Extensive intrafamilial and interfamilial phenotypic variation among patients with autosomal dominant retinal dystrophy and mutations in the human RDS/peripherin gene

E Apfelstedt-Sylla, M Theischen, K Rüther, H Wedemann, A Gal, E Zrenner

Abstract

Clinical phenotypes of patients with mutations in the human RDS/peripherin gene are described. A 67-year-old woman, who carried a 1 base pair deletion in codon 307, presented with typical late onset autosomal dominant retinitis pigmentosa (RP). In another autosomal dominant pedigree, a nonsense mutation at codon 46 caused 'inverse' retinitis pigmentosa-like fundus changes associated with progressive cone-rod degeneration in a 58-year-old man, whereas his 40-year-old son presented with yellow deposits in the retinal pigment epithelial layer resembling a pattern dystrophy, and with moderately reduced rod and cone function, as determined by two colour dark adapted threshold perimetry and electroretinography. It is suggested that both clinical pictures within this latter family may represent manifestations of fundus flavimaculatus. The clinical data of the three patients provide further evidence for the remarkable variety of disease expression within and between families with mutations in the RDS/peripherin gene. Currently, the most comprehensive statement could be that RDS/peripherin mutations are associated either with typical RP or with various forms of flecked retinal disease.

The gene for retinal degeneration slow (rds), a mouse model of human retinitis pigmentosa, was cloned in 1989. The normal gene product is called RDS/peripherin, a protein which localises to the disc rim of photoreceptor outer segments, and which is considered to play a role in maintaining the structure of outer segment discs. In the human RDS/peripherin gene, mutations were first identified in families with autosomal dominant retinitis pigmentosa (adRP, for review see Farrar et al). Subsequently, mutations were also found to be associated with fairly different phenotypes, such as macular dystrophy, pattern dystrophies of the retinal pigment epithelium (RPE), retinitis punctata albescens, and fundus flavimaculatus. In addition, clinically distinct entities such as retinitis pigmentosa, fundus flavimaculatus, and pattern dystrophy have recently been described to occur even within one family.

Here we present clinical findings of three patients from two autosomal dominant pedigrees, which provide further evidence for wide phenotypic variation between and within families with RDS/peripherin mutations.

Methods

OPHTHALMIC EXAMINATION

For each proband, an extended questionnaire was completed with respect to age at onset of nightblindness, side vision impairment, glare sensitivity, and reading difficulties. Ophthalmic examination included distance visual acuities, slit-lamp examination, direct and indirect
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An extended ERG procedure was performed in cases of non-detectable ERG signals: in the scotopic state we presented a 10 Hz stimulus at standard flash intensity (2.5 cd/m²) and cut off filters at 5 and 30 Hz and averaged 200 recordings at a time in order to detect remnant mixed rod/cone responses. In cases of non-recordable photopic amplitudes we applied a 30 Hz flicker stimulus of 3-6 cd/s/m² with cut off filters set at 30 and 100 Hz and averaged 300 recordings.

Molecular Genetic Analysis

The three exons of the RDS/peripherin gene were amplified by polymerase chain reaction (PCR) and analysed by single strand conformation polymorphism (SSCP) and heteroduplex analyses as described elsewhere.

Results

Family 1 (see pedigree in Fig 1A)

Report of cases

Individual I-2 was reported to have impaired night vision and visual acuity since about the age of 40, and marked side vision problems since her seventh decade. By this time the diagnosis of RP was established by a local ophthalmologist.

Individuals III-1, 2, and 3 (ages 47, 37, and 33, respectively), according to outside medical records, do not show any signs of retinal degeneration.

This 67-year-old woman (patient II-2) had noted a decrease of visual acuity and a constriction of her visual fields from the end of her fourth decade of life. Night vision problems did not become noticeable before the age of about 55.

On examination, the visual acuity was 20/100 right eye with a −1 D sphere and 5/150 left eye with a −1.5 D sphere. There was an exotropia of the left eye. Biomicroscopy revealed a small posterior subcapsular cataract in both eyes as well as a trace of cells, a posterior detachment, and a synchysis scintillans (left eye only) of the vitreous. On funduscopy (Fig 2A), both eyes showed optic atrophy, severe vessel attenuation, diffuse peripheral RPE, and choriocapillaris atrophy and sparse bone spicule pigmentation. Within the central vascular arcades of both eyes, there was some preserved pigment epithelium with atrophic foveal changes. Colour vision testing (right eye) revealed a marked tritan defect. Visual fields (Fig 3A) were constricted to

Figure 2  (A) Fundus (left eye) of patient II-2 (family I, age 67), showing optic atrophy, attenuated vessels, confluent RPE and choriocapillaris atrophy, sparse midperipheral bone spicule pigmentation, and foveal atrophy. *=artefacts. (B) Posterior pole (right eye) of 58-year-old patient III-4 (family 2), showing optic atrophy, attenuated vessels, confluent RPE loss, some choriocapillaris atrophy, sparse bone spicule-like or clumpy intraretinal pigmentation, and single yellowish deposits at the RPE level (arrowheads). The foveola shows a tiny preserved patch of hyperpigmented RPE (asterisk). The multiple greyish flecks are caused by dense synchysis scintillans. (C) Right fundus of 40-year-old patient IV-3 (family 2), showing large yellowish plaques within the foveal RPE, small round spots surrounding the macula, and fuzzy flecks anterior to the upper vascular arcades. (D) Fluorescein angiogram (same eye) of IV-3, demonstrating a patterned blockade of choroidal fluorescence in the fovea, and, outside the fovea, multiple hyperfluorescent granules the result of retinal pigment epithelial defects.
Family 2 (See Pedigree in Fig 1B)

Report of cases

Individuals I-1 and II-1 were reported to have progressive loss of visual function from their midlives on. Detailed information was not available. Individual II-4 had experienced a marked loss of visual fields and reading acuity in his fifties, and was reported to have become blind at about the age of 70. Individual III-2, a 57-year-old woman, was reported to have a macular degeneration. She lives abroad and was therefore not available for further investigation.

Proband III-4. This 58-year-old man first noted visual problems in mesopic environment and increased glare sensitivity in his second decade. Visual acuity and colour discrimination then deteriorated in his twenties. A loss of both night vision and visual fields became noticeable in his fourth decade of life. Since then he had experienced a progressive course of the disease finally leading on to an inability to read in his fifties.

At the age of 27, an ophthalmologist had established the diagnosis of Stargardt’s macular dystrophy, but 20 years later, when examined in another clinic, he was looked upon as suffering from retinitis pigmentosa. By that time, some white flecks at the posterior poles were recorded as peculiar features of the fundus.

On our examination, visual acuity was 20/200 right eye with −0·5 cyl axis 35° and 1/200 left eye with −0·25 sph. Biomicroscopy revealed clear lenses, but marked bilateral synchysis scintillans of the vitreous. On funduscopy (Fig 2B), he had optic atrophy, moderate vessel attenuation, and confluent RPE atrophy with some choroidal sclerosis extending from the posterior poles to the midperiphery, sparing only the foveola of the right eye where a tiny patch of hyperpigmented RPE could be seen. Within the atrophic RPE zones some bone spicule-like or clumpy pigmentations as well as single whitish flecks were scattered. The far fundus periphery appeared unchanged. Colour vision could not be tested because of poor vision. Kinetic perimetry (see Fig 4A) revealed a large central scotoma (left eye) and a ring scotoma (right eye), respectively, each extending to 30–40° peripherally and leaving a central island of about 1° diameter in the right eye only. Outer visual field borders were normal. On two colour dark adapted threshold perimetry of the right eye (Fig 5A), a regional degenerative pattern of both rods and cones was exhibited. There was an absolute scotoma corresponding to that found in photopic kinetic perimetry. Within the preserved temporal and nasal outer fields, rods detected the blue-green, and cones the red stimuli. Sensitivity losses to both stimuli ranged from 1·5 to 3 log units. In the ERG (Fig 6) there was a roughly equal reduction of rod and cone function, with amplitudes reduced to 10–15% of normal mean values. Peak times of isolated rod responses and scotopic combined rod/cone responses were slightly delayed, and those of 30 Hz flicker as well as single white and red flash cone responses were grossly delayed. Oscillatory potentials were nonrecordable.

Proband IV-3. This 40-year-old man denied having any visual problems. On examination,
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![Figure 3](image)

**Figure 3** (A) Kinetic visual fields of patient II-2 (family I), showing marked peripheral field loss. (B) ERG (right eye) of II-2, showing small residual responses to scotopic 10 Hz and photopic 30 Hz white flicker stimuli. Cone peak times following 30 Hz flicker stimulation are largely increased. Calibrations are 5 µV/div and 15 ms/div (10 Hz flicker) and 2.5 µV/div and 7.5 ms/div (30 Hz flicker), respectively.

Visual acuity was 25/20 with a correction of −0.5 cyl A 0° in both eyes. Biomicroscopy revealed clear lenses. On funduscoppy (Fig 2C), the optic discs and the retinal vessels appeared normal. However, multiple yellow-white, medium-sharp defined deposits varying in shape and size could be seen within the RPE layer. Large pliciform plaques were surrounding and partially invading the foveae. Smaller round deposits were scattered in the paramacular areas, and in several posterior midperipheral regions small linear flecks were arranged in a reticular pattern. No bone spicule pigmentation were seen. The equatorial and anterior fundus regions appeared normal. On fluorescein angiography of the right eye (Fig 2D) multiple hyperfluorescent spots appeared in the posterior pole, sparing only the fovea. They did not change during the transit nor stain in the late phases, thus being characteristic of RPE defects. In the fovea, there was a blockade of choroidal fluorescence corresponding to the large deposits seen on funduscoppy, which showed a remote resemblance to a butterfly lesion. The desaturated Farnsworth D15 test revealed mild tritanomaly in both eyes. Static perimetry (Fig 4B), performed with the Tubinger automated perimeter, revealed normal outer field borders, but scattered paracentral scotomas in the inferior fields of both eyes extending from the inferior foveal edge to about 25° peripherally. Marked sensitivity losses (>25 dB) predominated in the right eye which corresponded to the areas of major RPE defects detected by fluorescein angiography, whereas mild threshold elevations (>5-10 dB) were found in the left eye. Two colour dark adapted static perimetry along the vertical meridian of the right eye (Fig 5B) revealed a more widespread area of subnormal rod and cone sensitivity, covering the central 30° superiorly and the central 40° inferiorly. Rod sensitivity (to 500 nm) was reduced by about 0.5 log units at 30-40° eccentricity and by 1-1.5 log units within the central 20°. Cone sensitivity to 656 nm, however, was still within normal limits or near normal between 6° inferiorly and 10° superiorly. Cone threshold elevations in the remaining loci varied between 0.5 and 1.5 log units. Sensitivity differences confirmed a roughly equal functional loss of rods and cones with a slight preponderance of rod impairment in the perifoveal regions. In the ERG (Fig 6), isolated rod signals, scotopic mixed rod/cone amplitudes, oscillatory potentials, and light adapted 30 Hz flicker and single flash cone amplitudes were all reduced to about one third of normal mean values. Peak times of rod and cone signals were within the normal range.

Persons III-3 and IV-2 both have no subjective visual complaints and do not show any signs of retinal disease. The offspring of IV-3, individuals V-1 to 3 have not complained of visual problems and currently are not available for oculар examination.

**Molecular genetic findings**

DNA samples of III-4 and IV-3 showed a particular SSCP pattern for exon I of the RDS/peripherin gene. Direct sequencing revealed a C-to-T transition (CGA to TGA) at codon 46 creating an in frame translation stop signal (for details see Meins et al13).

**Discussion**

We studied the clinical expression of RDS/peripherin mutations in three individuals from two different pedigrees. Our data provide further evidence for the highly varying phenotypic expression of RDS/peripherin gene defects between and within families. In one of our patients, who carries a 1 bp deletion in codon 307, the phenotype is a late onset adRDP with typical features like disc pallor, vessel attenuation, bone spicule pigment deposits, and peripheral visual field loss. However, the widespread choriocapillaris atrophy observed is not a constant finding in RP. It may be concluded from the history of subjective symptoms in both the
patient and her affected mother that rod function loss occurred comparatively late and did not substantially exceed impairment of cone function, thus suggesting a 'type 2' RP. This is not unexpected, because RDS/peripherin is expressed both in rods and cones, and mutations can therefore affect both photoreceptor types. Comparable phenotypes with rather mild disease have been described in other patients with RDS/peripherin mutations. The 1 bp deletion causes a shift of the reading frame and thereby a substantial alteration of the carboxyl terminal amino acid sequence with a premature termination of translation at codon 323. This domain of the molecule is not conserved among different species, and its precise function is unknown.

The retinal disorders found in our patients with the Arg-46-stop mutation are more difficult to classify. One 40-year-old man (IV-3) shows yellow-white deposits at the RPE layer, which are most striking in the fovea of both eyes. An accumulation of yellowish lipofuscin-like material in the foveal RPE is a characteristic of autosomal dominant pattern dystrophies of the RPE. Nichols et al. found that patients with a missense mutation in codon 167 of the RDS/peripherin gene had butterfly-shaped pattern dystrophy. The fundus appearance of one of their patients does not differ substantially from that found in IV-3. As a rule however, individuals with pattern dystrophies have normal ERGs and so did the patients with the codon 167 mutation. In contrast, our proband had clearly abnormal ERG results, a finding which has been only sporadically described in pattern dystrophies. In addition, IV-3 has moderate but widespread rod and cone sensitivity loss in dark adapted threshold perimetry, a finding which would be unexpected in macular pattern dystrophies. Gass pointed out that the funduscopic findings in fundus flavimaculatus, a diffuse condition, may in some cases be hardly distinguishable from those in pattern dystrophies.

The older patient (III-4) with the Arg-46-stop mutation presents with RP-like fundus changes and, with respect to the sequence and pattern of his visual function loss, could be considered as having cone-rod dystrophy (CRD). Patients with CRD differ from those having typical RP by complaining of visual acuity loss rather than of nightblindness, by having pigmentary changes that are most prominent within the macular region and not in the midperipheral fundus, and by showing cone ERG impairment that is greater than or equal to rod impairment. On the other hand, cone-rod dystrophies tend to have early onset with severe visual impairment even at younger ages, and this obviously does not hold for patient III-4. In addition it is worth mentioning that at earlier stages of the disease he had a 'flecked retina', which is not a feature of CRD, and was diagnosed as having Stargardt's macular dystrophy. Therefore we suggest that the conditions of both patients are disparate manifestations of fundus flavimaculatus. The term 'fundus flavimaculatus' was introduced in 1962 by Franceschetti to describe the fundus of patients with linear and round, deep yellow, fleck-like deposits in the deeper retinal layers. Later it has been suggested that Stargardt dystrophy and fundus flavimaculatus are the same entity. Typical fundus flavimaculatus is a progressive autosomal recessive disease, which may or may not be associated with atrophic macular lesions and consecutive decrease of central vision; the ERG is usually normal or shows slight cone function impairment. However, fundus flavimaculatus rarely may evolve into a condition including disc pallor, retinal arteriolar attenuation, pigmentary deposits, diffuse pigment epithelial or retinochoroidal atrophy, disappearance of the yellow flecks, and marked cone and rod ERG impairment, thus mimicking a form of retinitis pigmentosa. In addition, there have been a few reports on autosomal dominant inheritance of fundus flavimaculatus. Krill observed considerable variation among affected members of autosomal dominant pedigrees, with the majority of patients showing a mild form and single members suffering from severe progressive cone-rod disease. This is also true for the family with the Arg-46-stop mutation presented here. We therefore believe that 'fundus flavimaculatus' is the most appropriate term to describe the clinical phenotype of this family.

Figure 4 (A) Kinetic visual fields of patient III-4 (family 2). There is a large ring scotoma in the right eye and a large central scotoma of the left eye respectively. Outer field borders are normal. (B) Static 30° visual fields of IV-3 (family 2) showing inferior paracentral scotomas.
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Similar findings – that is, a flecked retina in a younger patient and widespread photoreceptor function loss in her father, have been described in a family with a 2 bp deletion in codon 25 of the RDS/peripherin gene. The authors, however, used the term ‘retinitis punctata albescens’ to describe that condition.

RDS/peripherin is believed to play a role in maintaining the structure of outer segment discs. How can mutations in this protein compromise photoreceptor outer segment stability? In mice heterozygous for the rds mutation there should be a reduced amount of peri- pherin in the photoreceptor membrane (null allele). Apparently this insufficiency causes ballooned, swollen, and distorted rod outer segments. A similar pathomechanism may act in human RDS/peripherin null alleles. Thus far phenotypes of two presumed null mutations have been described in detail: one nonsense mutation at codon 258, presenting as adult onset vitelliform macular dystrophy, and a frameshift mutation at codon 25 which creates a translation stop at codon 42 and which is associated with ‘retinitis punctata albescens’. We suggest that the Arg-46-stop mutation described herein also represents a null allele, because the short length of the encoded protein should lead to an instability of the gene product. Thus one can consider fundus flavimaculatus to be another human phenotype comparable with rds in mice. The Arg-46-stop mutation has been previously reported to be associated with adRP. Detailed clinical data, however, have not been published. The extensive clinical variety of null allele phenotypes is surprising and unexplained. Additional genetic as well as non-genetic factors may play a role, which could be further studied once larger numbers of genetically characterised patients are available for examination.

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