PostScript

LETTERS

Dominant retinitis pigmentosa phenotype associated with a new mutation in the splicing factor PRPF31

Autosomal dominant retinitis pigmentosa results from mutations in 14 known proteins, and at least two further loci have been highlighted by genetic linkage in families (reviewed by the RetNet website; http://www.sph.uth.tmc.edu/Retnet/). The known genes include those encoding components of the phototransduction cascade, retinal transcription factors and retinal structural proteins.[1] The list also includes four ubiquitously expressed splicing factors: pre-mRNA processing factor 8 (PRPF8),[2] PRPF31,[3] PRPF3[4] and PAP-1, also known as RP9.[5 6]

Splicing is a complex process that involves the precise excision of introns from pre-mRNA by a macromolecular structure called the spliceosome. Three of the splicing factors implicated in autosomal dominant retinitis pigmentosa (ADRP) are components of the U4/U6-U5 tri-snRNP particle, an essential component of the spliceosome.[7 8] Mutations in one of these, PRPF31, have been reported to cause between 5 and 20% of ADRP.[9 10] In this report, a new mutation in the *PRPF31* gene is described, together with the clinical phenotype.

Cases

The proband was a 33-year-old female with a corrected visual acuity of 58 and 51 ETDRS letters in the right and left eye, respectively (approximate Snellen equivalents of 6/18 and 6/36). She had a myopic refraction with a spherical equivalence of −2 dioptres in each eye. Nyctalopia had been present since the middle of the second decade, and she had noticed a decrease in her central vision since the beginning of the third decade. At the most recent examination, she had early posterior subscapsular cataract, bone spicule formation in all four quadrants (fig 1a,b) attenuated arterioles and pale optic discs in each eye. The maculae appeared normal on clinical examination. On Goldman perimetry, the mean visual field to the V4e target measured 6.5° from fixation. Zeiss OCT 3 examination demonstrated a foveal thickness of 170 and 144 microns, respectively, in the right and left eyes, with absence of the third highly reflective band.[11]

Her younger sister had a similar clinical phenotype and age of onset. The 61-year-old mother was asymptomatic, with unaided visual acuities of 80 and 81 ETDRS letters (Snellen equivalent of 6/7.5). Fundus examination revealed mild bone spicule attenuation in the peripheral retina (fig 1c,d). Visual field to the V4e target on Goldman perimetry was slightly reduced from normal with a mean of 57.8° from fixation. Foveal thickness on Zeiss OCT 3 examination was 223 and 249 microns in the right and left eyes, respectively. The father of the proband was also asymptomatic with visual acuities of 85 ETDRS letters (Snellen 6/6) in both eyes and normal ocular examinations. No clinical information was available from any other living relative along the maternal line.

Mutation screening

DNA from the proband was included in a large cohort of retinal dystrophy DNAs, which were screened for mutations in a limited set of exons or parts of exons of known retinal degeneration genes. The exons screened were selected from the available literature because they were known mutation hotspots or locations of common founder mutations. Screening was carried out by radioactively labelled single-strand conformation polymorphism/heteroduplex analysis (SSCP/HA).[12]

One of the sequences screened was *PRPF31* exon 6. Screening of this sequence in the proband revealed a large mobility shift suggestive of a deletion. Sequencing revealed a novel 16 bp deletion present in the three female members of the family but absent from the father and from 120 control Caucasian genomic DNAs (240 chromosomes). This sequence change is denoted c.522–527del&IVS6+1to+10del[13] (fig 2). It deletes codons 175 and 176, the last two in exon 6, encoding glutamine and glycine residues. However, it also deletes the first 10 bp of intron 6, including the exon 6/intron 6 boundary and splice donor site, the mutation abolishing the exon 6 splice donor site. This may give rise to an mRNA transcript which includes intron 6, adding seven novel amino-acids then terminating the encoded protein, or could lead to the skipping of exon 6.

Discussion

This novel mutation in the *PRPF31* gene causes a severe phenotype in symptomatic cases, with the onset of nyctalopia in the second decade and loss of acuity from the third. Both the age of onset and the phenotype observed are similar to that described by Sato *et al*[14] in Japanese families. In addition, this report is the first to demonstrate variable penetrance of the phenotype in an asymptomatic carrier of the mutation. A high level of non-penetrance has been described previously, both in families with confirmed *PRPF31* mutations and in those linked to the RP11 locus before mutations in *PRPF31* were identified.[14–19] Evans *et al*[15] used the term bimodal expressivity to describe this phenomenon. Sato and colleagues also identified asymptomatic carriers of the mutations in the *PRPF31* gene by genetic analysis. One of these was an elderly relative of three generations of symptomatic RP sufferers, though he himself had no ocular abnormalities except for mild cataracts. In our report, the mother of the proband had definite retinal findings and a mildly reduced visual field on Goldman perimetry, though she was totally asymptomatic. This may perhaps imply that the range of phenotypes seen in *PRPF31*-RP could be better described as a spectrum of severity, rather than true bimodal expressivity.

The mutation described above is likely to result in a grossly abnormal transcript which may be subject to nonsense mediated decay.[20] This brings to 18 the number of published *PRPF31* mutations in the literature, comprising six deletions (ranging from one base pair to the whole gene), five splice-site mutations, two

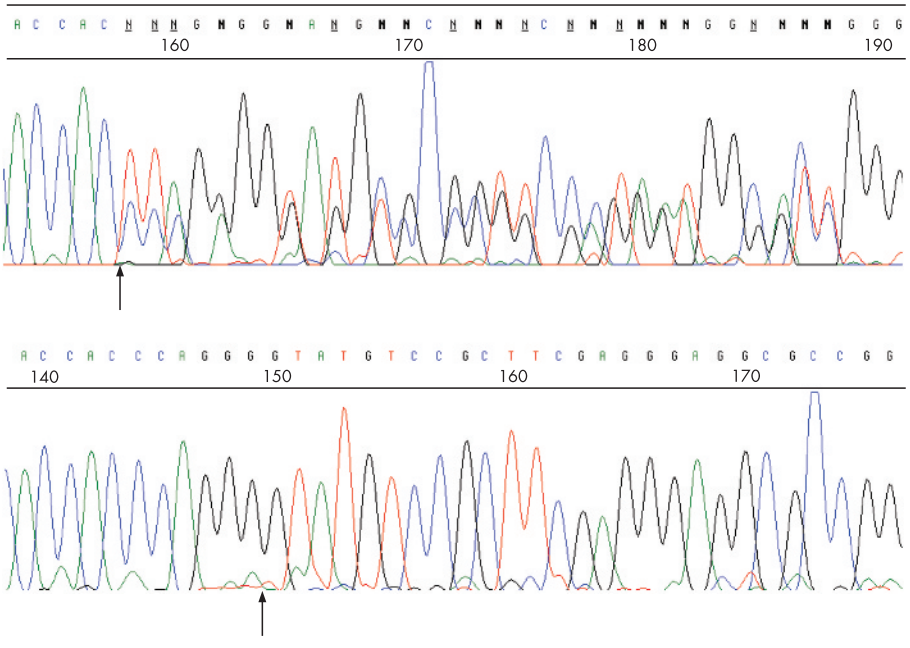

Figure 1 Electropherogram of the mutated (upper) and normal (lower) sequence of PRPF31 in. As the mutation is heterozygous, the upper image shows both the mutated and normal sequences superimposed. The arrow on the mutated sequence denotes the beginning of the deleted sequence, while the arrow on the normal sequence marks the boundary between exon 6 and intron 6. Sequence was generated from PCR-amplified DNA on a Pharmacia MegaBACE automated DNA sequencer.

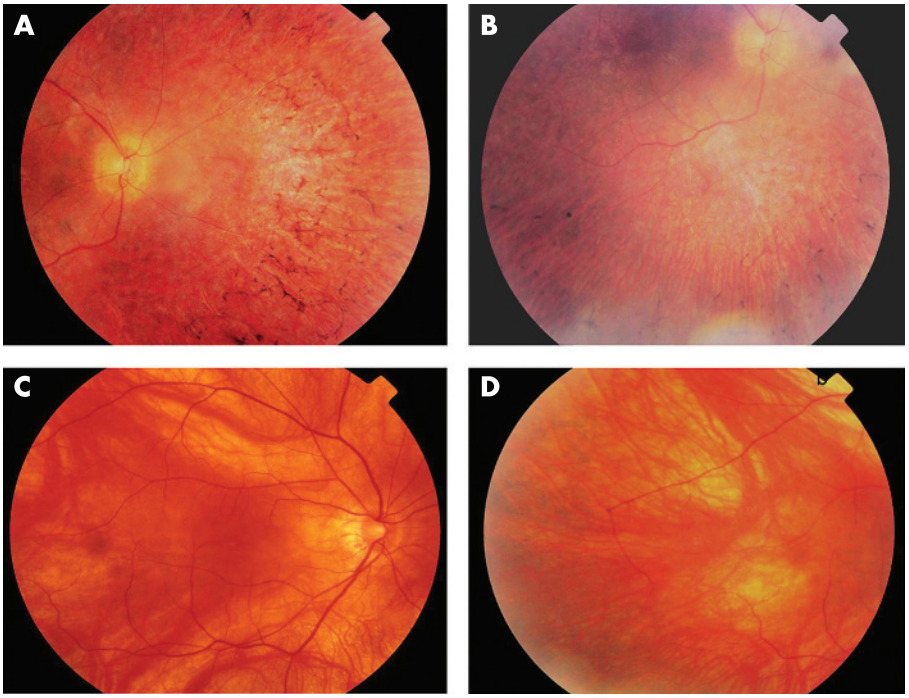

Figure 2 A and B, retinal photographs taken from the proband showing bone spicule attenuation. C and D, retinal photographs from the mother of the proband. The mild bone spicule attenuation was evident only on slit lamp biomicroscopy using a wide angle lens.

insertion/deletion events, one duplication, one insertion and only three missense mutations.[3 14 18 19 21–24] The lack of missense changes has led others to speculate that mutations in *PRPF31* cause RP due to haploinsufficiency and consequent insufficiency of splicing activity.[18] Wilkie *et al*[25] concluded that reduced mutant protein solubility in two of the known missense mutations, A194E and A216P, also led to splicing insufficiency.

This hypothesis is further supported by the finding that high-expressing alleles of *PRPF31* from the normal parent compensate for a potentially RP-causing mutation on the opposing chromosome.[26] This phenomenon accounts for the variation in severity described above and predicts that the normal second allele of *PRPF31* in the mother from the family described herein is a high-expressing variant which masks the RP symptoms. However, the alleles inherited by her daughters from their normal father are less well expressed, and so these individuals have a much more severe form of RP. To date, the mechanism controlling this level of expression remains unknown. A bimodal phenotype might be explained by a single diallelic polymorphism in a sequence involved in transcription regulation, whereas a spectrum of severity, as observed herein, might imply a more complex interplay between several such polymorphisms. Understanding the basis of this variation in severity, together with the finding of haploinsufficiency as a cause of disease, could have important implications for the testing of potential new treatments for this relatively common retinal degeneration.

Acknowledgements

We thank Yorkshire Eye Research (grant numbers 009 and 006) and the Leeds Teaching Hospitals Charitable Foundation for funding this research, and Mike Stockton in the medical illustration department, St. James's University Hospital, and of course the patient and her family for enabling us to carry out this research and share the information with the wider medical community.

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doi: 10.1136/bjo.2006.105163

Accepted 12 December 2006

Competing interests: None declared.

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Yoga can be dangerous—glaucomatous visual field defect worsening due to postural yoga

The relationship between the head-down body position and increased IOP is well known.[1–6] We present a 46-year old woman who presented with a worsening of glaucomatous visual field defects one year after starting to perform regularly a particular postural headstand yoga exercise, reversible after cessation of the exercise.

In 10 non-yoga-practising volunteers intraocular pressure (IOP) was measured by Tono-Pen in sitting and immediately after assuming a headstand position. A more than twofold increase of the IOP was measured in the headstand position. Therefore postural (head-down) yoga exercises are clearly not recommended for patients suffering from glaucoma.

Case report

A 46-year-old Caucasian woman followed at our clinic for a bilateral juvenile open-angle glaucoma presented on a routine examination a significant worsening of her visual field defects on both eyes (fig 1). Twenty years previously a bilateral trabeculectomy had been performed and since then intraocular pressures had always been stable without treatment (between 14 and 16 mm Hg). Slit-lamp examination revealed no apparent reason for the visual field deterioration. Detailed history taking finally showed that she had started one year previously (shortly after the last visual field examination) regularly to practise yoga, particularly a headstand position, called ''sirsasana''. Measurement of the IOP by Tono-Pen in the headstand position showed a twofold increase of IOP compared to IOP in the sitting position (32 compared to 16 mm Hg). We asked the patient to stop any yoga exercise with the head-down position and some months later the visual field defects improved significantly.

Comment

Postural yoga (''asanas''), including headstand posture (''sirsasana''), is along with breathing exercises (''pranayama'') and meditation (''dhyana'') one of the three basic components of hatha yoga, the system on which much of western yoga is based. Yoga has become a popular practice in the western world. In 1998 an estimated 15 million American adults had used yoga at least once in their lifetime, 7.4 million during the previous year.[7] Sirsasana is a preferred position that seems to induce euphoria and comfort after performing the posture.[6]

To evaluate the increase of IOP due to headstand position we measured IOP in 10 non-yoga-practising volunteers (4 women and 6 men, mean age 37.3 $\pm$ 11.3 years) in a sitting position and immediately after assuming a headstand position. IOP was measured by a single examiner using the Tono-Pen XL (Medtronic Solan, Jacksonville, Florida) in the left eye after application of oxybuprocaine 0.4% eye drops. IOP was measured four times consecutively and the mean IOP was calculated. All volunteers were in good health and did not present any known ocular pathology. The mean sitting IOP was 13.9 $\pm$ 1.76 mm Hg (range: 10.75–18.5). Immediately after assuming a headstand position the mean IOP increased to 31.8 $\pm$ 4.22 mm Hg (range: 23–38.75). These findings agree with a recent study (including 75 experienced yoga practitioners) that recorded a uniform twofold increase in the IOP during sirsasana, which was maintained during the posture and returned to near baseline level immediately after resuming a sitting posture.[6] Increased IOP has been explained with raised episcleral venous pressure[1] or increased choroidal volume by vascular engorgement.[8 9]

This case shows once more the importance of a good history taking and how sometimes unexpected personal habits can influence ophthalmologic pathologies. Patients suffering from glaucoma should be advised against practising postural (head-down) yoga exercises.

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doi: 10.1136/bjo.2007.114546
Accepted 11 January 2007

Competing interests: None.

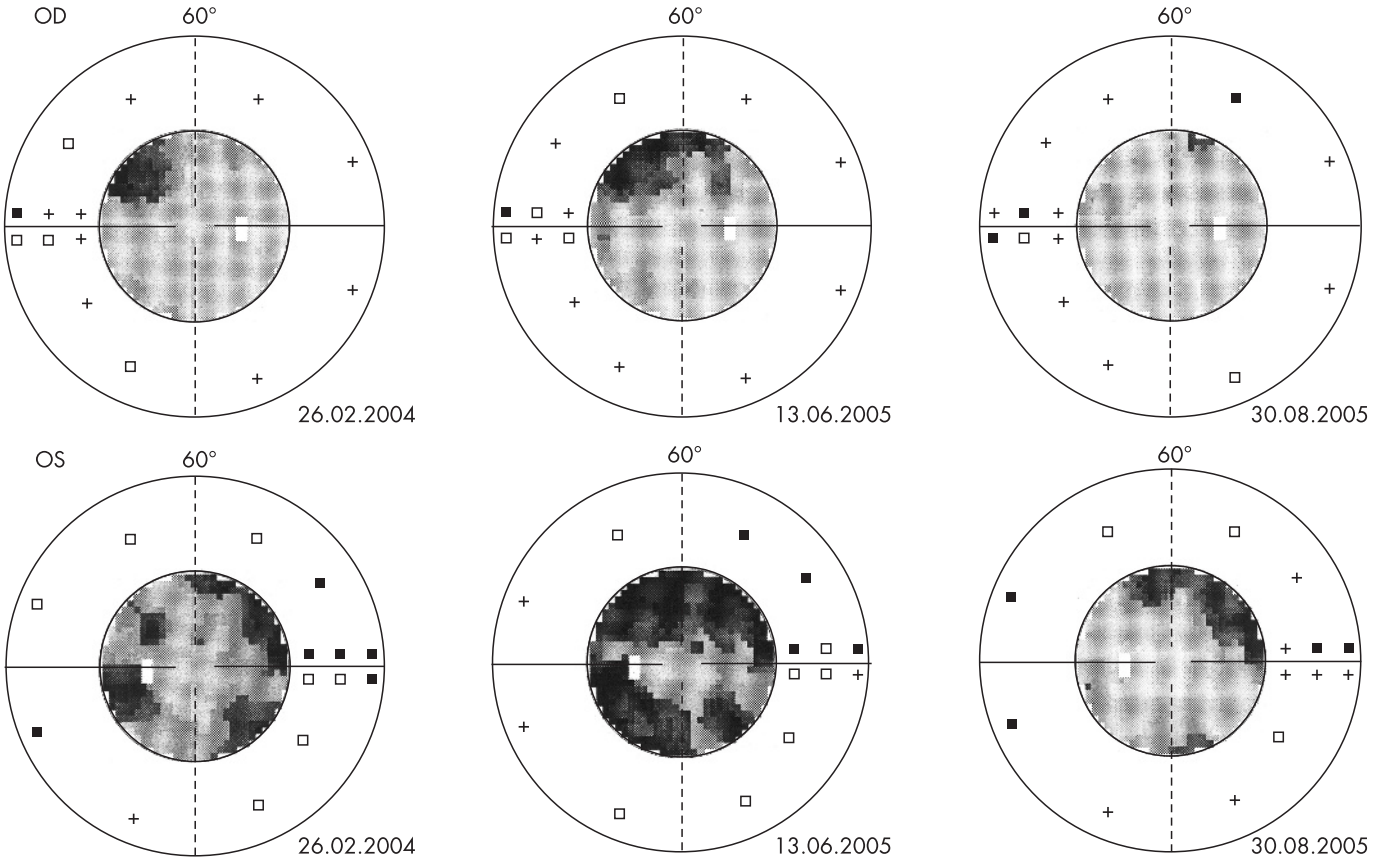

Figure 1 Visual field some months before and one year after starting postural yoga and one year after stopping it.