

EXTENDED REPORT

An atypical phenotype of macular and peripapillary retinal atrophy caused by a mutation in the *RP2* gene

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Aims: To determine the molecular basis and describe the phenotype of an atypical retinal dystrophy in a family presenting with bilateral, progressive central visual loss.**Methods:** Family members were examined. Investigations included Goldman perimetry, electrophysiology, and autofluorescence imaging. Candidate gene screening was performed using SSCP and sequence analysis. The proband's lymphoblastoid cells were examined for protein expression.**Results:** Fundal examination of the proband, his mother, and brother revealed peripapillary and macular atrophy. Autosomal dominant retinal dystrophy was suspected, but less severe disease in the mother led to screening for mutations in X linked genes. A 4 bp microdeletion in exon 3 of the *RP2* gene, segregating with disease, was identified. No *RP2* protein expression was detected.**Conclusion:** The distinct phenotype in this family, caused by this frameshifting mutation in *RP2*, broadens the phenotypic spectrum of X linked retinitis pigmentosa. The absence of *RP2* protein suggests that loss of protein function and not novel gain of function could account for the atypical phenotype. A definitive diagnosis of X linked retinitis pigmentosa permits appropriate genetic counselling with important implications for other family members. Clinicians should have a low threshold for screening *RP2* in families with retinal dystrophy, including posterior retinal disease, not immediately suggestive of X linked inheritance.

Retinitis pigmentosa (RP) defines a clinically and genetically diverse group of retinal dystrophies characterised by progressive photoreceptor cell degeneration. X linked retinitis pigmentosa (XLRP) affects about 10–20% of families with RP.^{1–3} It is a severe form of RP in terms of age of onset and progression, typically presenting with symptoms of night blindness and loss of dark adaptation within the first decade of life and reduction of visual fields in the second decade.¹ Disease may progress to complete blindness by the third or fourth decade. Premature cell death occurs initially in the mid-periphery of the retina and primarily affects rod photoreceptors. Clinically this is seen as bone spicules in the periphery caused by pigment epithelial atrophy and pigment migration into the retina. Other signs in the late stages of this degenerative disease include optic atrophy and attenuated blood vessels.^{1 2}

Five loci have been mapped for XLRP, of which *RP3* and *RP2* account for the majority of disease.^{4–7} Positional cloning resulted in the identification of the highly complex *RPGR* (RP guanosine triphosphatase (GTPase) regulator) gene in the *RP3* region^{8–10} and the *RP2* gene in the *RP2* region.¹¹ Many different mutations have been described in *RPGR* and *RP2* as a cause of XLRP. Mutations in *RPGR*, including the more recently identified *ORF15*, are the most common and account for approximately 70–90% of XLRP cases.^{8–10 12–18} *RP2*, the second most frequent cause of XLRP, accounts for approximately 6–20% of cases in the populations studied.^{11–13 19–22} The *RP2* gene has 5 exons and encodes a 350 amino acid polypeptide (RP2) which is ubiquitously expressed.^{23 24} *RP2* shows homology to cofactor C, which functions in the assembly of native tubulin heterodimers with other cofactors (A–E).^{25 26} Missense, nonsense, frameshift, insertion, and deletion mutations have been identified in the *RP2* gene, many of which occur at residues conserved with cofactor C, suggesting functional homology between these proteins.^{11 12 19} *RP2* has been localised to the plasma membrane

of a variety of cultured cells including rod and cone photoreceptors in the human retina.^{23 27–29} Although the function of *RP2* remains unknown, it has been shown to interact with GTP bound ADP ribosylation factor like 3 protein (Arl3).²⁷

We report a family with an atypical phenotype, initially thought to represent an autosomal dominant retinal dystrophy. Screening for mutations in X linked retinal disease genes were undertaken and the proband's leukocytes were examined for the presence of *RP2* protein.

MATERIALS AND METHODS

Clinical examination and investigations

The family pedigree is shown in figure 1. The proband, his mother, and younger brother were examined, and fundus photographs taken (Topcon imagenet digital imaging, Topcon Ltd, Newburg, UK). Investigations including Goldmann perimetry (see fig 3) and electrophysiology under ISCEV conditions were carried out on the proband and on the mother. Autofluorescence images using a confocal laser scanning ophthalmoscope (Zeiss, Oberkochen, Germany) were recorded on the younger brother using published techniques.³⁰

Mutation identification

Genomic DNA was extracted from peripheral blood leukocytes using the Nucleon II kit (Scotlab Limited, Strathclyde, UK) according to manufacturer's instructions. The five exons of the *RP2* gene were screened for sequence changes using SSCP as described.¹⁹ Exon 3 showed a distinct band shift in the proband compared with wild type control. This same

Abbreviations: ERG, electroretinogram; RP, retinitis pigmentosa; VA, visual acuity; XLRP, X linked retinitis pigmentosa

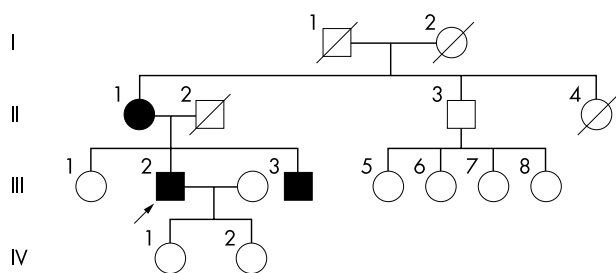


Figure 1 Pedigree structure. Affected individuals are drawn as blackened circles (females) and squares (males).

amplimer was bidirectionally sequenced for the proband, brother, and mother using the ABI Prism Ready Reaction Dye Terminator cycle sequencing kit (FS kit, Perkin-Elmer Boston, MA, USA) and fragments separated on an ABI 373A automated sequencer. Primer sequences and reaction conditions have been described previously.¹⁹

Cell culture

Human lymphoblastoid cell lines corresponding to the proband, (patient III:2), affected members of a different pedigree at Moorfields Eye Hospital with the Arg120stop nonsense mutation in RP2²⁸ and randomly selected control males were obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK). Cells were maintained in suspension culture in RPMI 1640 Glutamax-I (Life Technologies, Paisley, UK) supplemented with 10% fetal bovine serum (Sigma, Poole, UK) with media changes every 2 to 3 days.

Preparation of cell lysates

Lymphoblastoid cells were Dounce homogenised on ice in 20 mM Tris-HCL, pH 7.5, 500 mM NaCl, 12.5 mM KCl, 1 mM EDTA, 1 mM dithiothreitol containing a protease inhibitor cocktail (Sigma). The concentration of protein in the homogenates was determined using the Bio-Rad DC assay (Bio-Rad, Hemel Hempstead, UK), following the manufacturer's protocols.

Immunoblotting

The cell lysates were prepared for electrophoresis by the addition of sample buffer and heating to 96°C for 5 minutes as described previously.²⁸ Fifty µg or 100 µg of total protein were loaded on a 12% SDS polyacrylamide gel and, after electrophoresis, were electroblotted onto nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany). Non-specific binding sites on the nitrocellulose were blocked by incubation with 5% marvel, 1 × phosphate buffered saline, 0.05% Tween 20 overnight at 4°C. The blots were hybridised with sheep polyclonal antisera S974, and immunoreactive bands were visualised as described previously.²³

RESULTS

Clinical evaluation

Case III-2

The 46 year old proband first developed eye problems in childhood, with the onset of myopia. At age 16 his refraction was -12 dioptres in the right eye, and -13 dioptres in the left eye, and he was noted to have peripheral retinal myopic degeneration treated with cryotherapy. He reported reduced night vision from his teenage years, which was initially thought to be caused by his myopia, and his impaired acuity prevented him from being able to drive. He developed progressive loss of central vision over the following years with relative sparing of the peripheral field. His general

health was otherwise good and family history revealed an affected younger brother and mother (figs 1 and 2).

When examined aged 43, his Snellen visual acuity was 6/60 in both eyes. He was photophobic. There was no evidence of lens opacity or iris transillumination. Fundal examination revealed marked peripapillary chorioretinal atrophy in both eyes extending to the posterior pole and involving the macula (fig 2A–D). Cryotherapy scars were present peripherally. Field testing to confrontation and Goldmann perimetry demonstrated an inferior island of preserved visual field in both eyes (fig 3). Autofluorescence imaging showed no detectable retinal signal.

ISCEV standard Ganzfeld electroretinogram (ERG) responses were almost extinguished, but there was some very low amplitude, grossly delayed flicker response activity. Vision continued to deteriorate progressively, reducing to 1/60 in both eyes a year later.

Case III-3

The proband's younger brother lives in North America and had symptoms of night blindness since the age of 24. He required a low myopic prescription (-0.25/-1.25 × 32 RE, -0.25/-1.25 × 166 LE) and had Snellen visual acuities

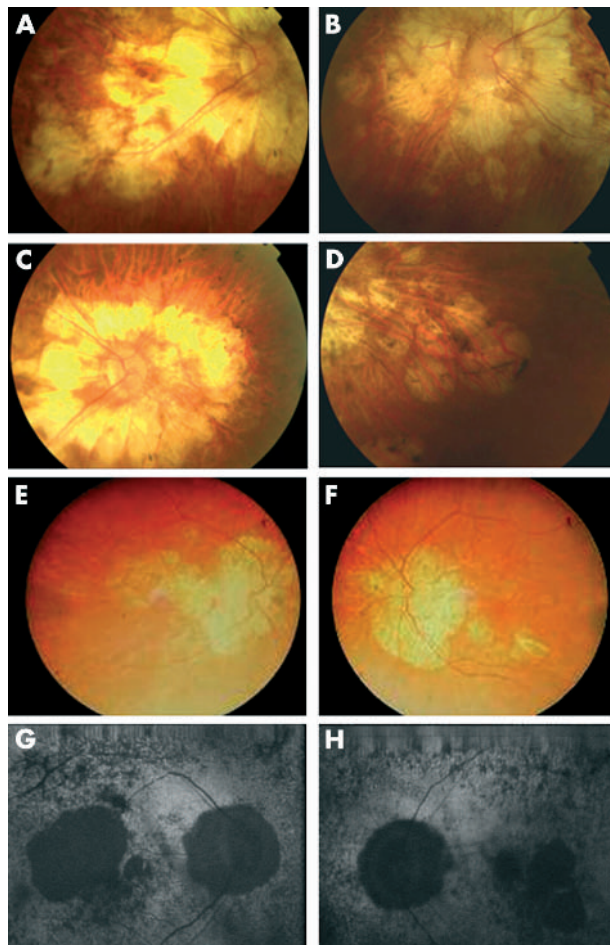


Figure 2 Fundus images for affected males and their carrier mother. (A) and (C) fundus photographs of right eye of proband III:2 (aged 45) visual acuity (VA) 1/60. (B) and (D) fundus photographs of left eye of proband III:2 (aged 45) VA 1/60. (E) and (F) fundus photographs of right and left eye of proband's mother II:1 (aged 68) VA 6/60 OU. (G) and (H) autofluorescence images of the right and left eyes of the proband's younger brother III:3 showing macular and peripapillary hypo-autofluorescence indicating atrophy of the retinal pigment epithelium (aged 39) VA 6/36 OU.

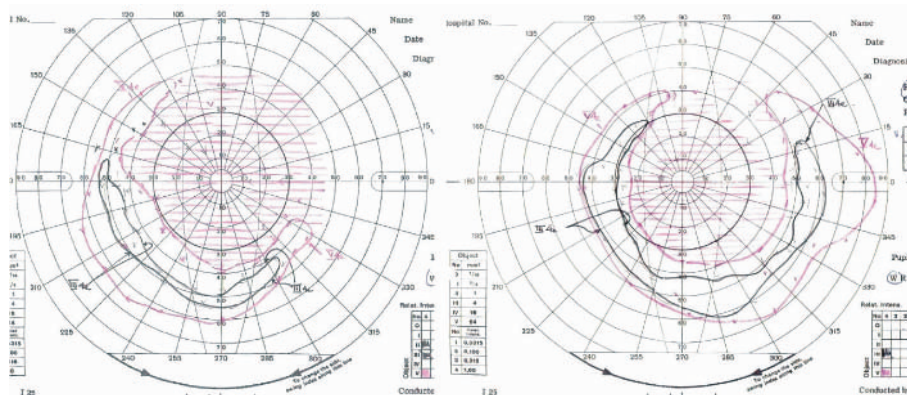


Figure 3 Goldmann perimetry of proband. Goldmann perimetry shows preserved inferior islands in both eyes to targets III4e and V4e (age 43) VA 6/60 OU.

recorded as 6/12 and 6/24 at age 32. He went on to develop progressive loss of his central and peripheral vision. Fundal examination revealed pallor of both optic discs, sparse bone spicule pigmentation peripherally and the macula was atrophic in both eyes. Autofluorescence imaging confirmed areas of atrophy (decreased autofluorescence) at the maculae and around the disc in both eyes. (fig 2G and H).

Case II-1

The proband's mother had experienced problems attributable to reduced acuity but, because of a previous stroke and dementia, history and examination were limited. At aged 68, her visual acuity was 6/60 in each eye with a hyperopic correction; right +4.25/−0.50×141 and left +5.00/−1.00×43. Examination of the fundus showed peripapillary atrophy with extension to the posterior pole, similar to that of the proband. (fig 2E and F).

Previous electrophysiology, when the patient was aged 59, had revealed rod ERGs reduced to 20–30% of normal, a maximal (mixed rod-cone) response reduced to 15% of normal, and delayed 30Hz flicker ERG reduced to a comparable level.

Genetic analysis

In view of the family history and fundoscopic appearance of the mother and proband, an initial diagnosis of autosomal dominant retinal dystrophy, primarily affecting central retina, with variable expressivity was suspected. The fact that the mother was less severely affected, and that no male to male transmission had occurred, led to screening for mutations in X linked retinal disease genes. A change was detected by SSCP analysis of the *RP2* gene, and following sequence analysis, a frameshifting, 4 bp microdeletion in exon 3 (796–799delCAGA) was found in the coding region of *RP2* (fig 4). This segregated with disease in the family, in that this maternal mutation was inherited by both affected sons (fig 4). This microdeletion was not detected in a screen of over 100 control chromosomes. It would be predicted to result in a frameshift at codon 266, causing a premature stop codon, three amino acids further downstream (Gln266fsTer269).

RP2 protein expression

RP2 protein is expressed in lymphoblastoid cells, and cells derived from patients of another family with an Arg120stop mutation, have previously been studied.²⁸ As the phenotype of the family presented here is atypical, we exploited the ubiquitous expression of RP2 and investigated the proband's (III:2) lymphoblastoid cell RP2 expression to test if the presence of an aberrant protein product could account for the

differences in phenotypic expression. A band of the predicted molecular mass of RP2, 39.6 kDa, was detected in the control male cell lines (fig 5). No RP2 protein, truncated or full length, was detected in the two different male patient cell lines (fig 5) by antibody S974, which can recognise the first 15 amino acids of RP2 alone.²⁸ The predicted mass of the truncated RP2 protein products were 14 kDa for the Arg120stop and 31 kDa for the proband (III:2) Gln266fs. An immunoreactive band corresponding to an unknown 68 kDa protein was observed in both patient and control lymphoblastoid cells (fig 5); this band is not a product of the *RP2* gene. However, the crossreactivity provided a useful internal loading and transfer control for the western blot assays.²⁸

DISCUSSION

The clinical phenotype and molecular pathology in a family presenting with bilateral, progressive central visual loss are

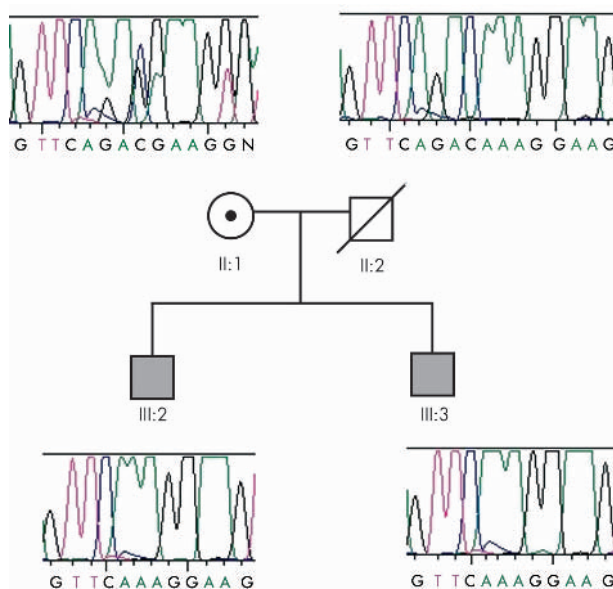


Figure 4 Segregation of a 4 bp deletion in the *RP2* gene with disease. Electropherograms depicting a 4 bp deletion in the *RP2* gene in affected males III:2 and III:3 (delCAGA). Wild type sequence is shown in the father (II:2), and the carrier mother (II:1) is heterozygote for the mutation. Note: the sequence software attempts to read through the carrier mother's chromosomes, but inspection of the raw data shown demonstrates the two alleles.

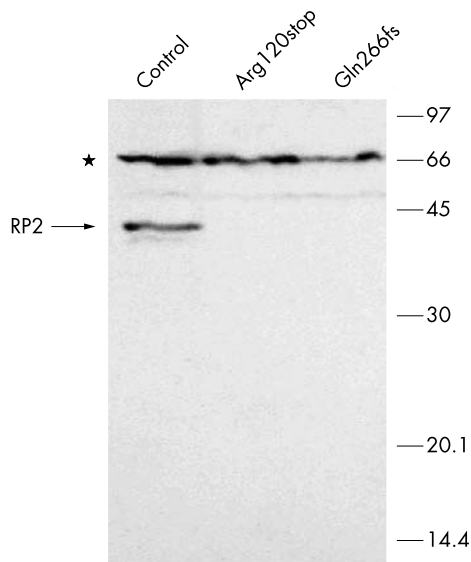


Figure 5 RP2 protein expression in patient and control lymphoblastoid cell lines. The western blot detects a band of the expected size in the control male cell line, but no RP2 protein was detected in the proband (Gln266fs) or a male patient with an Arg120stop mutation. The predicted molecular weight of RP2, 39.6 kDa, is marked on the left. *A 68 kDa band that crossreacts with sera S974 was seen in all cell lines.²⁹ Molecular weight markers are indicated on the right.

described. The macular and peripapillary atrophic changes in the family did not immediately suggest a diagnosis of RP. They are unlikely to be solely attributable to myopia as the mother and brother (II:1 and III:3), who showed similar posterior retinal signs, were hyperopic and mildly myopic respectively. Autosomal dominant retinal dystrophy with variable expressivity was initially suspected, but the milder phenotype in the mother and the severe ERG abnormality led to the screening for mutations in X linked retinal disease genes. This led to the detection of the microdeletion mutation in exon 3 of the RP2 gene.

The proband's mother showed an unusual phenotype for an XLRP carrier. Heterozygotes have been shown to vary from being asymptomatic with a normal fundus appearance (4%), to having characteristic retinal changes (87%).³¹ Profound visual loss has also been described in later life.³²⁻³³ Retinal changes include a tapetal reflex (52%) and peripheral pigmentary pigmentation (43%), especially inferiorly.³¹ These changes are present in 60% to 100% of carriers, but neither was seen in our case.¹⁻³²⁻³³ Electrophysiological testing can be useful in those carriers where the fundus appears normal, and is found to be reduced in amplitude in up to 86% of carriers.³¹ Peripapillary and macular atrophy however are not typical findings.

Several groups have studied the correlation between clinical characteristics and genotype with variable success. Rosenberg and colleagues³⁴ identified three RP2 mutations in families with XLRP in which they revealed striking inter-familial phenotypic differences and correlated disease severity to genotype. Those with a null mutation (Gln26stop) or a missense mutation (Arg118His) were associated with a severe phenotype. This was characterised by reduced visual acuity and subjectively impaired night vision in the first decade and progression to total blindness by the age of 50. Interestingly, the Gln26stop mutation was also associated with fundal lesions affecting the macular and peripapillary retinal pigment epithelium and choriocapillaris. The

Arg118His mutation was associated with a more typical phenotype including early onset of night blindness, peripheral visual field constriction, and bone spicule pigmentation. An in-frame deletion, Δ Ser6, was associated with a much milder phenotype.³⁴ Patients with this mutation had a later onset of disease in the second decade with preserved intact central vision, preserved visual fields for large targets, and residual cone ERG activity. It has been shown that this mutation interferes with the normal targeting of the RP2 protein to the plasma membrane.²³

One possible explanation for the distinct phenotype seen in this family is a gain of function or novel function of the truncated protein product. However, a lack of RP2 protein in the lymphoblastoid cells of the proband suggests that the mutation entirely abrogates protein expression and so an aberrant protein cannot be the cause of the atypical phenotype. Important modifying factors, genetic and/or environmental, may play a role in phenotypic expression of disease, and these may have been acting independently in each of the three family members. Alternatively, an important retinal modifier may have been co-inherited with the RP2 mutation on a nearby X chromosomal gene.

This family exemplifies the value of genetic testing in retinal disease that may show an atypical phenotype and highlights the spectrum of phenotypes that can occur due to a mutation in a single gene. Mutations in RP3 also appear to cause a broad clinical phenotype; as in a family with macular dystrophy found to be harbouring a mutation in RPGR gene.³⁵ In our family's case, the discovery of the genetic mutation not only gave a definitive diagnosis but allowed accurate genetic counselling with important implications for other family members, especially the proband's daughters who would be carriers for the mutation. Hence, the laboratory findings had a direct effect on clinical management.

We encourage clinicians to have a low threshold for screening RP2 and RPGR in families with retinal dystrophy, even when there is predominantly posterior retinal disease both in male and female affected family members, not immediately suggestive of X linked inheritance.

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