ORIGINAL ARTICLES – Laboratory science

Ocular findings in a family with autosomal dominant retinitis pigmentosa and a frameshift mutation altering the carboxyl terminal sequence of rhodopsin

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Figure 1 Pedigree of an adRP family with a deletion of 8 bp in the rhodopsin gene. Solid symbols are affected members, open symbols are unaffected members. Slashed symbols are deceased members. Arrows indicate persons examined ophthalmologically. Asterisks indicate members who underwent DNA analysis.

Abstract

A family is described in which an 8 base pair deletion (nucleotides 5252–5259, codons 341–343) of the rhodopsin gene cosegregates with autosomal dominant retinitis pigmentosa (adRP). The deletion results in a shift in the reading frame, causing a rhodopsin molecule extended by one residue and substantially altered at the carboxyl terminus. Phenotypic expression is relatively mild. In affected members, night blindness did not occur before the age of 16, and late onset of visual field loss was consistently reported. Even older individuals (59 and 76 years) had preserved central islands in the visual field; a younger female patient had normal visual fields until the age of 34. ERG and psychophysical tests showed well preserved cone function at stages of virtually abolished rod function. Phenotypic differences and similarities between this form of adRP and others associated with mutations at the carboxyl terminus of the rhodopsin molecule are discussed. The cause of RP by mutations in this region remains to be clarified. (*Br J Ophthalmol* 1993; 77: 495–501)

In a subset of families with autosomal dominant retinitis pigmentosa (adRP), a variety of mutations in the gene encoding rhodopsin have been shown to cosegregate with clinical symptoms,^{1–5}

and are therefore looked upon as the primary cause of the disease. Rhodopsin mutations are found in about one quarter of adRP families.^{2,5} The vast majority are point mutations, and only two inframe deletions of the rhodopsin gene have been reported.¹ In addition, only few of the mutations found are located in the cytoplasmic domain of the rhodopsin molecule or its carboxyl terminal end, respectively.^{1–3,5} By screening European adRP families for the presence of rhodopsin mutations, two deletions at the c (carboxyl) terminal end of the rhodopsin molecule resulting in a shift of the reading frame were identified in two families.⁶ Here we report on the clinical findings in one of the families that displays a deletion of 8 base pairs (bp) spanning codons 341–343 (nucleotides 5252–5259) of the rhodopsin gene. Because of this frameshift deletion, the c terminal end of rhodopsin is radically altered; nine 'new' amino acids appear. We were interested to determine the clinical phenotype of this mutation by electrophysiological and psychophysical testing of patients, as distinct rhodopsin mutations at the c terminal end reported thus far show a wide range of symptoms.^{7–10} The reason for this variety is not yet known. Detailed clinical assessment of affected persons, however, may help to improve the understanding of disease expression in patients with different rhodopsin mutations.

Patients and methods

The German adRP family presented here (see pedigree in Fig 1) was included in the screening programme for rhodopsin mutations at the department of human genetics of the Medical University Lübeck. Screening for mutations was carried out by restriction digestion, heteroduplex analysis, single strand conformation polymorphism (SSCP) analysis, and genomic sequencing following polymerase chain reaction (PCR) amplification of the five rhodopsin exons, as previously described.^{6,11}

Four affected members were seen for clinical investigation by the authors (EA-S, HG, or EZ respectively), whereas histories from another two affected members were obtained by questioning relatives.

For each proband, an extended questionnaire was completed with respect to age at onset of

Fig 2A

Figure 2 Fundus photographs from patients with an 8 base pair deletion of the rhodopsin gene. (A) 76-year-old patient II-8, showing optic atrophy, severe retinal vessel attenuation and choroid atrophy outside the macular area. There is diffuse loss of retinal pigment epithelium and mid-peripheral bone spicule pigmentation. (B) 34-year-old patient III-2, showing multiple yellowish spots in the retinal pigment epithelium layer. Vessels are only mildly attenuated; there are no bone spicule pigmentations.

night blindness, side vision impairment, glare sensitivity, and reading difficulties. Ophthalmic examination included distance visual acuities, slit-lamp examination with respect to lens opacities and vitreous changes, direct and indirect funduscopy, fundus photography, colour vision test, kinetic perimetry, electroretinography, and, in one patient, fluorescein angiography, dark adaptation, and two colour dark adapted threshold perimetry.

Fig 2B

On slit-lamp examination lens opacities were estimated following the classification of Fishman.¹² Colour vision was examined with the desaturated Lanthony Panel D 15 test. Kinetic visual fields were obtained with a Goldmann perimeter or a Tübinger perimeter, using 30' and 10' white test spots of 200 cd m⁻² on a background of 3.3 cd m⁻². Stimuli were moved from non-seeing to seeing areas. Dark adaptation was tested over a period of 40 minutes after dilating the pupils with tropicamide (0.5%) and after a preadaptation period of 10 minutes to a ganzfeld of 890 cd m⁻². The dark adaptation curve was obtained at 20° eccentricity on the horizontal meridian of one eye, determining the luminance necessary for the detection of a 106' white stimulus (stimulus duration, 500 ms) in a Tübinger perimeter, using 0.1 log increments. Thresholds were expressed in terms of attenuation relative to maximum luminance (272 cd m⁻²). Subsequently dark adapted two colour threshold perimetry was performed along the horizontal meridian with a 500 nm and a 656 nm 106' stimulus, following the method of Massof and Finkelstein.¹³ Thresholds were measured at the fovea at 2°, 6°, 10°, and then in 10° steps towards the nasal and temporal border. After 30 minutes of dark adaptation, full field ERGs were recorded from both eyes with a Nicolet Compact Four with ganzfeld controller (Nicolet Biomedical Instruments, Madison, Wisconsin, USA), and DTL electrodes¹⁴ according to the international ERG standard.¹⁵ ERG responses were amplified, bandpass filtered, and computer averaged. An artefact rejection was applied to eliminate disturbances by blinking reflexes. Rod responses were elicited by 0.5 Hz white stimuli (duration of 100 μs). A standard flash of 2.5 cd m⁻² intensity was attenuated by 2.5 log units with a Kodak Wratten neutral density filter. A mixed rod/cone response was obtained with a 0.2 Hz white flash at standard flash intensity. In cases of non-detectable responses, we presented a 4.5 Hz stimulus with maximum luminance and averaged 200 recordings in order to detect small remnant responses. Oscillatory potentials were recorded by using a standard flash and cut off filters at 100 and 250 Hz. Photopic responses were obtained after 10 minutes of light adaptation to a ganzfeld dome background illumination of 30 cd m⁻² with white 30 Hz flicker stimuli and white single flashes at standard flash intensity. Responses to red single flashes were obtained with a Kodak Wratten W 25 filter.

Results

REPORT OF CASES

Individual I-1

No medical records were available from this man. He was reported to have severely impaired visual fields and visual acuity from his mid 50s.

Individual I-4

This woman died at the age of 61. She appeared to be completely night blind, and to have constricted visual fields and reduced visual acuity in

Fig 2C

Figure 2 (C) Fluorescein angiogram of III-2. There are multiple hyperfluorescent granules surrounding the optic disc and the macula. (D) Fundus of 4-year-old patient IV-1. There are only minimal findings of nerve fibre layer dropout superior to the optic disc.

her last years. Medical records were not available.

Proband II-8

This 76-year-old man first noted difficulties with night vision at the beginning of his third decade of life and complained of increased glare sensitivity from about age 55. He stated that side vision impairment did not become noticeable until the age of 66, when the diagnosis of RP was

Fig 2D

established. Central vision and reading acuity had markedly deteriorated in the last year. On examination, the patient had a visual acuity of 20/60 in each eye. The refractive error was +2.5 sph -1 cyl axis 35° in the right eye and +3.0 sph -1 cyl axis 135° in the left eye. Slit-lamp examination revealed a small posterior subcapsular cataract in both eyes, as well as trace of cells and a posterior detachment of the vitreous.

On funduscopy (Fig 2A), he had optic atrophy and severe vessel attenuation in both eyes. There was an overall retinal pigment epithelium loss sparing only the fovea, some apparent choroidal sclerosis in the perimacular region as well as in the midperiphery, and typical bone spicule pigmentation scattered in all quadrants of the retina. Both maculae appeared to be atrophic. Colour vision testing revealed a tritan defect in both eyes. Visual fields (see Fig 3) were constricted to about 3° for the 30°/314 cd m⁻² and about 2° for the 10°/314 cd m⁻² target of the Tübingen perimeter, respectively. In contrast Goldmann perimetry performed at the age of 68 had shown a central field of 6–8° around fixation, and preserved temporal islands for the III/4e stimulus in both eyes. The rod and the cone ERG were non-recordable.

Proband II-10

He had noted poor night vision from the age of 45, and restricted visual fields from about 10 years later. He was initially diagnosed as having RP at the age of 59. On this ocular examination, visual acuity was correctable to 20/60 right eye with a -1 D sphere, and 20/100 left eye with a -1.5 D sphere. Slit-lamp findings were normal in each eye. The fundus examination showed optic atrophy and marked vessel attenuation. There was bone spicule pigmentation all over the mid-peripheral parts of the retina. The changes were bilaterally symmetrical. In Goldmann perimetry (Fig 3), the patient's visual fields were constricted in both eyes, to 20° around fixation for the IV/4e target and about 10° for the I/4e target, respectively. Retesting 3 years later showed central islands of 10° and 5° around fixation, respectively. Scotopic and photopic ERG responses had already been non-detectable at his first visit.

Proband III-2

In the 34-year-old daughter of II-8, a strabismus of the right eye had been detected at the age of 2. She first recognised problems with night vision at the age of 16, and, only recently, a slight increase in glare sensitivity. She was not conscious of any side vision or visual acuity impairment. At the age of 30 an abnormal ERG had led to the diagnosis of RP.

On examination, visual acuity was 20/25 right eye and 20/20 left eye. The refractive error was -0.5 cyl axis 35° right eye and -0.5 cyl axis 0° left eye. There was a slight esotropia of the right eye. Slit-lamp examination did not show lens opacities or vitreous changes.

On funduscopy (Fig 2B), the optic discs appeared normal, and retinal arterioles were only moderately attenuated. The maculae showed

Figure 3 Consecutive kinetic visual fields of patients II-8, II-10, and III-2 with an 8 bp deletion in the rhodopsin gene. For convenience, targets 30°–200 cd m^{-2} and 10°–200 cd m^{-2} of the Tübinger perimeter are referred to as targets III/4e and I/4e, since they are equivalent to the test spots of our Goldmann perimeter.

Figure 4 ERG rod and cone responses in individual III-2, age 34, with an 8 bp deletion in the rhodopsin gene (right) and a normal subject approximating normal mean values (left). In III-2, the rod b wave to dim white flashes is non-detectable, the scotopic mixed rod/cone response and oscillatory potentials are severely reduced. In contrast, cone amplitudes are in the low normal range; cone implicit times are slightly increased. Calibrations are: 120 $\mu\text{V/div}$ and 20 ms/div in traces 1) and 2); 25 $\mu\text{V/div}$ and 10 ms/div in trace 3); 50 $\mu\text{V/div}$ and 15 ms/div in trace 4); and 50 $\mu\text{V/div}$ and 10 ms/div in trace 5).

only some wrinkling of the inner limiting membrane. Bone spicule pigmentations were virtually absent. In the peripapillary as well as in the peripapillary and midperipheral regions, multiple irregular yellowish spots were present in the retinal pigment epithelium layer. On fluorescein angiography (Fig 2C), they appeared as spotty hyperfluorescences with the choroidal flush. Most of them did not show marked changes during the transit, while some showed a weak staining during the late phases. Thus the majority of the spotty lesions were characteristic of pigment epithelial defects, while some of them resembled drusen in Bruch's membrane.

The desaturated Farnsworth D15 test showed perfect colour vision. Perimetry (Fig 3), performed with the 30°/314 cd m^{-2} and the 10°/314 cd m^{-2} target of the Tübinger perimeter showed normal outer field borders, but the beginning of an arcuate scotoma from 10 to 30° eccentricity in the lower temporal, and, to a smaller extent, in the lower nasal quadrants of each eye. No scotoma had been detected on a previous test with a Goldmann perimeter at the age of 29. In the ERG (Fig 4), the amplitude of the isolated rod b wave was approximately zero. Scotopic mixed rod/cone b wave amplitudes were detectable, but reduced to 15–20% of mean normal amplitudes. Oscillatory potentials were barely

Figure 5 Dark adaptometry at 20° nasally with a 106' white stimulus in normal observers (shaded area, ± 2 SD) and patient III-2 (solid line).

recordable. In contrast, all light adapted cone amplitudes were still in the low normal range, with slightly increased implicit times.

Figure 6 Dark adapted two colour threshold profiles along the horizontal meridian in normal observers (upper curve, mean (± 2 SD)) and patient III-2 with an 8 base pair deletion in the rhodopsin gene (lower curve). Top, sensitivities to 500 nm; middle, sensitivities to 656 nm; bottom, sensitivity differences between the two stimuli. In case III-2, rods detect the 500 nm stimulus in the far periphery. In all other regions, cones detect both stimuli, indicated by a sensitivity difference near zero.

The dark adaptation curve (Fig 5), determined at 20° nasally on the horizontal meridian, was monophasic, revealing absence of rod function, impaired cone function, and a final threshold elevation of 4.5 log units.

On two colour (500 nm and 656 nm) dark adapted static perimetry along the horizontal meridian (Fig 6), rod sensitivity as determined by the blue-green stimulus was reduced in all regions. Threshold elevation, however, varied, being most prominent within 30° around fixation, where cones detected the 500 nm target. At greater eccentricities, thresholds were mediated by rods.

Cone sensitivity was considerably less reduced at all loci measured. Thresholds for 656 nm stimuli were all detected by cones. In the macular area, as well as in the far periphery, sensitivity was within the low normal range. Maximum sensitivity loss was between 30 and 40° eccentricity.

Proband IV-1

The 4-year-old son of III-1 had no noticeable visual impairment at the time of our evaluation. Visual acuity in the Sheridan Gardiner test was 6/6 each eye. Colour vision was normal. Funduscopy (Fig 2D) showed no alterations besides a broadened perimacular reflex of the inner limiting membrane and a nerve fibre layer dropout superior to the optic discs. Consent for ERG testing was not given by the parents.

Person III-4

This person had no visual complaints and did not show any signs of retinitis pigmentosa on an extended ocular examination (data not shown).

A summary of the findings in patients is provided in Table 1.

DNA ANALYSIS

In patients II-8, III-2, and IV-1, heteroduplex analysis of PCR amplified rhodopsin exon 5 revealed two bands of approximately similar intensity after ethidium bromide staining (not shown). The upper band corresponded to a fragment of the expected size (219 bp), and the lower one to a fragment being smaller by about 10 bp. The non affected relative studied (III-4) showed only one (homoduplex) band. Likewise, altered electrophoretic mobility of PCR fragments resulting in various band shifts was observed in the patients' DNA by single strand conformation polymorphism (SSCP) analysis (Fig 7; for details of the method see Horn *et al.*⁶). All these findings indicated a sequence change in exon 5 of the patients' rhodopsin gene. Indeed, direct sequencing of the PCR products from two patients revealed a deletion of 8 bp (nucleotides 5252–5259 in codons 341–343) predicting a protein with significantly different amino acid composition from codon 341 onwards and an overall length of 349 residues owing to the mutation setting the normal stop signal out of frame (see Fig 8). The deletion could not be detected in another 150 unrelated adRP patients.

Table 1 Ocular findings in patients with an 8 bp deletion in the rhodopsin gene

Patient, age (years)	Eye	Visual acuity	Refractive error	Lens PSC (grade)*	Macula (main finding)	Peripheral bone spicule pigment
II-8, 76	R	20/60	+2.50-1.0×35	+2	Atrophy	In all quadrants
	L	20/60	+3.0-1.0×135	+2	Atrophy	In all quadrants
II-10, 59	R	20/60	-1.0	0	Atrophy	In all quadrants
	L	20/100	-1.5	0	Atrophy	In all quadrants
III-2, 34	R	20/25	-0.5×35	0	Surface wrinkling	Very sparse
	L	20/20	-0.5×0	0	Surface wrinkling	Very sparse
IV-1, 4	R	6/6	Plano	0	Broadened perimacular reflexes	None
	L	6/6	Plano	0		None

Patient, age (years)	Eye	Colour vision	ERG rod b wave	Mixed rod/cone b wave (μV)	30 Hz flicker Amplitude (μV)	Implicit time (ms)
II-8, 76	R	Tritan defect	NR	NR	NR	-
	L	Tritan defect	NR	NR	NR	-
II-10, 59	R	NA	NR	NR	NR	-
	L	NA	NR	NR	NR	-
III-2, 34	R	Normal	NR	83	90	67
	L	Normal	NR	93	76	67
IV-1, 4	R	Normal	NA	NA	NA	-
	L	Normal	NA	NA	NA	-

*Following the grading of Fishman *et al.*¹³ NR=non-recordable, NA=not available. ERG normal ranges (5th through 95th percentile) are 117–272 μV for rod b wave, 292–622 μV for scotopic mixed rod/cone b waves, 75–179 μV for 30 Hz flicker amplitudes, and 58–63 ms for 30 Hz flicker latencies, respectively.

Discussion

We studied five members of a family in which an 8 bp deletion of the rhodopsin gene is cosegregating with the clinical expression of retinitis pigmentosa in persons heterozygous for the gene defect, whereas one person homozygous for the wild type allele is not clinically affected. In addition, there is consecutive transmission of the

disease through at least four generations. These data strongly suggest that this mutation is causative for autosomal dominantly inherited retinitis pigmentosa, like a variety of rhodopsin mutations found thus far.

There are some characteristics in this form of adRP, which distinguish it from phenotypes of other mutations located at the c terminal end of rhodopsin. Night vision impairment does not occur before the late teens. Likewise, in this pedigree there is late onset of visual field impairment with reasonable remaining field islands even in older individuals. Cone function is well maintained, even at a stage when the rod ERG is non-recordable. While these findings may be present in some other adRP families with various rhodopsin mutations, they are not common in patients with point mutations in codon 347.^{7,8} Individuals with proline 347 leucine mutations,^{7,8} as well as patients with a proline 347 arginine⁹ or a proline 347 serine mutation (Apfelstedt-Sylla *et al.*, unpublished observation), tend to have early onset of night blindness and visual field loss, and non-recordable ERGs even at younger ages.

On the other hand, Jacobson *et al.*¹⁰ reported a family with a stop codon mutation at the c terminal end of rhodopsin (glu 344) which demonstrates late onset and mild disease roughly comparable to the findings in our adRP family. Substantial differences between the families, however, concern rod function. No ERG rod responses could be detected in a 34-year-old individual with the 8 bp deletion, while in patients with the glu 344 mutation of comparable age, Jacobson *et al.* found only moderately reduced rod b waves. On two colour dark adapted threshold perimetry, rod sensitivity in these probands was measurable at most loci of the visual field, whereas in our patient III-2, rod mediated thresholds to the 500 nm stimulus could only be shown in the outer periphery. However, rod sensitivity loss in probands of both genotypes seems to be most prominent in the pericentral regions, and in a 44-year-old individual with glu 344 mutation at a more advanced stage of disease, only cone mediated thresholds are revealed. Cone function in younger individuals of both genotypes is well preserved, as shown by normal ERG cone amplitudes at only slightly increased implicit times, and by two colour threshold perimetry. So it cannot be concluded from these data whether different degenerative patterns are operating in both genotypes or whether the different findings rather reflect differences in onset or stage of the same pathogenetic process.

In the RP family reported here, the mutation found should result in a significant alteration of the amino acid composition at the c terminal end of rhodopsin, and in the molecule which is one amino acid longer than the wild type (see Fig 8). Nine 'new' amino acids appear; one negatively charged residue (341 glu) is lost, and one positively charged appears (347 arg). The effects of these alterations on rhodopsin function are not clear.

Phosphorylation of light activated rhodopsin by rhodopsin kinase and subsequent binding of arrestin to phosphorylated rhodopsin terminate

Figure 7 Detection of the 8 base pair deletion in exon 5 of the rhodopsin gene by gel electrophoresis of the DNA. Following PCR amplification, DNA fragments of patients II-8 (lane 2), III-2 (lane 3), IV-1 (lane 5), person III-4 (lane 4), and two non-related male controls (lanes 1 and 6) were analysed. Note the numerous shifts in the electrophoretic mobility of the three patients' DNA fragments. The two bands at the bottom (marked with arrows) correspond to double stranded DNA, while all others represent single stranded PCR fragments. M=molecular weight marker.

codons	340	341	342	343	344	345	346	347	348
nucleotides	5252-5259								
wild type	ACG	GAG	ACG	AGC	CAG	GTG	GCC	CCG	GCC TAA
	Thr	Glu	Thr	Ser	Gln	Val	Ala	Pro	Ala stop
		-	*	*					
8 bp deletion	ACG	CCA	GGT	GGC	CCC	GGC	CTA	AGA	CCT GCC TAG
	Thr	Pro	Gly	Gly	Pro	Gly	Leu	Arg	Pro Ala stop
								+	

-: negatively charged residue
+: positively charged residue
*: possible phosphorylation sites

GAGA: deleted bases

Figure 8 Eight base pair deletion in the human rhodopsin gene. Nucleotide and amino acid sequences for the wild types, and for the mutant genes and proteins, respectively.

rhodopsin's activation of transducin and thereby quench the phototransduction process (for review, see Hargrave and McDowell¹⁷). Rhodopsin kinase phosphorylates serine and threonine residues at the c terminal sequence of rhodopsin.¹⁸ Out of the seven threonines and serines within codons 344-343, only two are removed in the mutant molecule. Furthermore, it has been shown that not the c terminal end but the third cytoplasmic loop of rhodopsin is crucial for rhodopsin kinase binding,¹⁹ and that rhodopsin kinase apparently tolerates some variety of amino acid sequence surrounding its substrates - that is, the threonines and serines, without impairment of its activity.²⁰ From these data it does not seem very likely that the mutation in this family has a strong effect on the efficacy of rhodopsin phosphorylation.

There are hints from tissue cell culture experiments, that some rhodopsin mutants may lead to disturbed transport of synthesised molecules to the plasma membrane, but this apparently does not hold for c terminal mutants pro 347 leu and glu 344 stop.²¹ The 8 bp mutant displayed in this adRP family has to be constructed and tested to resolve the question of whether inefficient rhodopsin transport may be a cause of RP in this genotype.

Interestingly, the last two residues of the mutant rhodopsin molecule in our family are identical with those of the wild type - that is, there is a proline next to the final residue. As mentioned above, point mutations at codon 347 resulting in the loss of proline are associated with relatively severe clinical disease. One might speculate that the presence of a proline residue at the rhodopsin c terminal end has an effect on determining clinical severity, but this is in contrast to the mild phenotype displayed in the family with the stop codon mutation at codon 344 reported by Jacobson *et al.*¹⁰

At the present time, one certainly has to be cautious in drawing conclusions concerning phenotypical characteristics and disease processes in various genotypes. Our knowledge may

expand when larger numbers of individuals can be tested clinically, and when in vitro experiments lead to better understanding of the functional defects at the molecular and cellular level caused by alterations of the rhodopsin molecule.

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