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.

**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 imagnet 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.

**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. 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
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 RP2 28 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.26 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, 1/6 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 electoretinogram (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
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 macular 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. 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. 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.

DISCUSSION
The clinical phenotype and molecular pathology in a family presenting with bilateral, progressive central visual loss are
Atypical RP2 phenotype

In our family’s case, the discovery of the genetic mutation not immediately suggestive of X linked inheritance. 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. In our family’s case, the discovery of the genetic mutation not only gave a definitive diagnosis but also showed 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.

**REFRENCES**


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