

Ocular findings associated with a 3 base pair deletion in the peripherin-RDS gene in autosomal dominant retinitis pigmentosa

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Abstract

Affected members of a family with autosomal dominant retinitis pigmentosa were found to have a 3 base pair deletion at codon 118 or 119 of the retinal degeneration slow gene. This mutation causes the loss of a highly conserved cysteine residue in the predicted third transmembrane domain of peripherin-rds, a photoreceptor specific structural glycoprotein localised to both rod and cone outer segment disc membranes. Four of these individuals underwent detailed clinical, psychophysical, and electroretinographic testing in order to characterise their photoreceptor dysfunction. Nyctalopia was reported early in the second decade by all patients. Global rod and cone dysfunction was recorded by the third decade with severe reduction of both photopic and scotopic function by age 30 years. This retinal degeneration slow gene mutation may lead to the primary loss of both rod and cone photoreceptor function.

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Retinitis pigmentosa denotes a group of hereditary retinal disorders, causing degeneration of retinal photoreceptors which begins in the mid-peripheral fundus. Classically, the diagnosis of retinitis pigmentosa has been used to describe an unknown number of clinically similar but genetically distinct conditions,^{1,2} causing a recognisable pattern of functional and ophthalmoscopic changes. Population surveys have reported that 22 to 25% of all cases of retinitis pigmentosa are inherited in an autosomal dominant fashion.^{3,4} Since the first recognition that mutations in the rhodopsin gene caused autosomal dominant retinitis pigmentosa^{5,6} more than 30 mutations have been detected in the rhodopsin gene, and it appears that this gene accounts for about 25% of autosomal dominant retinitis pigmentosa.⁷⁻¹⁰

In several families with autosomal dominant disease, there is no linkage with markers in the long arm of chromosome 3,¹¹ and genes other than that for rhodopsin have been shown to be responsible for autosomal dominant retinitis pigmentosa.¹²⁻¹⁵ One of these is the retinal degeneration slow gene (RDS) on chromosome 6p,¹³⁻¹⁵ which is of particular interest since this gene is responsible for the photoreceptor degeneration in the retinal degeneration slow mouse.^{16,17} The product of the RDS gene, peripherin-rds, is a photoreceptor specific glycoprotein that is localised to the outer segment disc

membranes of both rods and cones, and is thought to be essential for normal retinal photoreceptor outer segment disc assembly and orientation.¹⁸⁻²⁰

Recently it has been reported that a wide variety of phenotypes are associated with RDS gene mutations including central as well as peripheral dystrophies, and disease with outer retinal atrophy as well as those presenting with accumulation of material at the level of the retinal pigment epithelium.²¹⁻²³ One of these mutations, a 3 base pair deletion at codon 118 or 119 leading to the loss of a highly conserved cysteine residue in the predicted third transmembrane domain of peripherin-rds (cysteine 118/119 deletion), has been identified as the cause of autosomal dominant retinitis pigmentosa in families of Irish origin.^{13,14,21} We describe the clinical, psychophysical, and electroretinographic findings of affected members in one of these families.²¹

Patients and methods

Families with peripheral retinal degenerations and clear evidence of autosomal dominant inheritance were screened for genetic mutations in the RDS gene. Affected members of one family were found to have a cysteine 118/119 deletion mutation.²¹ This mutation was not found by heteroduplex analysis in 60 unrelated control subjects with normal vision.²⁴

CLINICAL EVALUATION

Patients were questioned as to their subjective complaints of poor night vision, decreased central acuity, and constriction of visual field. Measurement of Snellen visual acuity, complete ophthalmoscopic examination, colour fundus photographs, and fluorescein angiography were performed to document the retinal findings.

PSYCHOPHYSICS

Static threshold perimetry was used to measure retinal sensitivities in one eye of all patients. A modified Humphrey visual field analyser²⁵ using the 30-2 and 30/60-2 programs was used under photopic and scotopic conditions. Photopic visual field testing was performed using a white Goldmann size III test spot. Scotopic perimetry was performed with a dilated pupil after 45 minutes of dark adaptation using blue (450 nm) and red (650 nm) Goldmann size V test stimuli to isolate rod and cone responses respectively.

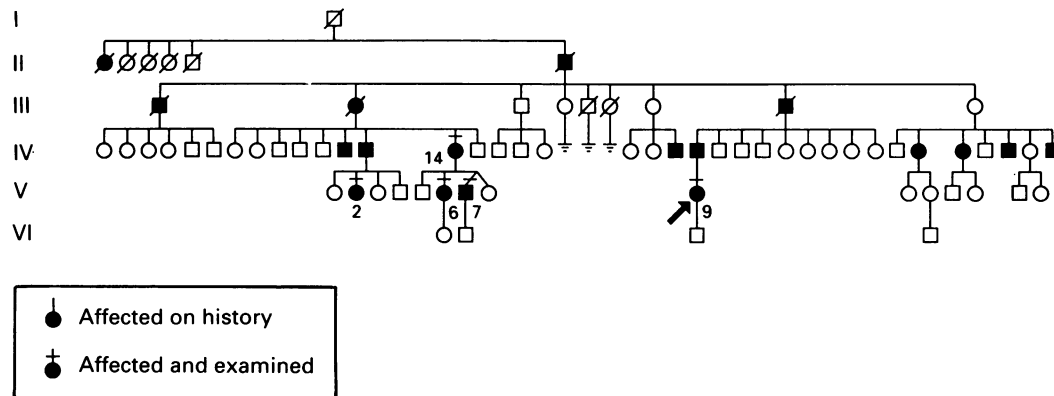
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Figure 1 Pedigree shows males (■) and female (●) known to be affected in a family with autosomal dominant retinitis pigmentosa. Arrow (↗) indicates proband. Bar = examined by the authors. Diagonal line = the patient is deceased.



Dark adaptometry was performed by measuring recovery of retinal sensitivity to white Goldmann size III and V test stimuli at two locations eccentric to fixation after intense light adaptation of 7.5 Troland seconds sufficient to bleach 95% of rhodopsin.²⁶ Baseline dark adapted sensitivity thresholds were established before bleaching. Measurements were continued until prebleach thresholds were reached.

COLOUR VISION

Colour contrast sensitivity was measured by a system fully described elsewhere.²⁷ The minimum colour contrast between letter/annulus and background at which identification of the letter/annulus is possible is 6% centrally and 25% peripherally (22° of eccentricity) for the protan, deutan, and tritan colour confusion axes in normal subjects.

ELECTRORETINOGRAPHY

The electroretinogram was recorded using standard testing protocols^{28,29} with gold foil electrodes and a Grass PS22 stroboscope as a stimulator. Further electroretinographic responses were obtained using a series of coloured flashes against different coloured backgrounds. Green (550 nm) and red (660 nm) light emitting diodes were used as a stimulus against an opposite background. This system has been fully described elsewhere.³⁰ The pattern evoked electroretinogram was also recorded as described in other reports.³¹

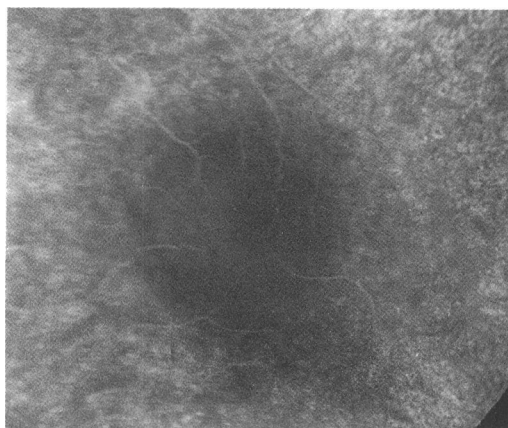


Fig 2A

Table 1 Age at examination, age at onset of symptoms, and visual acuity of subjects included in the study

Subject	Age at examination (years)	Onset of symptoms (years)		Visual acuity	
		Night vision	Visual field	Right eye	Left eye
V-2	30	<10	13	6/12	6/12
V-6	24	<10	24	6/9	6/9
V-7	29	<10	11	6/18	6/9
V-9	22	<10	20	6/9	6/12

Results

Of the family shown to have a cysteine 118/119 deletion mutation, 18 members are known to be affected (Fig 1). All affected members were aware of difficulty with night vision by the age of 10 years, and reduced visual fields during the second or third decades of life (Table 1). Reading vision deteriorated after the age of 50 years, and the oldest patient examined had no useful vision at the age of 60 years. By the third decade of life there was irregularity of pigmentation at the level of the retinal pigment epithelium in the mid-peripheral fundus which was confirmed by fluorescein angiography (Fig 2A), but little intraretinal pigmentation. Patient V-2 also had mild macular oedema (Fig 2B). By 60 years there was profound atrophy of the retina and choroid.

There was sufficient residual function in four members between the ages of 22 and 30 years (V-2, V-6, V-7, V-9) to characterise the loss (Table 1). In all the central sensitivities were within 2 log units of normal (Figs 3, 4) except in case V-7. In V-7 cone and rod thresholds were

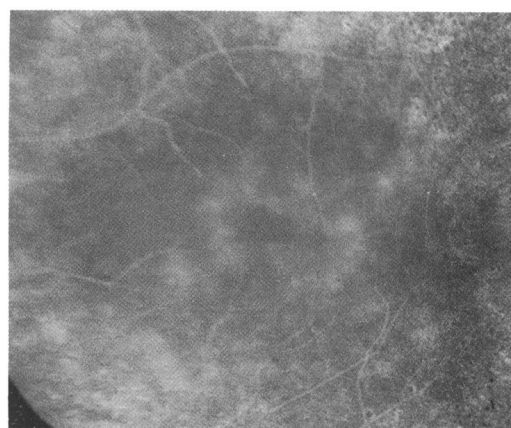


Fig 2B

Figure 2 (A) Angiogram of V-9 confirms widespread subtle retinal pigment epithelial alteration. (B) Fluorescein angiogram of patient V-2 revealing cystoid macular oedema.

Figure 3 Scotopic perimetry of the central 60° with a blue (450 nm) Goldmann size V test stimulus (A,C,E) and of the central 30° with a red (650 nm) test stimulus (B,D). (A) Patients V-2; (B,C) patient V-6; 44 (E,F) patient V-9.

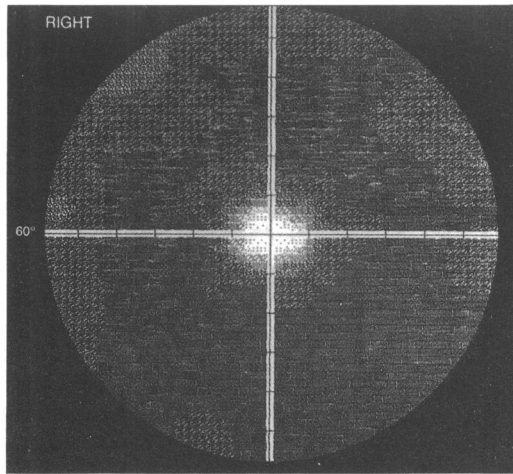


Fig 3A

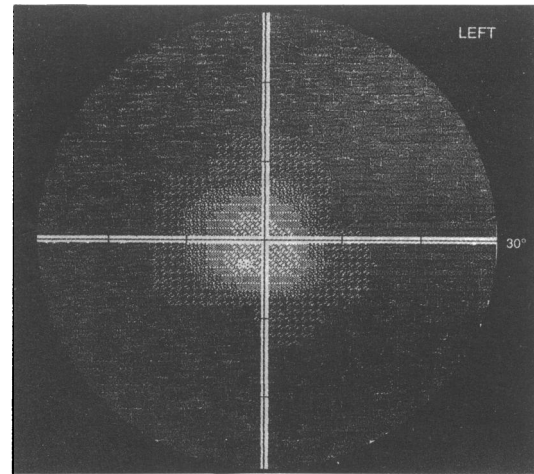


Fig 3D

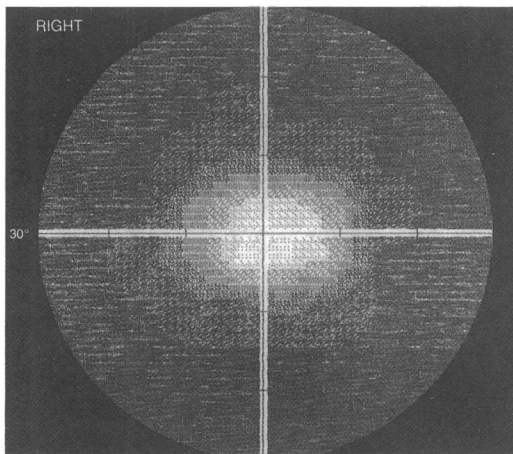


Fig 3B

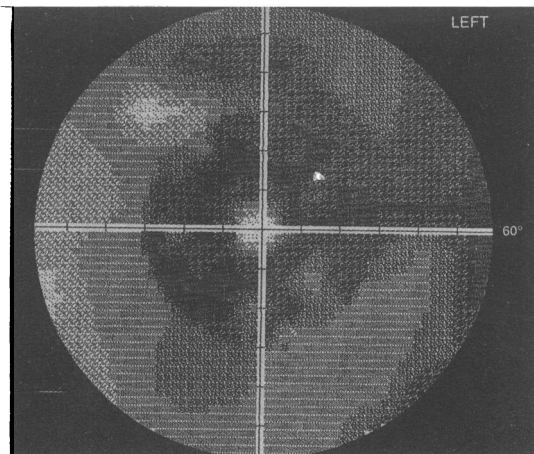


Fig 3E

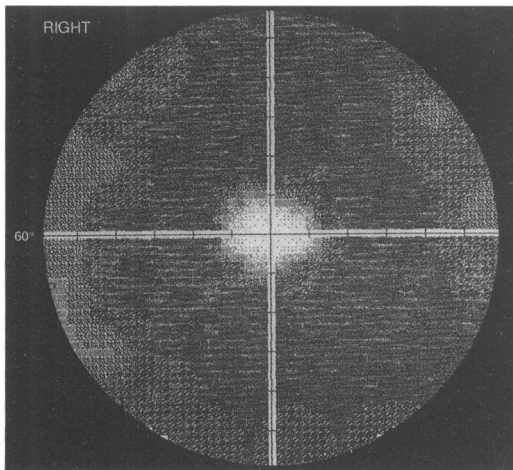


Fig 3C

elevated by 2 and 3–5 log units respectively. Depression of sensitivities was less marked under photopic than under scotopic conditions, and cone function was detectable throughout the visual field in two subjects (V-2, V-9), depression of cone sensitivities of 1–2 log units being less marked than those of rods (1–4 log units). In the other two little function was detectable outside 9°. Recovery from bleach was recorded at two paracentral points in each subject (Fig 4). At 3° of eccentricity the prebleach thresholds were elevated by 1–2 log units, the rod-cone break occurred at the normal time and final thresholds were achieved by 45 minutes. In V-2 at 9° of

eccentricity the prebleach threshold was elevated by 4 log units and the recovery was monophasic without evidence of rod contribution.

Colour contrast sensitivities were normal centrally in all four subjects but beyond 6° the objects were not seen at 100% contrast (Fig 5).

In V-7, the electroretinogram was extinguished (Fig 6) and the pattern electroretinogram was just detectable (Fig 7). The electroretinograms were severely depressed in the other three for every testing condition. Minimal cone responses were registered with the 30 Hz flicker and to a red (660 nm) flash on a green (550 nm) background. The pattern electroretinogram was also abnormal, the P50 amplitude being reduced to 50% of the normal value.

Discussion

In our family with a cysteine 118/119 del mutation in the RDS gene, affected members consistently had early onset nyctalopia, and a mild reduction of central acuity with advanced visual field constriction by the third decade of life. The findings are characteristic of retinitis pigmentosa and contrast with central retinal dystrophies which have been recorded with other mutations in the RDS gene.^{21,22}

The disease appears to be more severe than implied by initial description by Farrar *et al* of mild functional loss and late onset of symptoms with this 3 base pair deletion^{13,14} although the findings are qualitatively similar. Nightblind-

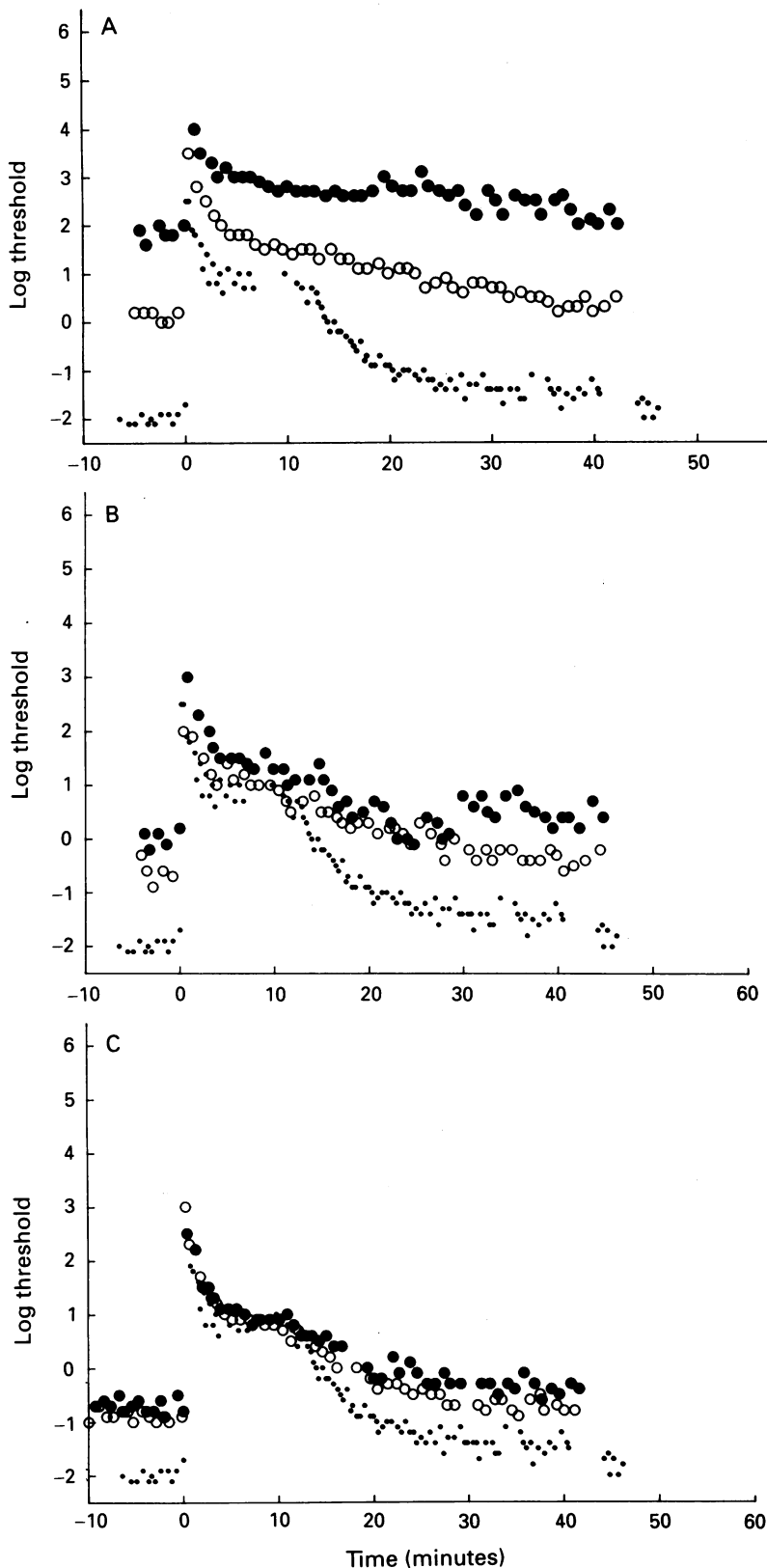


Figure 4 Dark adaptation curves. (A) Patient V-2, at 3° (○) and 9° (●) from fixation; (B) patient V-6 at 3° (○) and 90° (●) from fixation; (C) patient V-9, both symbols at 3° from fixation. The small symbols indicate normal recovery from bleach at 3° of eccentricity.

ness was described as occurring in the fourth decade with rod and cone responses lost by the fifth or sixth decades implying the type II form of RP.³²⁻³⁴ Some variability of disease expression was detected in our family as is common in autosomal dominant disease, and the inconsistency may be due to chance alone. Testing corroborated the severity of disease in our family with profound loss of both photopic and scotopic function. Isolated rod and cone sensitivities under all conditions were markedly abnormal

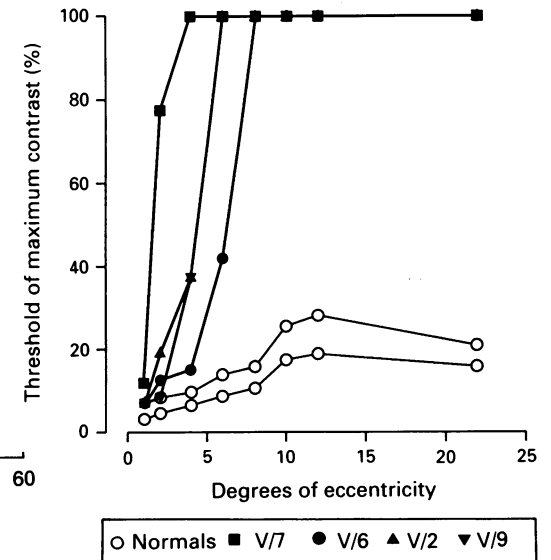


Figure 5 Colour contrast sensitivity plots of the protan axis from affected patients. Beyond 6° of eccentricity, none of the patients were able to identify coloured images at 100% contrast between image and background.

outside the central 10° of visual field in all patients tested, and by the late twenties, profound photoreceptor sensitivity threshold elevations were recorded as close as 3° from fixation. Cone dysfunction occurred concomitantly with rod dysfunction but was slightly less severe, the sensitivity of central cones in light adapted conditions being less depressed than in dark adapted conditions. This is explained by the persistence of cone-mediated contrast detection in the photopic state, but a loss of absolute cone sensitivity in the scotopic state. Colour contrast sensitivity testing detected abnormal pericentral cone function in areas where rod and cone sensitivities were thought to be relatively normal based upon static perimetric and adaptometric testing. The flash electroretinogram responses were reduced to a minimal cone response in three patients and extinguished in the fourth.

The areas of scotopic and photopic sensitivity loss are generally the same implying that both rods and cones are affected by disease. The data do not make it clear whether both photoreceptor classes are affected primarily or the cones are lost as a consequence of demise of rods. This distinction formed the theoretical basis for the division between the two classes of dominant disease.³²⁻³⁴ In those subjects with residual function sufficient to make measurement reliable it appears that the photopic system was less involved than the scotopic implying the possibility that cones may be lost as a secondary event.

Because peripherin-rds is expressed in both rods and cones, it is feasible that both rod and cone photoreceptor function are primarily affected by this mutation. To date, five mutations in the RDS gene have been identified which are associated with autosomal dominant retinitis pigmentosa phenotypes.^{14 15 21 22} In four of these, cone responses in young affected patients were significantly delayed and moderately reduced in amplitude. Furthermore, four additional RDS gene mutations, each with proved autosomal dominant inheritance have been identified as causing macular dystrophy.^{21 23} Affected patients

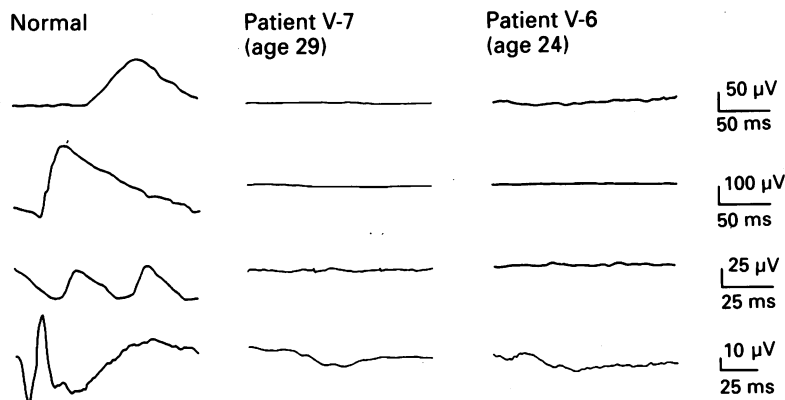


Figure 6 Electrorretinographic data from patients V-6 and V-7. Upper row: blue 460 nm flash semisaturating for rods. Second row: intense blue flash, with rod and cone activity. Third row: 30 Hz flicker. Fourth row: red 660 nm flash on green 550 nm background.

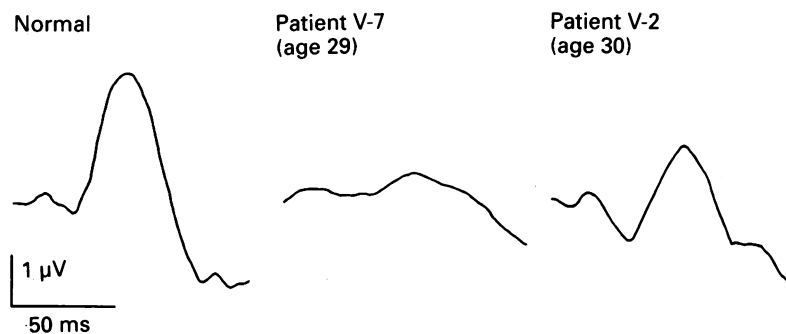


Figure 7 Pattern evoked electroretinograms from two patients. The P50 amplitude was reduced to below 25% of the normal value in patient V-7 and to 50% of the normal value in patient V-2.

with either of the arginine 172 mutations demonstrated central rod and cone sensitivity loss, there being some evidence to suggest that the cones may be a primary target of disease.³⁵

The putative function of peripherin-rds may provide an explanation for the differential involvement of both rods and cones with different mutations. Peripherin-rds is a photoreceptor protein with a primary structure of 346 amino acids, four transmembrane hydrophobic domains, and 2 N-linked glycosylation sites, one of which is conserved across four species.^{19,36} It is believed to be important to the physical structure of the outer segments, and may be responsible for the stability of the edge of the discs or maintenance of the parallel arrangement of the outer segment membranes.³⁷ It has been suggested that non-covalent bonding of peripherin-rds to ROM-1, a rod specific protein structurally related to peripherin-rds may be important in rods but this cannot be the case in cones since ROM-1 is found only in rods.³⁸ Therefore, it is conceivable that a mutation which would interfere with the binding of ROM-1 would disrupt rod structure but not that of cones. Such a distinction does not explain the pathogenesis of central and peripheral degenerations, unless functional differences exist between central and peripheral photoreceptors with respect to peripherin-rds. Such differences have yet to be sought. Consequently, a mutation of the RDS gene could theoretically alter the structural stability of rods or cones alone, or both, and produce many different phenotypes.

The expression of the RDS gene differs

markedly from that of rhodopsin in that rhodopsin expresses only in rod photoreceptors. However, from our data there appears to be no fundamental difference in disease caused by the codon 118 mutation in this family when compared with retinal dystrophy consequent upon some rhodopsin mutations.

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