A splice-site variant in *FLVCR1* produces retinitis pigmentosa without posterior column ataxia

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Abstract

FLVCR1 (feline leukaemia virus subgroup c receptor 1) is a transmembrane protein involved in the trafficking of intracellular heme. Homozygous variants in *FLVCR1* have been described in association with a clinical syndrome of posterior column ataxia with retinitis pigmentosa (PCARP). Here we describe a patient with non-syndromic retinitis pigmentosa homozygous for a splice-site variant in *FLVCR1* (c.1092+5G>A) without evidence of posterior column ataxia or cerebellar degeneration. We suggest an association between intronic splice-site variants in *FLVCR1* and the absence of posterior column degeneration and suggest an hypothesis to explain this observation. Should this association be proven, it would provide valuable prognostic information for patients. Retinal degeneration appears to be the sole clinical manifestation of this *FLVCR1* variant; gene therapy approaches using an adeno-associated viral vector with sub-retinal delivery may therefore represent a therapeutic approach to halting retinal degeneration in this patient group.

Key words

FLVCR1; feline leukaemia virus subgroup c receptor 1; retinitis pigmentosa; PCARP; posterior column ataxia with retinitis pigmentosa

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Retinitis pigmentosa (RP) is the most common monogenic cause of blindness. Variants in over 100 genes have been associated with the retinitis pigmentosa phenotype. Homozygous variants in *FLVCR1* (feline leukaemia virus subgroup c receptor 1), which encodes a transmembrane heme transporter, have been described in association with a clinical syndrome of posterior column ataxia with retinitis pigmentosa (PCARP), [1-4] a syndrome first described in 1997-8.[5, 6] Herein we describe a patient with a splice-site (intronic) variant in *FLVCR1* who exhibited retinitis pigmentosa without posterior column degeneration (therefore, without PCARP). In addition, a novel missense variant was identified in *FLVCR1* in the same patient, although with uncertain pathogenicity.[7] We suggest a link between intronic splice-site variants in *FLVCR1* and the absence of posterior column degeneration and suggest an hypothesis to explain this observation.

The affected 32-year-old female was referred to a specialist retinal genetics clinic for an opinion on the management of bilateral cataracts and cystoid macular oedema associated with retinitis pigmentosa. She was first aware of visual symptoms aged 5, with nyctalopia noted during her teenage years. There was no reported family history of retinitis pigmentosa. She has one older sister who is unaffected and her parents are unrelated. Prior to this presentation, her genetic diagnosis was uncharacterised. She had no symptoms of ataxia, and preserved light touch and vibration sensation in her legs.

On examination, her visual acuity was 20/200 in her right eye and 20/100 in her left. She had bilateral posterior subcapsular cataracts, more prominent in the right eye than the left. Retinal examination revealed advanced mid-peripheral reticular pigmentary changes consistent with retinitis pigmentosa bilaterally **(Figure 1)**. Fundus autofluorescence imaging revealed widespread patchy hypoautofluoresence in the mid

periphery in both eyes with cystoid macular oedema. Optical coherence tomography imaging confirmed cystoid macular oedema in the both eyes **(Figure 1)**.

She underwent right phacoemulsification cataract surgery with 1 milligram of intravitreal triamcinolone. One month post-operatively, her vision had improved to 20/120 OD and the cystoid macular oedema had regressed. However, 5 months following surgery, cystoid macular oedema was identified in both eyes. She underwent left cataract surgery with intravitreal dexamethasone implant (700 micrograms) with improvement in vision to 20/120 and resolution of cystoid macular oedema. She underwent right intravitreal dexamethasone implant, followed by bilateral YAG laser capsulotomy procedures. Her vision was measured at 20/120 right eye and 20/80 left eye, two years following surgery. Two further recurrences of cystoid macular oedema were treated successfully with topical dexamethasone 0.1% four times daily.

Next generation sequencing of a panel of 111 genes associated with RP or an RP-like phenotype identified a homozygous splice-site variant in *FLVCR1*, (c.1092+5G>A; genomic-co-ordinate Chr1.hg19:g.213,056,785) **(Figure 2a)**, and a novel heterozygous missense variant in *FLVCR1* (c.1285T>C, p.Phe429Leu genomic-co-ordinate Chr1.hg19:g.213,061,321) **(Figure 2b)**. In order to confirm true homozygosity for *FLVCR1* variants, Sanger sequencing was performed to detect the presence of the detected variants in all immediate family members **(Figure 2a-b)**. The patient's mother and father were confirmed to be heterozygous for *FLVCR1* variant c.1092+5G>A. The father was shown to have a complex *FLVCR1* allele, with the splice-site variant *in cis* with the missense variant c.1285T>C p.Phe429Leu. Her sister did not have either familial *FLVCR1* variant. All first-degree relatives were visually asymptomatic with a normal

ophthalmic examination. These results are consistent with a diagnosis of autosomal recessive *FLVCR1*-related retinal degeneration.

FLVCR1 (feline leukaemia virus subgroup c receptor 1) is a gene located on the long arm of chromosome 1 (1q.32.3). It encodes a 555 amino acid protein with 12 transmembrane domains that functions to export cytoplasmic heme.[4] Free heme is toxic to cells; *Flvcr*1-/- mice die in mid-gestation.[8] FLVCR1 is expressed widely, although most prominently in retina followed by posterior columns of the spinal cord, cerebellum and other central nervous system tissues.[2] Neurodegeneration has been attributed to impaired heme export from neuronal cells in the retina and posterior columns where FLVCR1 expression is highest resulting in retinitis pigmentosa with posterior column ataxia (PCARP) in some patients with homozygous *FLVCR1* variants. Survival of patients with homozygous *FLVCR1* variants into late adulthood suggests sufficient FLVCR1 function to maintain heme transport for erythropoiesis.

To date eight variants in *FLVCR1* have been reported to cause retinitis pigmentosa, some of which also result in a syndrome of PCARP **(Table 1)**.[1-3, 7, 9, 10] The reported patient is homozygous for a previously reported *FLVCR1* variant (c.1092+5G>A) situated within the consensus splice donor site. Functional work in blood has shown skipping of exon 4 resulting in a frameshift deletion of 68 base pairs and the introduction of a premature termination codon in the mRNA.[7] The c.1092+5G>A variant has been reported in 16 of 23,168 European individuals (0.07%) and in 5 of 7,023 South Asian individuals (0.07%) in the Exome Aggregation (ExAC). Splice-site variants in *FLVCR1* are likely to result in reduced protein function since exon skipping results in a frameshift deletion and a premature stop codon in the mRNA, which is subsequently likely to be targeted for

nonsense mediated decay.[7] Two individuals within the same study were reported to have this variant (c.1092+5G>A), one of whom was homozygous and the other a compound heterozygote **(Table 1)**. Neither patient (aged 9 and 34) in this reported series demonstrated posterior column degeneration or ataxia, which typically manifests in the second decade of life.[7] Null *FLVCR1* variants are embryonically lethal.[8] It is likely therefore that all variants reported **(Table 1)** are likely to result in the translation of some functional FLVCR1 protein.

Some splice-site variants in *FLVCR1* have been reported in patients with retinitis pigmentosa in the absence of posterior column degeneration (c.1092+5G>A; **(Table 1))**.[7] Splice-site *FLVCR1* variants may result in the translation of some fully functional FLVCR1 protein product which is sufficient for tissues in which FLVCR1 is expressed at a lower level (posterior columns and cerebellum), but which is insufficient to protect against the toxic effects of excess intracellular heme in tissues which express FLVCR1 highly (retina). Whilst the *FLVCR1* 1092+5 G>A mutation has been reported before as a cause of retinitis pigmentosa (RP) in the absence of posterior column ataxia,[7] the effects of deep splice site base changes are difficult to predict, particularly when the base change is a transition (in this case between purines). Whilst the canonical 5′GU-AG 3′ sequences are almost 100% conserved, the 5'+5 splice donor position whilst usually G, may also be A.[11] This makes the interpretation of the variant difficult, and in this case particularly challenging, since the *FLVCR1* mutations are normally associated with posterior column ataxia.[1-3, 6] The authors of a previous report describing the *FLVCR-1* 1092+5 G>A variant in a patient with RP in the absence of posterior column ataxia acknowledge that it is difficult to comment on the whether the variant described is associated with the unusual clinical phenotype. These data from our case provide

additional independent evidence that this novel phenotype does indeed exist. It is important to verify this fact because the ataxia develops later. Furthermore, the absence of ataxia in the context of this *FLVCR-1* variant will help guide counselling for affected patients.

The heterozygous missense variant in *FLVCR1* (c.1285T>C, p.Phe429Leu) is of uncertain clinical significance. It appears sufficient to prevent the RP phenotype because there is no evidence of haploinsufficency in the unaffected heterozygous father, and some FLVCR1 protein is produced in the proband to prevent the onset of ataxia. This almost certainly indicates that the two copies of the *FLVCR1* gene in this patient are heterozygous, despite the 1092+5 G>A mutation being homozygous. This provides further evidence that the splice site variant (1092+5 G>A) is likely to be disease causing, and not from elsewhere in the gene that is linked to this position.

Several human clinical trials have demonstrated short-term safety and efficacy of gene therapy in patients with inherited retinal dystrophies (such as choroideremia), using an adeno-associated viral (AAV) vector.[12, 13] X-linked retinitis pigmentosa secondary to RPGR variants are currently being evaluated in human clinical trials. *FLVCR1* is an ideal therapeutic target for gene therapy. The small size of the gene (2.6kb) is suitable for AAV vector encoding, an approach shown in phase 1 human clinical trials to target photoreceptor degeneration.[14] Furthermore, widespread expression of FLVCR1 will permit experimental *ex vivo* testing of the AAV vector in human fibroblasts of affected patients, supporting the development of *FLVCR1* gene therapy.

PCARP involves central nervous system degeneration, particularly of the dorsal columns of the spinal cord.[1, 2, 4, 15] AAV vector based gene therapy has demonstrated promising results in mouse models of central nervous system degeneration.[16] AAV vectors delivered directly into the cerebrospinal fluid space have shown efficacy at expressing green fluorescent protein within the central nervous system in mouse models.[17] Moreover, the AAV-B1 capsid has demonstrated a favourable transduction profile with widespread gene transfer demonstrated *in vivo* throughout the central nervous system.[18] Further optimisation of AAV capsid variants and tissue-specific promoters through *in vivo* studies of gene therapy in neurodegenerative disease may reveal whether gene replacement targeting the dorsal columns is possible.

The mechanism by which variants in *FLVCR1* result in retinal degeneration is unclear. Accumulation of iron has been suggested as a mechanism of retinal degeneration; transferrin is endogenously secreted by retinal cells and investigated as an intravitreal neuroprotective strategy.[19] Systemic iron chelation has been demonstrated protective against light-induced and iron-induced retinal degeneration.[20, 21] Intravenous iron administration has been associated with the development of age-related macular degeneration reported in humans, and further characterised in mouse models.[22, 23] Genetic causes of iron accumulation have been described in association with retinitis pigmentosa as part of a spectrum of central nervous system manifestations.[19] Impaired heme transport in *FLVCR1* homozygotes is likely to result in increased intracellular iron within the neurosensory retina, and consequent retinal degeneration. Demonstrating iron accumulation within the retina of patients with *FLVCR1* variants may facilitate investigation into the effect of local and/or systemic iron chelation therapy to reduce the rate of retinal and posterior column degeneration in this patient group.

The development of a mouse model of PCARP with missense *FLVCR1* variants may allow further investigation of *FLVCR1* gene therapy applied to the retina and spinal cord. Identification of *FLVCR1* related retinitis pigmentosa early in the clinical course may permit a therapeutic opportunity to slow or halt photoreceptor degeneration. Symptoms of ataxia begin in the third decade in PCARP: this represents a therapeutic window to treat the CNS with gene therapy before the onset of posterior column degeneration.

Method

Enrichment for *FLVCR1* was achieved as part of a customised HaloPlex enrichment system kit (Agilent Technologies) designed to capture the coding exons and 10bp of the flanking introns of 111retinal genes. HaloPlex reactions were prepared as per manufacturer's instructions. Libraries were pooled into batches of 14 and sequenced on an Illumina MiSeq instrument (Illumina) using a MiSeq v3 kit as per manufacturer's instructions. Reads were aligned using BWA[24] and variants called using Platypus.[25] All variants identified by next generation sequencing were confirmed by Sanger sequencing.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Figures

Table 1. FLVCR-1 variants reported. Each row represents an individual patient reported in the literature.

Figure 1. Retinal imaging studies. **(a&b)** Colour photographs of right and left macula, **(c&d)** widefield fundus autofluorescence imaging (55 degrees) demonstrating symmetrical, widespread patchy hypoautofluorescence in the mid-peripheral retina, **(e&f)** fundus autofluorescence (30 degrees) imaging of both maculae demonstrating likely cystoid macular oedema, **(g&h)** optical coherence imaging demonstrating bilateral cystoid macular oedema, worse on the right than the left. All images were taken with Spectralis, Heidelberg Engineering, Heidelberg, Germany.

Figure 2. (a) Sanger sequencing of proband, mother, father and sister. The father and mother are both heterozygous for the *FLVCR1* c.1092+5G>A variant. The sister is unaffected. **(b)** Sanger sequencing demonstrating the c.1285T>C p.Phe429Leu variant in the father. Individual genotypes are as follows: Proband's alleles (c.1092+5A)/(c.1092+5A) and (c.1285C)/WT; father's alleles: (c.1092+5A)/WT and (c.1285C)/WT; mother's alleles: (c.1092+5A)/WT and WT/WT; sister's alleles: WT/WT and WT/WT.

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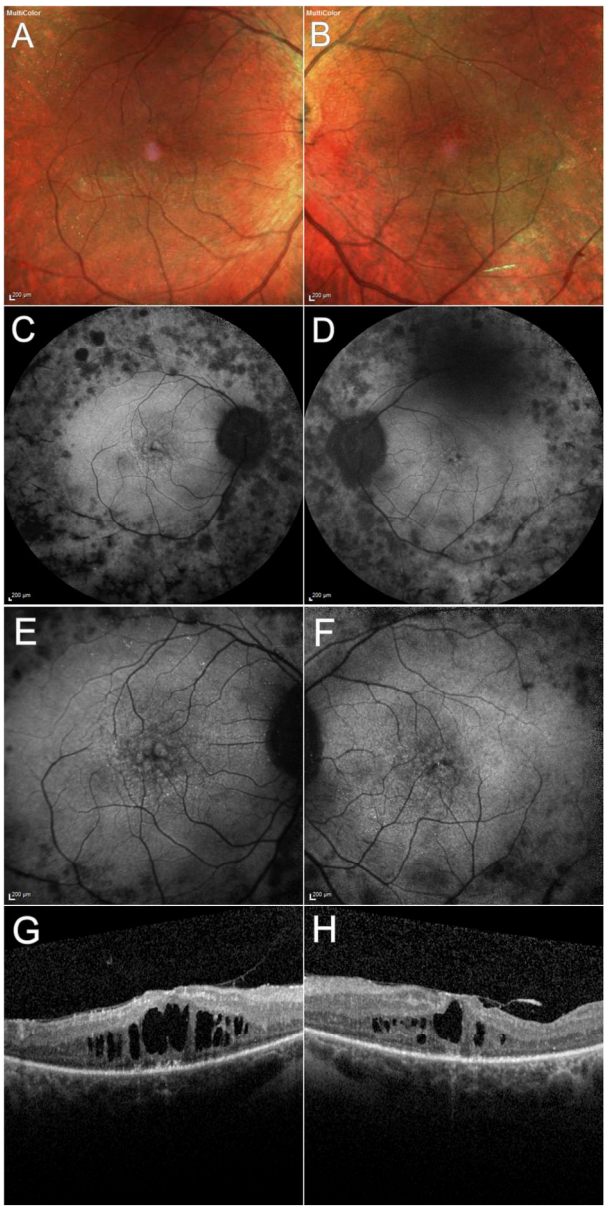
Table 1. FLVCR-1 variants reported. Each row represents an individual patient reported in the literature.

Variant Allele 1	Variant Allele 2	Clinical features	OMIM	Intron/ Exon	Protein structure	Reference
c.361A>G p.Asn121Asp	c.361A>G p.Asn121Asp	RP Sensory ataxia Areflexia	609144.0001	Exon	Transmembrane domain (1)	Rajadhyaksha, 2010[2] Puffenberger, 2012[23]
c.721G>A p.Ala241Thr	c.721G>A p.Ala241Thr	RP Sensory ataxia	609144.0002	Exon	Transmembrane domain (5)	Rajadhyaksha, 2010[2]
c.574T>C p.Cys192Arg	c.574T>C p.Cys192Arg	RP Sensory ataxia	609144.0003	Exon	Transmembrane domain (3)	Rajadhyaksha, 2010[2]
c.1477G>C p.Gly493Arg	c.1477G>C p.Gly493Arg	RP Sensory ataxia Learning difficulties	609144.0004	Exon	Transmembrane domain (12)	Ishuira, 2011[1] (2 patients reported)
c.1547G>A p.Arg516Gln	c.1593+5_+8 delGTAA	RP Sensory ataxia Muscle weakness and atrophy	Not specified	Exon Intron	Topological domain	Shaibani, 2015[3]
c.1092+5G>A	c.1092+5G>A	RP No ataxia	Not specified	Intron	Splice-site variant	Tiwari, 2016[7] Glocke, 2014[24] This study
c.479T>C p.Leu160Pro	c.1092+5G>A	RP No ataxia	Not specified	Exon Intron	Transmembrane domain (11) Splice site	Tiwari, 2016
c.1285 T>C p.Phe429Leu *in cis* with c.1092+5G>A	c.1092+5G>A	RP No ataxia	Not specified	Exon Intron	Transmembrane domain (10) Splice site	This study



Retinal imaging studies. (a&b) Colour photographs of right and left macula, (c&d) widefield fundus autofluorescence imaging (55 degrees) demonstrating symmetrical, widespread patchy hypoautofluorescence in the mid-peripheral retina, (e&f) fundus autofluorescence (30 degrees) imaging of both maculae demonstrating likely cystoid macular oedema, (g&h) optical coherence imaging demonstrating bilateral cystoid macular oedema, worse on the right than the left. All images were taken with Spectralis, Heidelberg Engineering, Heidelberg, Germany.

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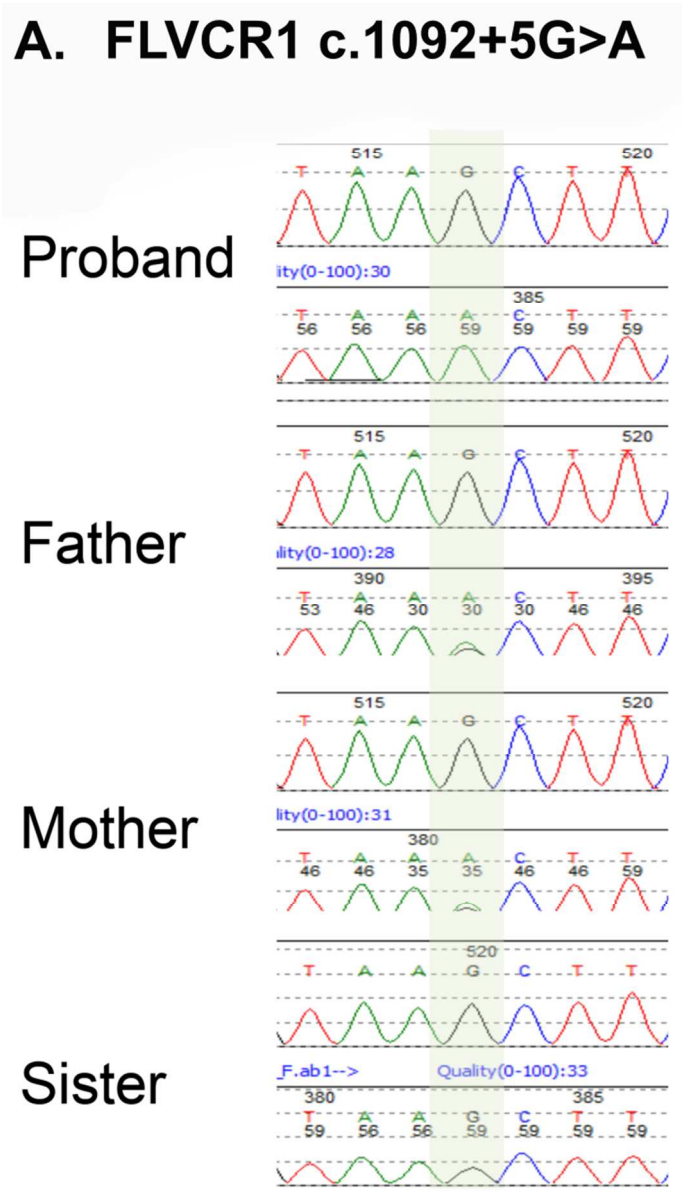

Figure 2. (a) Sanger sequencing of proband, mother, father and sister. The father and mother are both heterozygous for the FLVCR1 c.1092+5G>A variant. The sister is unaffected.

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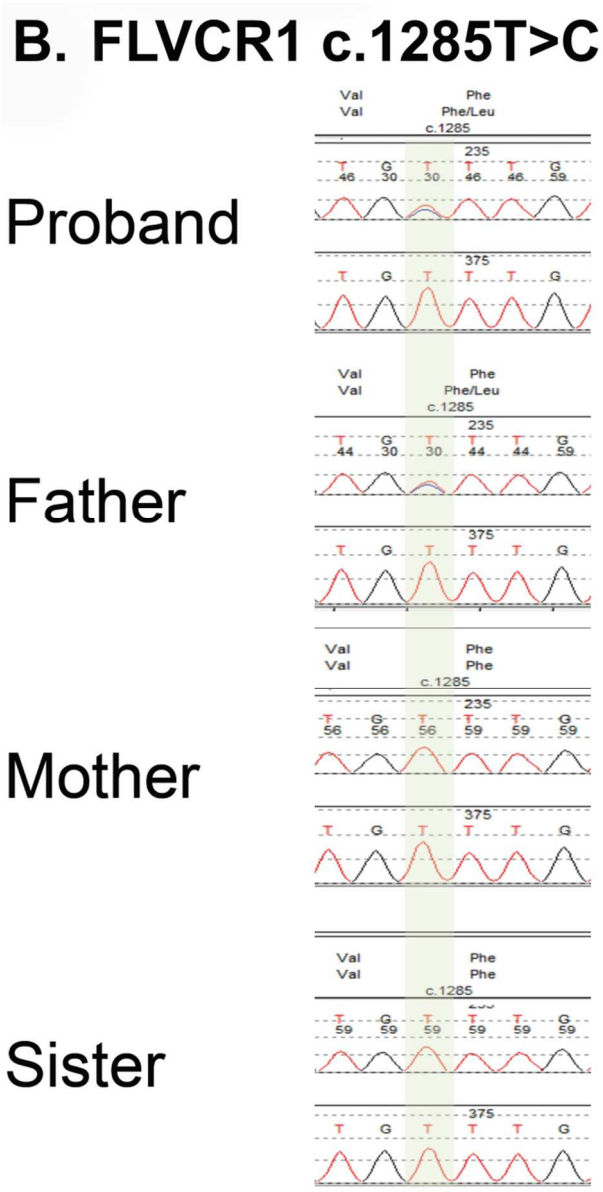

(b) Sanger sequencing demonstrating the c.1285T>C p.Phe429Leu variant in the father. Individual genotypes are as follows: Proband's alleles (c.1092+5A)/(c.1092+5A) and (c.1285C)/WT; father's alleles: (c.1092+5A)/WT and (c.1285C)/WT; mother's alleles: (c.1092+5A)/WT and WT/WT; sister's alleles: WT/WT and WT/WT.

60x121mm (300 x 300 DPI)