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Clinical and molecular characterization of familial exudative vitreoretinopathy associated with microcephaly

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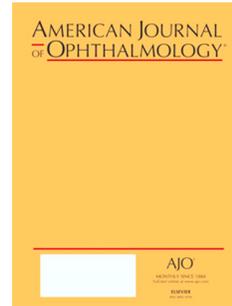
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Abstract

Purpose

Familial exudative vitreoretinopathy (FEVR) is a rare finding in patients with genetic forms of microcephaly. This study documents the detailed phenotype and expands the range of genetic heterogeneity.

Design

Retrospective case-series

Methods

Twelve patients (ten families) with a diagnosis of FEVR and microcephaly were ascertained from pediatric genetic eye clinics and underwent full clinical assessment including retinal imaging. Molecular investigations included candidate gene Sanger sequencing, whole-exome sequencing (WES) and whole-genome sequencing (WGS).

Results

All patients had reduced vision and nystagmus. Six were legally blind.

Two probands carried bi-allelic *LRP5* variants, both presenting with bilateral retinal folds. A novel homozygous splice variant, and two missense variants were identified. Subsequent bone density measurement, identified osteoporosis in one proband.

Four families had heterozygous *KIF11* variants. Two probands had a retinal fold in one eye and chorioretinal atrophy in the other; the other two had bilateral retinal folds. Four heterozygous variants were found, including two large deletions not identified on Sanger sequencing or WES.

Finally, a family of two children with learning difficulties, abnormal peripheral retinal vasculogenesis and rod-cone dystrophy were investigated. They were found to have bi-allelic splicing variants in *TUBGCP6*.

Three families remain unsolved following WES and WGS.

Conclusions

Molecular diagnosis has been achieved in seven of ten families investigated including a previously unrecognized association with *LRP5*. WGS enabled molecular diagnosis in three families after prior negative Sanger sequencing of the causative gene. This has enabled patient-specific care with targeted investigations and accurate family counseling.

Clinical and molecular characterization of familial exudative vitreoretinopathy associated with microcephaly

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Introduction

Familial exudative vitreoretinopathy (FEVR) is a disorder of abnormal vascular development with manifestations including incomplete peripheral vasculogenesis, retinal folds and total retinal detachment.[1] Disease severity is highly variable within families.[1] FEVR is genetically heterogeneous with associated genes including *LRP5* (autosomal dominant, AD; autosomal recessive, AR), *FZD4* (AD, AR), *NDP* (X-linked recessive), *TSPAN12* (AD, AR), *ZNF408* (AD), and *CTNNB1* (AD).[2-10] The majority of these gene products are involved in the canonical Wnt signaling pathway, a transmembrane pathway that activates β-catenin/TCF transcription, involved in retinal development/angiogenesis.[11, 12] The exception, *ZNF408* is a zinc finger transcription factor identified in three families to date.[2, 9]

Rarely, FEVR has been reported in association with microcephaly.[13-18] *KIF11* has been reported as one cause of this phenotype complex, allelic with microcephaly with or without chorioretinopathy, lymphedema or mental retardation (MCLMR, MIM#152950).[19-21] In addition, one patient with bi-allelic variants in *TUBGCP6* has been reported with microcephaly, retinopathy and retinal folds; one patient with a heterozygous nonsense variant in *CTNNB1* with retinal detachment, microcephaly and limb abnormalities; and one patient with bi-allelic variants in *TUBGCP4* and microcephaly and a retinal fold.[16-18] The aim of this study was to characterize a series of twelve children from ten families with microcephaly and FEVR and investigate the extent of any genetic heterogeneity. Additionally, the potential benefits of a molecular diagnosis in informing both genetic counseling and relevant systemic investigations were studied.

Methods

Ascertainment of patients

This was a retrospective case series of patients all recruited from the pediatric genetic clinics at Moorfields Eye Hospital. The study protocol adhered to the tenets of the Declaration of Helsinki and received approval from the local ethics committee. Written, informed consent was obtained from all participants prior to their inclusion in this study, with parental written consent provided on behalf of the children involved in this study.

Clinical assessment

In total, ten probands and two affected siblings were ascertained for detailed phenotyping. All patients had retinal imaging and systemic assessment including growth parameters by a pediatrician. Microcephaly was defined as an occipitofrontal circumference (OFC) two standard deviations (SD) or more below the mean (≤ -2 SD) for gender, age and ethnicity.[22] Based on the growth charts used within UK practice, this corresponded to ≤ the 2nd centile.[23] When necessary, patients were referred for further specialist opinion, for instance with *LRP5* variants known to be associated with reduced bone mass.[24] Available parents were examined and fundus fluorescein angiography (FFA) performed if indicated.

Color fundus imaging and FFA were obtained by 35-degree fundus imaging (Topcon Great Britain Ltd, Berkshire, UK) or ultra-widefield (200 degrees) confocal scanning laser imaging (Optos plc, Dunfermline, UK) or by RetCam imaging (Clarity Medical Systems Inc, California, USA). RetCam was used for examination under general anesthesia using a corneal contact probe with a field of approximately 130 degrees.

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FFA was performed with an intravenous injection of fluorescein. Fundus autofluorescence (FAF) imaging and spectral domain optical coherence tomography (OCT) scans were performed using Spectralis (Heidelberg Engineering Ltd, Heidelberg, Germany). Full field electroretinography was performed using gold foil electrodes to incorporate the ISCEV standards but in infants and young children skin electrodes were used with modified protocols.[25, 26]

Molecular investigations

In total, eight probands had candidate gene Sanger sequencing including *LRP5* (n=7), *KIF11* (n=7), *FZD4* (n=1), *TSPAN12* (n=1), *NDP* (n=3), and *TUBGCP6* (n=1) with *NR2E3* also screened in six probands following ERG findings suggestive of enhanced S-cone syndrome. This identified causative variants in four probands (*LRP5* n=2, *TUBGCP6* n=1, *KIF11* n=1). Identified variants were segregated with available family members. Variant nomenclature was assigned in accordance with GenBank Accession numbers NM_002335.3 for *LRP5,* NM_004523.3 for *KIF11*, and NM_020431.3 for *TUBGCP6* with nucleotide position 1 corresponding to the A of the ATG initiation codon.

Five families underwent WES analysis, four through collaborators at St George's University of London (UK) using SureSelect All Exon kit v.4.0 (Agilent Technologies) and one at AROS Applied Biotechnology (Aarhus, Denmark) using Agilent's SureSelectXT Human All Exon version 5 exon capture. All samples were sequenced on a HiSeq2000 platform (Illumina Inc) as previously described.[27-29] Further study was performed on five families with WGS on proband and available parents, four performed at Edinburgh Genomics and one as part of the 100,000 genomes project as previously described.[30] Examination of the sequencing reads was performed with Integrative Genomics Viewer (IGV).[31]

Variants were identified as novel if not previously reported in the literature and if absent from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/); NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (http://evs.gs.washington.edu/EVS/); 1000 genomes project (http://www.1000genomes.org/); the Exome Aggregation Consortium (ExAC) Cambridge, MA (http://exac.broadinstitute.org); and the Genome aggregation Database (gnomAD, http://gnomad.broadinstitute.org).[32] The likely pathogenicity of novel missense variants was assessed using the predictive algorithms of 'Sorting Intolerant from Tolerant' (SIFT, http://sift.jcvi.org) and Polymorphism Phenotyping v2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2).[33, 34] Where relevant, potential splice site disruption was assessed using Splice Site Prediction by Neural Network (http://www.fruitfly.org/seq_tools/splice.html).[35]

RNA analysis was performed on a blood sample from patient 8. Lymphoblastoid cell lines were established by EBV transformation and cultured in RPMI, 15% FCS 100 U/mL penicillin, and 100 µg/mL streptomycin. Total RNA was isolated and RT-PCR was performed using standard protocols across the exons flanking the intron harbouring putative splice altering variants in *TUBGCP6* (TUBGCP6_ex10F AGAAGGACTGTGCCGTCTAC; TUBGCP6_ex12R GCCATCCGTTCTGACATCTG; TUBGCP6_ex19F CTCATGAAGCGCTCCATCAC; TUBGCP6_ex20R TGCAGCTCCACGAAGAAGTA). Resulting PCR amplicons were electrophoresed to determine intron inclusion and some samples were subcloned for direct sequencing.

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Results

Twelve patients (8 male, 4 female) from ten families were investigated (Table 1, Figure 1). All had FEVR of variable severity with visual impairment and nystagmus. Visual acuity was most reduced for patients with retinal folds and detachment, and least reduced for chorioretinal atrophy and abnormal peripheral retinal vascularisation (Table 2). Age ranged from 4 months to 17 years at last review with duration of follow up ranging from 0 to 95 months (mean 48 months). OFC ranged from below the 0.4th centile to the 2nd centile. Developmental delay was present in eight patients.

Patient 1 (GC16755) presented with eye poking at 2 months of age and was found to have a total detachment in the left eye and a retinal fold with superotemporal tractional retinal detachment and avascular nasal retina in the right eye. *NDP* screen was negative. Microcephaly was identified at 9 years of age in the ophthalmology clinic. Autozygosity mapping identified a region of homozygosity at the EVR1 locus encompassing both *FZD4* and *LRP5*.[7] Sanger sequencing of *FZD4* and *LRP5* identified a novel homozygous variant c.4112-3C>G in *LRP5* in the proband predicted to abolish a splice acceptor site. Segregation found this variant to be homozygous in his affected sister, and heterozygous in the unaffected sister and parents. Due to the molecular diagnosis, bone density was being monitored with a normal DEXA scan to date. The younger affected sister was not under our care but was reported to have unilateral peripheral non-perfusion only.

Patient 2 (GC19160) presented in early infancy with nystagmus, poor vision and bilateral retinal folds. Microcephaly was observed at birth. Sanger sequencing identified a heterozygous novel missense variant in *LRP5*, c.3914G>A (p.Cys1305Tyr), predicted damaging *in silico* (Sift 0, Polyphen2 1.0). This was also identified in the mother who had a normal dilated fundus examination but subtle abnormal peripheral retinal perfusion on Optos widefield FFA. A bone density scan in the proband age 5 years was abnormal, with a Z-score of -2.1 indicating risk of osteoporosis.[36] Further family segregation found the proband's sister, whose dilated retinal examination was normal, was not a carrier of the variant. Further retinal screening for this at-risk family member was therefore not performed. Due to the disparity between proband and maternal retinal findings, whole genome sequencing was performed on the proband and mother. This identified a further missense variant in *LRP5*, c.2254C>T (p.Arg752Trp) that was not present in the mother and therefore likely to be in trans. Paternal DNA was unavailable. This variant has previously been reported in a compound heterozygous state in a patient with osteoporosis pseudoglioma syndrome and in a heterozygous state in the patient's mother who had low bone density.[37] In light of the presence of a second missense *LRP5* variant, we reviewed the initial Sanger sequencing data and could not detect this second variant despite having detected the first from the same DNA sample. It is possible that a SNP within the original primer led to amplification of the wildtype strand only. Independent NHS accredited laboratory (Manchester Centre for Genomic Medicine) verification of both variants was performed with Sanger sequencing and confirmed both variants in the proband and the p.Cys1305Tyr variant only in the mother.

Patient 3 (GC21489) presented in infancy with nystagmus, poor vision and microcephaly. Examination under anesthesia at 6 months of age identified bilateral retinal folds, and flash ERG evidence of generalized rod and cone dysfunction. The ERGs recorded with corneal electrodes to stroboscopic non-Ganzfeld flashes,

revealed abnormal features similar to those associated with enhanced S-cone syndrome. The photopic 30Hz flicker ERGs were delayed and smaller than photopic single flash ERG a-waves and the latter were delayed and simplified with a low b:a ratio, similar in shape to larger responses obtained following a short period of dark adaptation.[38] This finding prompted screening of *NR2E3* in this patient without identifying any pathogenic variants. WES was subsequently performed on the proband and both parents identifying a de novo, nonsense variant, c.247C>T (p.Arg83*) in *KIF11* that has been reported twice in dbSNP (rs1064796738).

Patient 4 (GC20377) presented in infancy with reduced vision and was found to have a retinal fold in the left eye with areas of chorioretinal atrophy in the right eye. Microcephaly was diagnosed in the ophthalmology clinic at 6 years of age. The ERGs, recorded at 6 years of age with corneal electrodes to non-Ganzfeld flashes under anesthesia, indicated generalized rod and cone system dysfunction. Screening of *KIF11* by Sanger sequencing identified a novel, heterozygous frameshift deletion, c.2910_2914del (p.Glu970Aspfs*17).

Patient 5 (GC18797) with bilateral retinal folds underwent Sanger sequencing of candidate genes, including *KIF11,* which was negative. Subsequent trio WGS of proband and parents identified a de novo 762bp deletion spanning exon 4 of *KIF11* chr10:94,366,561_94,367,322del (Figure 3).

Patient 6 (GC19303) had a retinal fold and peripheral chorioretinal atrophy in the right eye and focal chorioretinal atrophy in the left. International standard full-field ERGs, performed at the age of 7 years, were undetectable in the right eye and showed evidence of moderately severe generalized rod and cone photoreceptor dysfunction in the left. His father also had microcephaly and incomplete peripheral vascularisation. Screening of the most likely candidate gene, *KIF11*, was negative by Sanger sequencing and by WES. However, subsequent WGS identified a heterozygous 14kb deletion spanning exon 1 of *KIF11* (chr10:94,345,322_94,359,501del) in both proband and father (Figure 3).

Patients 7 and 8 (GC21033) were siblings with learning difficulties, microcephaly and rod-cone dysfunction on ERG. Dilated fundus examination identified abnormal peripheral retinal vasculogenesis with peripheral scarred ridges in both eyes (Figure 1). FFA in patient 8 identified vascular malformations anterior to this ridge. Based on the combination of microcephaly, learning difficulties and rod-cone dysfunction, candidate gene sequencing was performed by colleagues in Edinburgh. They identified bi-allelic variants in *TUBGCP6*, c.2066-6A>G (reported in 15 of 246266 alleles on gnomAD, no homozygotes) and c.4485-21A>C (novel) predicted to both create cryptic splice sites and out of frame transcripts. It was confirmed by RNA analysis that the intronic mutation c.2066-6A>G causes the insertion of 5 intronic nucleotides and the sequence change at RNA level is therefore r.2065_2066ins2066-1_2066-5, which will lead to a truncated protein, p.(D689Vfs*2) (Figure 4A). The c.4485-21A>C variant was also shown to generate alternative spliced product (Figure 4B). Direct sequencing of PCR product was unavailable for this latter variant.

Four patients (three families) remain unsolved. Patient 9 (GC19713) had a heterozygous variant identified on WES in *LRP5*, c.2116G>A (p.Gly706Arg) found in 1 in 31348 alleles on gnomAD. Segregation found this variant to be homozygous in her unaffected mother who had a normal dilated fundus examination and an OFC on

the 9th centile. Given the lack of phenotype in the mother it was thought to be an unlikely cause of the proband's condition. Proband and mother have been recruited to a national whole genome study (100,000 genomes), paternal DNA has been unavailable. Paternal head circumference is reportedly small but unconfirmed due to non-attendance. Patient 10 (GC19208) with right peripheral non-perfusion and telangiectasia and left total retinal detachment, underwent WES and subsequently trio WGS without any probable causative variants found in known genes. Patients 11 and 12 (GC20924) are brothers. Patient 11 had bilateral retinal folds and patient 12 right total retinal detachment and left retinal fold with a tractional detachment that progressed to complete detachment and loss of vision. Both carry a paternally inherited copy number gain at 4q22.2 (0.68Mb), identified by array comparative genomic hybridization. Within this region are seven genes (*HPGDS, PDLIM5, SMARCAD1, BMPR1B, UNC5C, PDHA2,* and *STPG2*) one of which, bone morphogenetic protein receptor type 1B (*BMPR1B)* has been shown in a mouse model to be required for normal ventral ganglion cell axon targeting to the optic nerve head as well as controlling inner retinal apoptosis.[39] Given the normal fundus examination and FFA in the father, and the lack of known ophthalmic phenotype with any of these genes, it is unlikely that this copy number gain is pathogenic although non-penetrance remains possible. WES in patient 11 and WGS in both affected patients and their father have not found any probable causative variants to date.

Discussion

In this series of ten families with FEVR and microcephaly, seven (70%) have been molecularly solved. Previously published solved rates for isolated FEVR is typically 50% and in this series the addition of WGS in the unsolved families has aided the molecular diagnosis.[2] Two families were identified to have *LRP5* related disease and this has not previously been recognized as a potential cause of FEVR and microcephaly.

All patients presented in early childhood with nystagmus and visual impairment. Fundus features were variable, in keeping with the known spectrum of FEVR, ranging from incomplete peripheral vasculogenesis, to retinal folds, to retinal detachment.[1] In addition, there were two patients with unilateral retinal folds and contralateral chorioretinal atrophy which has been seldom reported in the literature.[15, 20, 40] Visual acuity correlated with fundus features, being most severe in those patients with retinal folds or detachment.

ERG was recorded in six patients, four with *KIF11* variants and two with *TUBGCP6* variants*,* and identified generalized retinal dysfunction. The *TUBGCP6* patients had evidence of rod and cone dysfunction and retinal degeneration on retinal imaging, with loss of photoreceptor structure on OCT and abnormal autofluorescence consistent with an underlying retinal dystrophy. For the other patients, detailed retinal imaging was not possible due to the severity of the retinal abnormalities precluding precise structure-function comparison. It is likely that in the majority of patients with FEVR, there would be measurable photoreceptor dysfunction secondary to abnormal retinal development, but this hypothesis and the determination of whether the natural history is stationary or progressive needs to be further investigated with interval ERG. There is some evidence of disease progression in *KIF11* related retinal dystrophy and chorioretinopathy based on visual acuity and serial imaging.[41, 42] Previously reported ERG findings indicate a range of severity of rod and cone dysfunction on ERG including normal ERG.[41, 43] A detailed characterization of

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MCMLR due to *KIF11* variants in six patients found rod and cone dysfunction, with abnormal macular function in those with available pattern ERG results.[44]

FEVR with microcephaly has been reported in association with *KIF11,* and *TUBGCP6,* but has not been previously reported with *LRP5.*[18, 20] Microcephaly in this series ranged from the 2nd centile (-2 SD) to <<0.4th centile (<-5SD). In two patients it was only observed after measurements in the ophthalmology clinic. One limitation of our study is the lack of complete data on parental OFC and peripheral retinal examinations, which may be helpful in determining the mode of inheritance. This could facilitate filtering whole genome data from the unsolved families.

LRP5 (MIM 603506) was initially found to cause osteoporosis-pseudoglioma syndrome, a recessive disorder of severe retinal dysplasia and juvenile osteoporosis, and subsequently associated with dominant high bone density from missense variants located within the N-terminal YWTD-EGF domain.[24, 45, 46] *LRP5* has also been associated with both dominant and recessive forms of FEVR, with variants distributed throughout the gene, and with evidence of non-penetrance in dominant disease.[7, 47] Of the two families identified with *LRP5* variants in this study, both have recessive disease. For patient two, the second missense variant was only identified after WGS. For this family, the molecular diagnosis has enabled detection of reduced bone density in the proband and negative molecular screening in the younger sister, which avoided repeat dilated examinations. There appears to be a spectrum of disease associated with recessive *LRP5* variants, with variable ophthalmic and systemic features. As yet, a clear genotype-phenotype correlation is not apparent.[24]

Kinesin family member 11 (*KIF11* MIM 148760) encodes Eg5, a microtubule motor involved in mitosis.[48] Eg5 also has non-mitotic functions such as involvement in endothelial cell lineage proliferation, secretory protein transportation and protein translation.[49-51] Its exact role in retinal development has not yet been elucidated although Kif11 has been found to localize to the modified primary cilium of photoreceptors in murine retina.[41] Variants were first identified in association with MCLMR and later with microcephaly and FEVR and with retinal dystrophy although not all patients have microcephaly.[19-22, 41] Variants have been reported without obvious mutational hotspots and without apparent difference in variant distribution between patients with retinal folds and those with chorioretinal atrophy.[19, 52] At least 40% of variants arise de novo. Reduced penetrance has been reported in carrier parents.[19] Patient 3 in this report has a de novo nonsense variant and patient 4 a novel frameshift variant with segregation unavailable. Patients 5 and 6 have single exon spanning deletions, undetected by Sanger sequencing as well as WES in the latter patient. For patient 3's family, the molecular diagnosis identifying de novo disease has allowed accurate genetic counseling on the risk of recurrence in future children.

Pathogenic variants in *TUBGCP6* (MIM 610053) were first reported in a patient with microcephaly and chorioretinopathy, without additional details of the ophthalmic examination.[53] Subsequently, disease-causing variants have been identified in four patients, all with extreme microcephaly (-7.2 to -11.1 SD), short stature (-2.3 to -3.45 SD), and retinopathy; with one patient additionally described to have retinal folds.[18] In one patient, the ERG was not recordable. The siblings in our series had abnormal retinal vascularization, identified in conjunction with a rod-cone dystrophy, but with less severe microcephaly, and without short stature. The variants in our cases create cryptic splice sites leading to out of frame transcripts.

Our series of patients illustrates several key clinical and molecular considerations. In patients presenting with FEVR, systemic evaluation is recommended to identify those with associated findings, as microcephaly may not be immediately apparent. Variants in *LRP5* have not previously been reported with FEVR and microcephaly. This molecular diagnosis necessitates bone density investigations. WGS is particularly useful for detecting structural rearrangements often missed with Sanger or WES, and identified causality in *KIF11*-associated disease. Molecular diagnosis is especially relevant in de novo *KIF11* related disease where there is a low risk of recurrence for future siblings but for the proband, significant future risk to progeny. Our study also illustrates that whilst the majority of FEVR with microcephaly has been solved, there remain further molecular mechanisms yet to be identified, comprising novel genes or non-coding variants affecting known genes. Further interrogation of WGS data from this cohort may reveal candidate variants in the future.

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Conflict of Interest: There are no conflicts of interest for any author.

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Figure captions

Figure 1: Pedigrees and variant segregation for 10 FEVR families

Black circles and squares indicate affected individuals
M, mutation; WT, wild-type; Pt, patient

Figure 2: Retinal imaging in FEVR with microcephaly

A: Patient 2 age 9 months, LE RetCam colour and FFA images demonstrating retinal fold from optic disc to ora serrata with limited retinal vascularisation elsewhere

B: Patient 2's mother, LE Optos colour and FFA images, no obvious abnormalities on colour, but abnormal peripheral vascularisation on FFA

C: Patient 4 age 7 years**,** RetCam images, RE inferior, circumscribed chorioretinal atrophy lesions, LE retinal fold

D: Patient 7 age 16 years, LE colour montage, mid-peripheral RPE hypopigmentation, peripheral scarred ridge; FAF imaging foveal and macular rings of increased autofluorescence with reduced signal in-between; OCT, outer retinal atrophy with centrally preserved inner segment ellipsoid band

E: Patient 8 age 13 years, RE Optos colour and FFA demonstrating macular atrophy, peripheral scarred ridge with lack of vascularisation anterior to it and vascular malformations

F: Patient 11 age 13 years, Optos colour images of RE retinal fold with exudative detachment, LE smaller retinal fold with macular atrophy

LE, left eye; RE, right eye; FFA, fundus fluorescein angiogram; RPE, retinal pigment epithelium

Figure 3: Whole genome reads using IGV from patients 5 and 6 demonstrating heterozygous deletions

PB, proband; F, father; M, mother.

Figure 4: Consequences of *TUBGCP6* splicing variants on RNA transcripts

A) Sequence electropherograms of the exon 11–exon 12 junction of control and c.2066-6A>G variant *TUBGCP6* transcripts generated by RT-PCR in lymphoblastoid cell lines (LCL). The c.2066-6A>G variant led to inclusion of 5bp of intron 11.

B) Gel electrophoresis of *TUBGCP6* transcripts generated by RT-PCR using primers in exon 19 and exon 20. Alternative splice products were demonstrated in patient 8's LCL with the c.4485-21A>C variant. Control-RT and c.4485-21A>C-RT lanes were experiments performed without reverse transcriptase (RT) to demonstrate no genomic DNA contamination.

Table 1: Clinical summary for patients with FEVR and microcephaly

Patient, gender, family	Gene	Age last review	Latest VA logMAR (Snellen)	Fundus features	Growth parameters, centile			Other findings
					OFC	Ht	Wt	
Pt 1 (M) GC16755	LRP5	9 yrs	R CF L NPL	RE retinal fold and tractional RD, nasally avascular, LE total RD with no fundal view	$\ll0.4^{th}$	$0.4^{th}-2^{nd}$	$2^{nd}-9^{th}$	Normal DEXA scan Normal MRI brain
Pt 2 (F) GC19160	LRP5	8 yrs	R 1.4 (20/500) L 1.5 (20/640)	BE retinal folds with partial retinal vascular preservation on FFA	$<0.4^{th}$	95th	95th	Reduced bone mass on DEXA scan, maternal OFC 95th centile
Pt 3 (M) GC21489	KIF11	4 mths	Fixing and following	BE retinal folds with partial retinal vascular preservation on FFA. Rod-cone dysfunction on ERG	$<0.4^{th}$	9th	2nd	Normal MRI brain
Pt 4 (M) GC20377	KIF11	10 yrs	R 0.48 (20/60) L 1.4 (20/500)	RE chorioretinopathy with focal atrophy, LE retinal fold. Rod-cone dysfunction	<0.4th	N/A	N/A	Developmental delay
Pt 5 (M) GC18797	KIF11	4 yrs	BEO 1.0 (20/200)	BE retinal folds. Reduced ERG responses R>L, tested age 6 weeks	2nd	N/A	N/A	Normal MRI brain
Pt 6 (M) GC19303	KIF11	14 yrs	R NPL L 0.80 (20/125)	RE retinal fold with peripheral chorioretinal atrophy and pigment, LE focal chorioretinopathy ERG R undetectable L rod-cone dysfunction	$\ll0.4^{th}$	25th	50th	Normal MRI brain Father microcephalic
Pt 7 (M) GC21033	TUBGCP6	17 yrs	R 0.56 (20/70) L 0.52 (20/63)	Loss of photoreceptors outside of fovea on OCT, lack of peripheral vascularisation with fibrotic ridge. Rod-cone dysfunction	$\ll0.4^{th}$	2nd	$9-25^{th}$	Moderate learning difficulties
Pt 8 (F) GC21033	TUBGCP6	14 yrs	R&L 0.7 (20/100)	Lack of peripheral vascularisation on FFA with fibrotic ridge. Rod-cone dysfunction	$\ll0.4^{th}$	$9-25^{th}$	$2^{nd}-9^{th}$	Moderate learning difficulties
Pt 9 (F) GC19713	WES negative	6 yrs	R 0.7 (20/100) L NPL	RE Large temporal retinal fold, LE Large retinal fold with partial RD	$\ll0.4^{th}$	0.4-2nd	25-50th	Global developmental delay Maternal OFC 9th centile
Pt 10 (F) GC19208	WES and WGS negative	11 yrs	R 1.0 (20/200) L NPL	RE macular atrophy, temporal peripheral non-perfusion, telangiectasia with shallow exudative RD, LE total RD	$<2^{nd}$	N/A	N/A	Developmental delay, learning difficulties
Pt 11 (M) GC20924	WES and WGS negative	15 yrs	R 1.3 (20/400) L 1.25 (20/320)	RE retinal fold with exudate, LE retinal fold with macular scar	0.4^{th}	N/A	N/A	Parents normal FFAs, paternal OFC 91st centile R early cataract
Pt 12 (M) GC20924	WGS negative	10 yrs	R NPL L NPL	RE total RD no fundal view, LE retinal fold, tractional RD and exudate progressing to total RD	$<0.4^{th}$	N/A	N/A	Developmental delay

VA, visual acuity; OFC, occipitofrontal circumference; Ht, height; wt, weight; R, right; L, left; CF, counting fingers; NPL, no perception of light; RD, retinal detachment; DEXA, dual energy X-ray absorptiometry; FFA, fundus fluorescein angiogram; BEO, both eyes open; BE, both eyes; WES, whole-exome sequencing; WGS, whole-genome sequencing

Table 2: Visual acuity based on retinal phenotype

Retinal abnormality	Number of eyes	Vision logMAR (Snellen)
Chorioretinal atrophy	2	0.48-0.80 (20/60-20/125)
Peripheral non-perfusion	5	0.52-1.0 (20/63-20/200)
Retinal fold	13	0.7-NPL (20/100-NPL)
Retinal detachment	4	NPL

NPL, no perception of light

1

Patient 5

Patient 6

GC16755
LRP5
c.4112-3C>G
novel

M/WT WT/M

M/M   M/WT M/M
Pt 1

GC19160
LRP5
c.3914G>A (p.Cys1305Tyr)
c.2254C>T (p.Arg752Trp)
First novel

M1/WT

M1/M2 WT/?
Pt 2

GC21489
KIF11
c.247C>T
(p.Arg83*)

WT/WT WT/WT

M/WT
Pt 3

GC20377
KIF11
c.2910_2914del
(p.Glu970Aspfs*17)
novel

M/WT
Pt 4

GC18797
KIF11
chr10:94366561_
94367322del

WT/WT   WT/WT

M/WT
Pt 5

GC19303
KIF11
chr10:94345322_
94359501del

M1/WT

M1/WT
Pt 6

GC21033
TUBGCP6
c.2066-6A>G
c.4485-21A>C
Second novel

M1/WT

M1/M2 M1/M2
Pt 7   Pt 8

GC19713
WES negative

Pt 9

GC19208
WGS negative

Pt 10

GC20924
WGS negative

Pt 11 Pt 12

A

Exon 11 → ← Exon 12

T G A G T G A C C G G C

Control

Exon 11 → Intron 11 ← Exon 12

T G A G T G T G T A G A C C G G C

c.2066-6A>G

B

Control

c.4485-21A>C

Control - RT

c.4485-21A>C - RT

bp

1000
800
600
500
400
300
200
100

Highlights

All patients had reduced vision and nystagmus and half were legally blind

Retinal findings ranged from peripheral non-perfusion to total retinal detachment

Systemic associations included low bone density and learning difficulties

Variants in *LRP5*, *KIF11*, and *TUBGCP6* were identified in seven of ten families