Mutations in *KIF11* cause autosomal dominant microcephaly variably associated with congenital lymphedema and chorioretinopathy

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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 (MLCRD) 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 impact on the 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 central nervous system development. Moreover, identification of *KIF11* mutations in patients with chorioretinopathy and lymphedema suggests a previously undescribed involvement of EG5 in the development and maintenance of retinal and lymphatic structures.

There is substantial phenotypic overlap between microcephaly, primary lymphedema and chorioretinal dysplasia syndrome (MLCRD, MIM 152950) and chorioretinal dysplasia, microcephaly and mental retardation syndrome (CDMMR, MIM 156590).[1] Both have been observed to segregate with autosomal dominant inheritance and present with an overlapping, yet variable, spectrum of central nervous system (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] (Figure 1A,B). 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, typically confined to the dorsum of the feet (Figure 1D) and lymphoscintigraphy reveals the absence of uptake from the web spaces between the toes (Figure 1E). We have identified causative variants for this complex disease and show that MLCRD and CDMMR can be caused by mutation of *KIF11*, suggesting that both syndromes should be considered as a single entity with variable clinical features but a unified molecular basis.

Whole exome sequencing has proved to be a successful approach to the identification of causative mutations in primary lymphoedema.[6,7,8] We therefore sought to establish the molecular genetic basis of MLCRD with whole exome sequencing of five unrelated probands with microcephaly and lymphedema (individuals MLCRD01:II-2, MLCRD02:II-2, MLCRD03:III-2, MLCRD04:I-1 and MLCRD05:I-1 in Table 1 and Table S1). Subjects for this study were recruited through genetic, lymphovascular and ophthalmic clinics in Europe. Informed consent was obtained from all subjects. All affected individuals and family members underwent a detailed physical examination. Ethical approval for this study was obtained from the South West London Research Ethics Committee (REC Ref: 05/Q0803/257 'Analysis of genes and their functions in patients with primary lymphoedema').

Whole exome capture was performed by in-solution hybridisation followed by massively parallel sequencing using the SureSelect All Exon CCDS Target Enrichment System (Agilent)[9] and sequencing on a Genome AnalyserIIx (Illumina) with 76bp 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 was calculated with custom scripts and the BedTools package.[10] Over 5.1 gigabases 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 insertion/deletion 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] Filtering of variants for novelty was performed by comparison to dbSNP132 and 1000 Genomes SNP calls (December 2010) and variants identified in 250 control exomes, primarily of European origin, 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, requiring 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 a prior expectation of genetic heterogeneity among these five cases revealed *KIF11* as the only gene to harbour previously unobserved variants in three of the five individuals (Table S4). All three mutant alleles are predicted to lead to premature termination of the *KIF11* protein product; a nonsense variant (p.R387X), a single nucleotide deletion (p.I1006LfsX62) and a dinucleotide deletion (p.L347EfsX8) (Table 1). All were confirmed by Sanger sequencing (primer sequences are listed in Table S5). Sequencing in the two subjects, in which exome sequencing did not reveal novel *KIF11* variants, confirmed the wild-type coding sequence. Our findings indicated 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 kindreds, including a large multi-generational pedigree (MLCRD11, Figure S1). Sequencing revealed a further seven independent and novel *KIF11* variants in a heterozygous state in those probands: three frameshift insertion 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 10 identified novel *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 a spectrum of eye and lymphatic abnormalities (Table 1). In total we identified *KIF11* mutations in ten of the 14 MLCRD kindreds examined.

Within the extended MLCRD families, we observed individuals with heterozygous mutations in *KIF11,* in whom there was no evidence of lymphatic involvement (Table 1). Given the considerable and recognised overlap between the two conditions, this led us to address the hypothesis that MLCRD and CDMMR are allelic disorders. Sequencing of *KIF11* in a cohort of six independent kindreds in which microcephaly and eye abnormalities had been observed in the absence of lymphedema revealed five independent novel variants; two missense substitutions and three variants predicted to lead to premature termination of the protein product - including one nonsense mutation (p.R387X) that we had previously identified in pedigree MLCRD01 (Table 1). In total we identified 27 carriers of 14 novel mutant *KIF11* alleles in 15

independent kindreds. 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 kindreds, from which four probands had previously been diagnosed with MLCRD and one with CDMMR (Table S1). These cases may represent *KIF11* alleles comprising non-coding or undetected coding variants. They may represent phenocopies of MLCRD and CDMMR, or be explained by locus heterogeneity. We were unable to investigate the latter as 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 anti-parallel fashion so that the resulting molecule possesses two motor domains and two non-motor tails at each end of a central stalk. The contact of EG5 with microtubules is established through the C-terminal tails, while the subsequent sliding of the anti-parallel microtubules is driven by the motor domains.[17] Seven of the identified mutant alleles are predicted to lead to premature termination of the protein; two nonsense and five frameshift, inducing insertions and deletions. 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 1056 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 splicing of the 5kb 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.F144L, p.R234C and p.S235C) are located within the motor domain[15] and the fourth (p.R944C) 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, with signs of a proliferation defect 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 regulating axonal outgrowth[22] and CNS development.[23] Several genes mutated in recessively inherited microcephaly have products with roles in centrosome formation and spindle development: *CENPJ, MCPH1, ASPM, CDK5RAP2, STIL, CEP152,* and *WDR62* (MIM: 608393, 251200, 608716, 604804, 612703, 604321 and 604317). Our identification of heterozygous

mutations in *KIF11* 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 for a previously undescribed role of EG5 in the development and maintenance of the lymphatic and retinal structures. It is currently unclear if MLCRD and CDMMR result from disruption of the mitotic function of EG5 or 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 to speculate that the dominant mutations observed in this study may 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. The microcephaly was primary (congenital) in all cases and there was some correlation between the degree of microcephaly and the severity of the learning disorder. Whilst all probands were ascertained by the presence of microcephaly, there was marked intra-familial variation (Table 1) including one parent with 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 mutations in *KIF11* but none of the patients had retinal folds or microphthalmia suggesting that the condition originally described by Jarmas et al[5] might be non-allelic. A number of patients with additional abnormalities (e.g. thrombocytopenia, craniosynostosis) were not found to have mutations in *KIF11*. It could be of relevance for clinical differentiation that chorioretinopathy was not observed in any of the *KIF11* mutation negative cases (Table S1), although numbers are too small for this to be a definitive observation.

The lymphedema in the mutation positive cases was present at birth, restricted to both lower limbs and rarely extended above the knees. The dorsum of the feet was particularly involved with small, dysplastic nails and deep inter-phalangeal creases. There were often large calibre 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 intra-familial consistency of the clinical features regardless of the type of mutation but this is difficult to verify with such small numbers.

In conclusion, we have identified *KIF11* mutations causative of 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 may 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 previously undescribed 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 for EG5 during development.

Supplemental Data

Supplemental data include 6 tables and 3 figures.

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Web Resources

Online Mendelian Inheritance in man (OMIM), http://www.omim.org/

Consensus Coding Sequence Project (CCDS) project, http://www.ncbi.nlm.nih.gov/projects/CCDS/

dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/

1000 Genomes Project, http://www.1000genomes.org

Human Splicing Finder, http://www.umd.be/HSF/

SIFT, http://sift.jcvi.org/

PolyPhen, http://genetics.bwh.harvard.edu/pph2/

Accession Numbers

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

References

1. Fryns, J.P., Smeets, E., Van den Berghe, H. (1995). On the nosology of the 'primary true microcephaly, chorioretinal dysplasia, lymphoedema' association. Clin. Genet. *48*, 131-133.
2. Leung, A.K.C. (1985). Dominantly inherited syndrome of microcephaly and congenital lymphedema. Clin. Genet. *27*, 611-612.
3. Warburg, M., and Heuer, H.E. (1994). Chorioretinal dysplasia-microcephaly-mental retardation syndrome (letter). Am. J. Med. Genet. *52*, 117.
4. Vasudevan, P.C., Garcia-Minaur, S., Botella, M.P., Perez-Aytes, A., Shannon, N.L., Quarrell, O.W.J. (2005). Microcephaly-lymphoedema-chorioretinal dysplasia: three cases to delineate the facial phenotype and review of the literature. Clin. Dysmorphol. *14*, 109-116.
5. Jarmas, A.L., Weaver, D.D., Ellis, F.D., Davis, A. (1981). Microcephaly, microphthalmia, falciform retinal folds, and blindness: a new syndrome. Am. J. Dis. Child. *135*, 930-933.
6. Ostergaard, P., Simpson, M.A., Brice, G., Mansour, S., Connell, F.C., Onoufriadis, A., Child, A.H., Hwang, J., Kalidas, K., Mortimer, P.S., et al. (2011). Rapid identification of mutations in GJC2 in primary lymphedema using whole exome sequencing combined with linkage analysis with delineation of the phenotype. J. Med. Genet. doi:10.1136/jmg.2010.085563.
7. Ostergaard, P., Simpson, M.A., Jeffery, S. (2011). Massively parallel sequencing and identification of genes for primary lymphedema: a perfect fit. Clin. Genet. *80*, 110-116.
8. Ostergaard, P., Simpson, M.A., Connell, F.C., Steward, C.G., Brice, G., Woollard, W.J., Dafou, D., Kilo, T., Smithson, S., Lunt, P., et al. (2011). Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). Nat. Genet. *43*, 929-931.
9. Coffey, AJ., Kokocinski, F., Calafato, MS., Scott, CE., Palta, P., Drury, E., Joyce, CJ., LeProust, EM., Harrow, J., Hunt, S., et al. (2011). The GENCODE exome: sequencing the complete human exome. Eur. J. Hum. Genet. *19*, 827-831.
10. Quinlan, AR., and Hall, IM., (2010). BEDTools: a flexible suite of utilities for comparing genomic feature. Bioinformatics *26*, 841-842.
11. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., and 1000 Genome Project Data Processing Subgroup (2009). The sequence alignment/Map format and SAMtools. Bioinformatics, *25*, 2078-2079.
12. Simpson, M.A., Irving, M.D., Asilmaz, E., Gray, M.J., Dafou, D., Elmslie, F.V., Mansour, S., Holder, S.E., Brain, C.E., Burton, B.K., et al. (2011). Mutations in *NOTCH2* cause Hajdu-Cheney syndrome, a disorder of severe and progressive bone loss. Nat. Genet. *43*, 303-305.
13. Li, K., and Stockwell, TB., (2010). Variant Classifier: A hierarchical variant classifier for annotated genomes. BMC Res. Notes, *3*, 191.

14. Valentine M.T., Fordyce P.M., Krzysiak T.C., Gilbert, S.P., Block, S.M. (2006). Individual dimers of the mitotic kinesin motor Eg5 step processively and support substantial loads *in vitro*. Nat. Cell. Biol. *8*, 470-476.
15. Sawin, K.E., and Mitchison, T.J. (1995). Mutations in the kinesin-like protein Eg5 disrupting localization to the mitotic spindle. Proc. Natl. Acad. Sci. USA *92*, 4289-4293.
16. Rapley, J., Nicolas, M., Groen, A., Regue, L., Bertran, MT., Caelles, C., Avruch J., Roig J. (2008). The NIMA-family kinase Nek6 phosphorylates the kinesin Eg5 at a novel site necessary for mitotic spindle formation. J. Cell Sci. *121*, 3912-3921.
17. Weinger, J.S., Minhua Q., Yang, G., Kapoor, T.M. (2011). A nonmotor microtubule binding site in Kinesin-5 is required for filament crosslinking and sliding. Curr. Biol. *21*,154-160.
18. Desmet, FO., Hamroun, D., Lalande, M., Collod-Beroud, G., Claustres, M., Beroud C. (2009). Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucl. Acids. Res. *37*, e67.
19. Castillo, A., and Justice, M.J. (2007). The kinesin related motor protein, Eg5, is essential for maintenance of the pre-implantation embryogenesis. Biochem. Biophys. Res. Commun. *57*, 694-699.
20. Chauviere, M., Kress, C., Kress, M. (2008). Disruption of the mitotic kinesin Eg5 gene (*Knsl1*) results in early embryonic lethality. Biochem. Biophys. Res. Commun. *372*, 513-519.
21. Sawin, K.E., LeCuellec, K., Phillippe, M., Mitchison, T.J. (1992). Mitotic spindle organization by a plus-end-directed microtubule motor. Nature *359*, 540-543.
22. Myers, K.A. and Bass, P.W. (2007). Kinesin-5 regulates the growth of the axon by acting as a brake on its microtubule array. J. Cell Biol. *178*, 1081-1091.
23. Ferhat, L., Cook, C., Chauviere, M., Harper M., Kress, M., Lyons, G.E., Baas, P.W. (1998) Expression of the mitotic motor protein Eg5 in postmitotic neurons: Implications for neuronal development. J. Neurosci. *18*, 7822-7835.
24. Eventon-Friedman, S., Singer, A., Shinwell, E.S. (2009). Microcephaly, lymphedema, chorioretinopathy and atrial septal defect: a case report and review of the literature. Acta Paediatr. *98*, 758-762.

FIGURE TITLES AND LEGENDS

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 facies in (A) and (B) are characteristic with upslanting palpebral fissures, a broad nose with rounded tip, long philtrum with a thin upper lip, prominent chin and ears.

(C) Composite color photograph of left fundus in patient CDMMR05 II-1 showing focal areas of peripheral chorioretinal atrophy.

(D) Bilateral congenital lower limb primary lymphedema in cases with MLCRD, involving the dorsum of the feet with pitting edema, deep inter-phalangeal creases, small dysplastic nails and wide calibre veins.

(E) Comparison of lower limb lymphoscintigraphy at 2 hours in patient MLCRD01 II-2 and an unaffected control, showing no significant main tract filling suggestive of initial lymphatic vessel dysfunction.

Figure 2. Location of the identified mutations in *KIF11*

Upper panel: Mutations indicated with respect to the genomic organisation of the *KIF11* gene.

Lower panel: KIF11 protein domain structure excluding the two identified splice site mutations.

Table 1. Clinical and genetic findings in *KIF11* mutation positive patients.

Pedigree	ID	Gender	Head Circumference	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.R387X
	II-2[a]	Female	-7.2	congenital, bilateral, lower limb	none	mild LD, dysmorphic features	c.1159C>T	10	p.R387X
MLCRD02	I-1	Female	-1.6	none	diabetic retinopathy	mild LD	c.3016delA	21	p.I1006LfsX62
	II-2[a]	Female	-4.0	congenital, bilateral, lower limb	none	mild dysmorphic features	c.3016delA	21	p.I1006LfsX62
MLCRD03	II-1	Male	-2.0	none	none	mild LD	c.1039_1040delCT	9	p.L347EfsX8
	III-2[a]	Female	-7.5	congenital, bilateral, lower limb plus pleural effusions	hypermetropic astigmatism, chorioretinopathy	mild LD	c.1039_1040delCT	9	p.L347EfsX8
MLCRD06	I-1	Male	-4.3	congenital, bilateral, lower limb	none	low birth weight, failure to thrive	c.432T>G	5	p.F144L
MLCRD07	I-1[b]	Male	-2.3	congenital, bilateral, lower limb	bilateral chorioretinopathy	mild LD, ASD, myoclonic epilepsy	c.2830C>T (*de novo*)	20	p.R944C
MLCRD08	I-1	Male	-3.5	none	none	none	c.1425_1426delinsAAA	12	p.V476NfsX2
	II-1	Male	-5.5	congenital, mild, bilateral, lower limb	myopia	moderate LD	c.1425_1426delinsAAA	12	p.V476NfsX2
MLCRD09	I-1	Female	-3.0	none	none	none	c.1592delA	13	p.Q531RfsX8
	II-1[c]	Male	-4.0	congenital, bilateral, lower limb	bilateral chorioretinopathy	ASD, dysmorphic features	c.1592delA	13	p.Q531RfsX8
MLCRD10	I-1	Male	-5.5	congenital, mild, bilateral, lower limb mild (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, 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, lower limb plus mild in hands	none	moderate LD, hypospadias	c.2547+2T>C	18/19	donor splice site
	IV-3	Male	-3.7	congenital, mild, bilateral, 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
MLCRD12	I-1	Male	-6.4	congenital, mild, bilateral, lower limb	bilateral chorioretinopathy	moderate LD	c.1963_1964dupAA	15	H656SfsX8
CDMMR01	II-1	Female	-5.0	adult onset, post traumatic mild edema,	chorioretinopathy	mild LD	c.1159C>T(*de novo*)	10	p.R387X
	III-1	Male	-5.5	none	hypermetropic astigmatism, chorioretinopathy	moderate LD	c.1159C>T	10	p.R387X
CDMMR02	I-1	Female	low by history	none	bilateral chorioretinopathy	none	c.704C>G	7	p.S235C
	II-1	Female	low by history	none	bilateral chorioretinopathy	moderate LD	c.704C>G	7	p.S235C
CDMMR03	I-1	Male	-3.4	none	no vision in right eye (retinal detachment), peripheral retinal atrophy in left eye	mild LD	c.2304_2305delCA (*de novo*)	18	p.H768QfsX7
CDMMR04	I-1	Male	-5.1	none	chorioretinopathy, nystagmus, exotropia	moderate LD	c.700C>T	7	p.R234C
CDMMR05	II-1	Female	-3.9	none	chorioretinopathy	mild LD	c.1804C>T	14	Q602X
	II-2	Female	-6.1	none	hypermetropic astigmatism	none	c.1804C>T	14	Q602X

MLCRD - microcephaly lymphedema chorioretinal dysplasia syndrome, CDMMR - chorioretinal dysplasia microcephaly mental retardation, LD - learning difficulties, ASD - atrial septal defect.

[a] Individuals exome sequenced in primary analysis.

[b] Case description in Vasudevan et al (2005).[4]

[c] Case description in Eventon-Friedman et al (2009).[24]

Head circumference measured as occipitofrontal head circumference and corrected for age and sex.

Figure 1

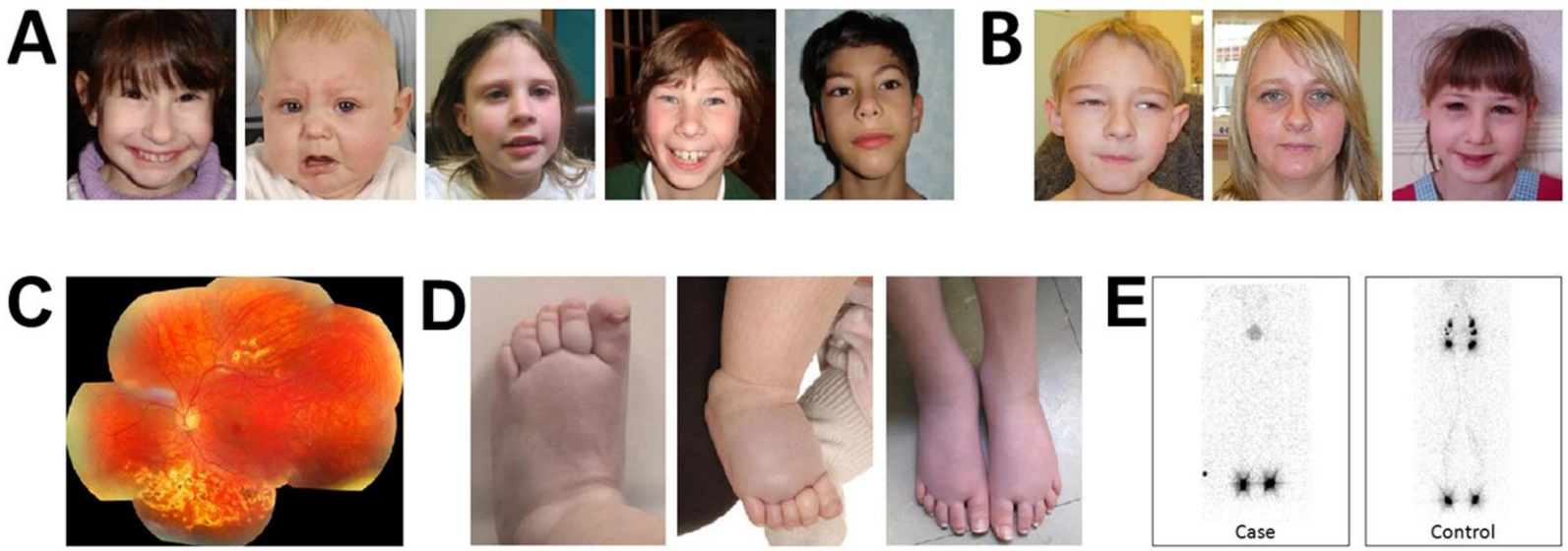
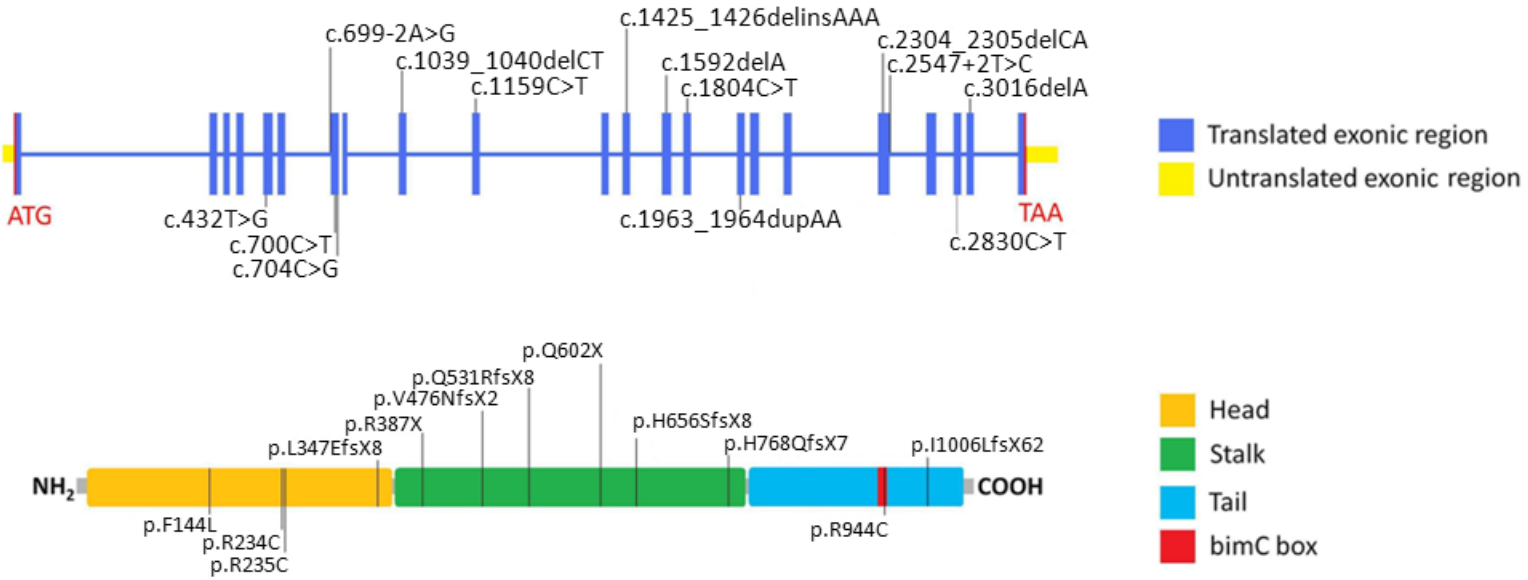


Figure 2


c.699-2A>G
c.1039_1040delCT
c.1159C>T
c.1425_1426delinsAAA
c.1592delA
c.1804C>T
c.2304_2305delCA
c.2547+2T>C
c.3016delA
ATG
c.432T>G
c.700C>T
c.704C>G
c.1963_1964dupAA
TAA
c.2830C>T
Translated exonic region
Untranslated exonic region
p.Q602X
p.Q531RfsX8
p.V476NfsX2
p.R387X
p.L347EfsX8
p.H656SfsX8
p.H768QfsX7
p.I1006LfsX62
NH2
COOH
p.F144L
p.R234C
p.R235C
p.R944C
Head
Stalk
Tail
bimC box