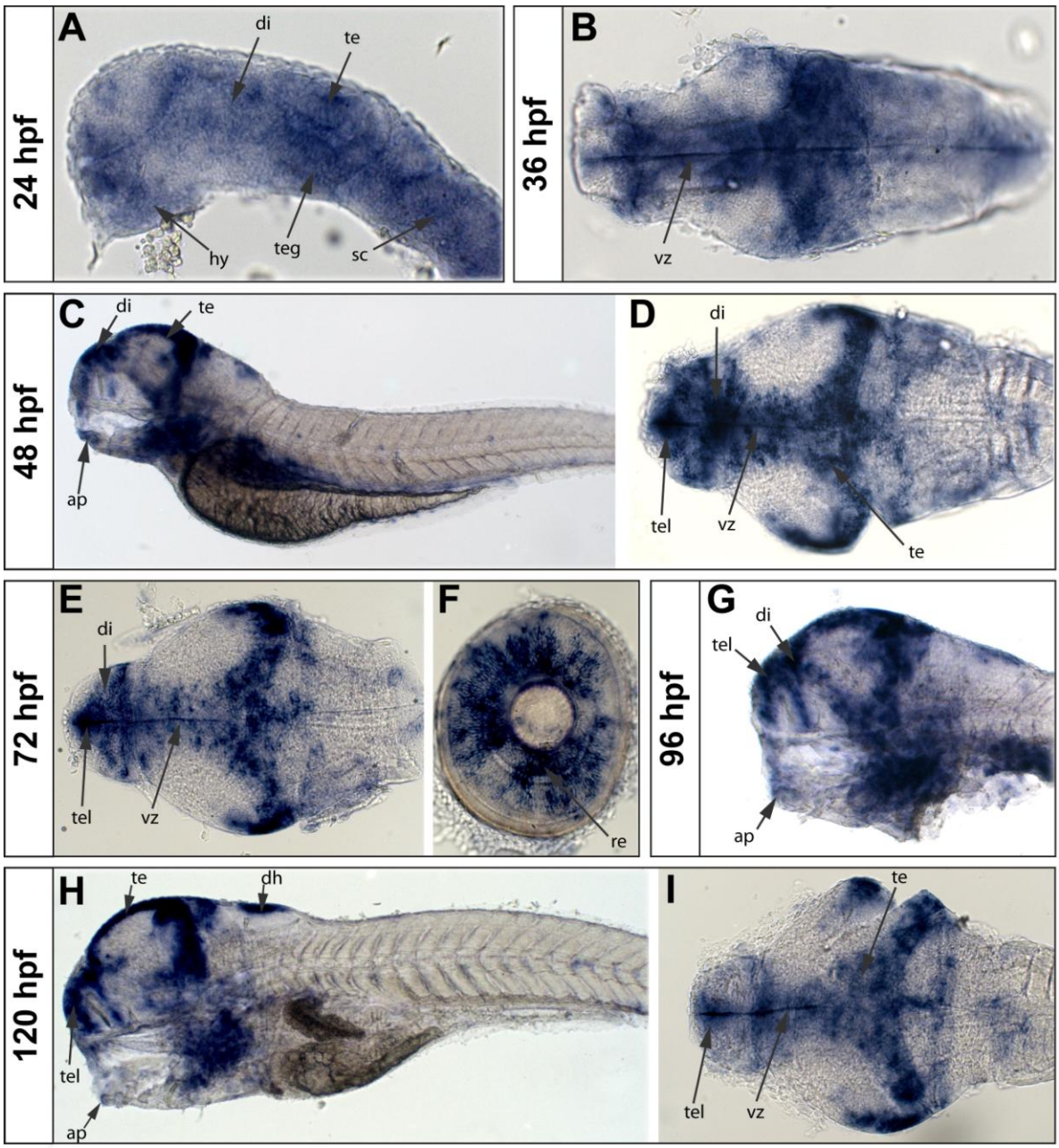

Figure S3. Zebrafish *kif11* Is Expressed in Tissues Associated with High Mitotic Activity

Whole mount in situ hybridization of albino zebrafish embryos at 24 hours post fertilization (A), 36 hpf (B), 48 hpf (C, D), 72 hpf (E, F), 96 hpf (G), and 120 hpf (H, I). Shown are lateral (A, C, F, G, H) and dorsal (B, D, E, I) views. Embryos were dissected and flat-mounted in glycerol for imaging. *kif11* mRNA is expressed in a highly dynamic fashion during embryonic development. Consistent with the mitotic role of *kif11*, the gene is strongly expressed in proliferating tissues of the embryo including various parts of the brain and the spinal cord after 24 hpf (A). From 36 hpf onwards, the brain specific expression pattern of *kif11* becomes more restricted and transcripts can continuously be detected within the ventricular zone of the brain (B-E, G-I). In addition, *kif11* mRNA is evident in a subset of retina cells. This expression seems to be confined to a proliferative region at the periphery of the retina, called the ciliary marginal zone (F). ap: anterior pitiuary, di: diencephalon, dh: dorsal hindbrain hy: hypothalamus, re: retina, sc: spinal cord, te: tectum, teg: tegmentum, tel: telencephalon, vz: ventricular zone.

Table S1. Clinical Observations in the Nine Subjects in whom No *KIF11* Coding Sequence Variant Was Identified

Pedigree	ID	Gender	Head circumference (SD)	Lymphedema	Eye abnormalities	Additional notes
MLCRD04	I-1[a]	female	-3.4	congenital, bilateral, lower limb	none	mild LD
MLCRD05	I-1[a]	male	-3.0	congenital, bilateral, lower limb	none	ASD, hypospadias, atypical dysmorphism
MLCRD13	I-1	female	-3.0	onset age 6, bilateral, lower limb	alternating convergent squint	mild LD
MLCRD14	I-1	male	-2.5	congenital, bilateral, lower limb	none	mild LD, thrombocytopenia, persistent warts
CDMMR06	I-1	female	by history	none	left retinal detachment	mild LD, ASD

MLCRD – microcephaly-lymphedema-chorioretinal dysplasia syndrome, CDMMR - chorioretinal dysplasia-microcephaly-mental retardation, LD - learning difficulties, ASD - atrial septal defect. Head Circumference - standard deviations from the mean of occipitofrontal head circumference, and corrected for age and sex.

[a] Individuals exome sequenced in primary analysis.

Table S2. Summary Statistics for Exome Sequencing - Mapping and Coverage

Sequenced Exomes	MLCRD01	MLCRD02	MLCRD03	MLCRD04	MLCRD05
Uniquely mapped reads	77649450	67741032	69344296	38731048	68231238
Uniquely mapped to target +/- 150bp reads	52443413	44210462	45807664	27879495	43115963
Uniquely mapped to target reads	40683900	33622720	35346690	21068627	32330469
CCDS bases with coverage >1	26964657	27079271	27036220	26952834	27065091
CCDS bases with coverage >5	25522583	25666954	25572227	24758173	25689190
CCDS bases with coverage >10	23888785	23972814	23850746	22021708	24016992
CCDS bases with coverage >20	21159028	20930306	20913016	17498285	20912346
Mean coverage	72.86	59.67	63.42	37.49	57.92

Total number of mapped reads and resulting coverage of the CCDS exome.

Table S3. Summary Statistics for Exome Sequencing - Variant Calling

	MLCRD01		MLCRD02		MLCRD03		MLCRD04		MLCRD05	
Variant type	known	novel	known	novel	known	novel	known	novel	known	novel
All variants	18320	322	18220	215	18431	182	18448	187	17834	204
heterozygous	11004	314	10705	210	11108	181	10905	183	10764	197
homozygous	7316	8	7515	5	7323	1	7543	4	7070	7
Indels	196	9	217	10	221	10	233	13	199	7
heterozygous	109	9	126	10	129	10	127	12	115	7
homozygous	87	0	91	0	92	0	106	1	84	0
3n indels	124	6	137	5	137	5	139	8	121	6
heterozygous	69	6	81	5	76	5	84	7	70	6
homozygous	55	0	56	0	61	0	55	1	51	0
Non 3n indels	72	3	80	5	84	5	94	5	78	1
heterozygous	40	3	45	5	53	5	43	5	45	1
homozygous	32	0	35	0	31	0	51	0	33	0
SNVs	18124	313	18003	205	18210	172	18215	174	17635	197
heterozygous	10895	305	10579	200	10979	171	10778	171	10649	190
homozygous	7229	8	7424	5	7231	1	7437	3	6986	7
Synonymous_SNVs	8409	98	8372	63	8398	51	8351	48	8196	75
Heterozygous	5035	98	4871	63	4940	51	4869	48	4894	75
Homozygous	3374	0	3501	0	3458	0	3482	0	3302	0
Nonsynonymous_SNVs	9715	215	9631	142	9812	121	9864	126	9439	122
heterozygous	5860	207	5708	137	6039	120	5909	123	5755	115
homozygous	3855	8	3923	5	3773	1	3955	3	3684	7
Splice site (10bp)	2163	30	2076	22	2152	21	2052	18	2060	18
heterozygous	1287	28	1195	19	1325	21	1205	17	1221	16
homozygous	876	2	881	3	827	0	847	1	839	2
Transition:Transversion ratio	3.1	2.32	3.07	2.58	3.09	2.14	2.98	2.42	3.1	3.1
heterozygous	3.21	2.36	3.05	2.59	3.07	2.12	2.97	2.43	3.07	3.15
homozygous	2.94	1.33	3.11	2	3.12	n/a	2.99	2	3.14	2

Numbers of variants of different classes identified by exome sequencing in the five sequenced cases. SNV - single nucleotide variant, 3n - multiples of three nucleotides.

Table S4. Numbers of Candidate Genes Highlighted by Exome Sequencing

Any x of 5 individuals	1	2	3	4	5
Genes with heterozygous NS/SS/I variants	8047	5191	3139	1442	436
Not in dbSNP, 1000G or 250 control exomes	698	33	1	0	0

NS - nonsynonymous, SS - splice site, I –indels.

Table S5. Primers Used for Sanger Sequencing and In Situ Hybridization

Primers used for amplification and sequence analysis of human *KIF11* covering the coding regions and associated splice sites.

Exon	Forward	Reverse	Amplicon length (bp)
1	GCCAGAGTACCGGGTAGAGA	tattgaacagaacgcggaga	386
2	tctccatcaccaacaatgtg	tcagcccactagaagccatc	389
3	gatggcttctagtgggctga	aaagaaacatgcacccatatga	620
4	tcatatgggtgcatgtttcttt	gacagaggccctcaaacaag	209
5	acagactgttgtaaatgacttctctaa	caaagcctcacacattaaaattc	413
6	GTTTGATGATCCCCGTAACAA	gtgacccacataccctgacc	426
7	ttcttgatatctcctgtctgatgtt	tgtattaaatcgccagcaaaa	394
8	tgggtgattagctttgtagtgg	tgtgagtcactgtgtccatcc	500
9	aaaggttgggcatagtggtc	ccttccatgggcaatttaac	360
10	ggtttttcttgggaagtcca	gcactggatataaagattttac	432
11	acttgggtatcaagtgatggtga	gcttttcacataacagtaaatatc	257
12	tgttaacctaccgggtaacttctt	ccagtgagaaaaacatggatga	474
13	ggctggaaaatttactagttct	tgcatggtagagagtattggtga	493
14	ttgctttgtagtgagccagttc	aaagctcaggaagaaagaatagca	448
15 + 16	tcatttagaaaggtttacagaagtgg	tcaagtcatatcaaatcatcctaaaac	496
17	cacttccactgctttcctctg	cagccaggtttttcaatgtct	493
18	ccagactgtccacgttcagat	catttgttttgaaatctattcttagcc	482
19	agcactactcatgccactgc	ttccagttttcccattttgag	525
20	caggtagcaagactgatcctca	cggggtaagattgaggggta	399
21	accatgggcattgtgttatactc	tgctcaccacgaacaatacc	295
22	gttggctcaaacttgtgctg	atctatacacaaggctcaagtt	365

Primers used to generate antisense probe for in-situ hybridization

kif11_probe_forward	cctccatcaatctggaggaa
kif11_probe_revese	CATTAACCCTCACTAAAGGGAAtgcaaacactgaaccagctc

Table S6. Intronic Mutations in *KIF11* that Lead to Splicing Defects

Mutation	WT CV	Mutant CV	CV variation (%)
c.699-2A>G	79.05	50.1	-39.62
c.2547+2T>C	83.59	56.75	-32.1

Prediction of the effect of the splice site mutations on splicing using HSF (Human Splicing Finder v.2.4.1.). CV - consensus value. A negative CV is predictive of the mutation will induce exon skipping.