SCIENTIFIC REPORT

Novel rhodopsin mutations and genotype-phenotype correlation in patients with autosomal dominant retinitis pigmentosa

A Schuster, N Weisschuh, H Jägle, D Besch, A R Janecke, H Zierler, S Tippmann, E Zrenner, B Wissinger

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Aim: To identify novel or rare rhodopsin gene mutations in patients with autosomal dominant retinitis pigmentosa and description of their clinical phenotype.

Methods: The complete rhodopsin gene was screened for mutations by DNA sequencing in index patients. Mutation specific assays were used for segregation analysis and screening for controls. Eight patients from five families and their relatives were diagnosed with autosomal dominant retinitis pigmentosa (adRP) by means of clinical evaluation.

Results: Mutation screening identified five different rhodopsin mutations including three novel mutations: Ser176Phe, Arg314fs16, and Val20Gly and two missense mutations, Pro215Leu and Thr289Pro, that were only reported once in a mutation report. Electrophysiological and psychophysical testings provide evidence of an impaired rod system with additionally affected cone system in subjects from each genotype group. Visual function tended to be less affected in subjects with the Arg314fs16 and Val20Gly mutations than in the Ser176Phe phenotype. In contrast, Pro215Leu and Thr289Pro mutations caused a remarkably severe phenotype.

Conclusion: The ophthalmic findings support a correlation between disease expression and structural alteration: (1) extracellular/intradiscal Val20Gly and cytoplasmic Arg314fs16 mutation—mild adRP phenotype; (2) Ser176Phe mutation—"mostly type 1" disease; (3) predicted alteration of transmembrane domains TM V and TM VII induced by Pro215Leu and Thr289Pro—severe phenotype. However, variation of phenotype expression in identical genotypes may still be a typical feature of RHO mutations.

R etinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of inherited retinal degenerations.[1] Patients with RP experience night blindness, visual field constriction, and eventually loss of central vision, in most cases caused by degeneration of the photoreceptor cells of the retina.[1] [2] There are autosomal dominant (adRP), autosomal recessive (arRP), X linked (xlRP), and rare mitochondrial or digenic forms.[1] To date, 13 gene loci are known for adRP,[3–13] 21 for arRP, and five for xlRP (RetNet http://www.sph.uth.tmc.edu/RetNet/).

The first photoreceptor specific gene found to be mutated in adRP was rhodopsin (RHO).[14] [15] Rhodopsin is the light absorbing molecule that initiates the signal transduction cascade in rod photoreceptors. Mutations of the rhodopsin gene account for approximately 25% of adRP.[16] Numerous mutations in the RHO gene have been identified, most of them being point mutations.[15] The analysis of mutant rhodopsins suggest that some RP mutations impair protein folding, 11-*cis* retinal chromophore binding, G-protein coupling/activation, and/or cellular trafficking of the rhodopsin protein.[17] [18]

In this study we report the results of the RHO gene screening in five independent adRP families.

PATIENTS AND METHODS

Recruitment of patients, DNA isolation, and mutation analysis

Eight patients were included into the study, all suffering from adRP. The study was conducted in accordance with the tenets of the Declaration of Helsinki.

Mutation detection by direct sequencing

Patient DNA was extracted using a standard salting out procedure.[19] For mutation detection by sequencing, PCR was performed using corresponding sense and antisense primers and 1 U Taq polymerase (Eppendorf, Hamburg, Germany). PCR products were separated on a DNA capillary sequencer (ABI 3100 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA).

Mutation detection by DHPLC

Denaturing high performance liquid chromatography (DHPLC) analysis was conducted with the WAVE nucleic acid fragment analysis system equipped with a L-7400 UV detector (Transgenomic, Omaha, NE, USA). Samples with aberrant profiles were sequenced.

Mutation detection by RFLP

A 588 bp PCR product encompassing Exon 1 was digested with 1 U *RsaI* restriction enzyme (NEB, Beverly, MA, USA). The Val20Gly missense change results in the loss of one of three genuine *RsaI* restriction sites. Restriction digest for normal individuals results in two fragments of about 420 bp and 160 bp that can be visualised on a 4% agarose gel. For individuals heterozygous for the Val20Gly mutation, restriction digest results in an additional fragment of 178 bp.

Clinical studies of the *RHO* mutation phenotype

Phenotype analysis comprised clinical examination, Goldmann perimetry, Panel D15 testing, dark adapted final thresholds, Ganzfeld electroretinography (UTAS 2000 system; LKC Technologies, Gaithersburg, USA), according to ISCEV standard[20] and multifocal electroretinography using the VERIS system (EDI, San Francisco, CA, USA).[21] [22]

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Table 1 *RHO* mutations

Nucleotide sequence alteration	Consequence	Location	Mutation carriers/ families tested	Mutation carriers/ controls	Previously reported
c.59T>G	Val20Gly	Exon 1	6/10	0/100	No
c.527C>T	Ser176Phe	Exon 2	1/1	0/100	No
c.644C>T	Pro215Leu	Exon 3	5/12	0/100	See ref 23
c.865°>C	Thr289Pro	Exon 4	1/1	0/100	See ref 23
c.942insG	Arg314fs16	Exon 5	1/1	Not performed	No

## RESULTS

Mutation analysis

The index patient of each family was screened for mutations in the *RHO* gene. Table 1 summarises the respective sequence alterations. Three of them were novel mutations (Val20Gly, Ser176Phe, Arg314fs16), and the other two mutations (Pro215Leu, Thr289Pro) have only been reported once in a brief mutation report without clinical details.[23]
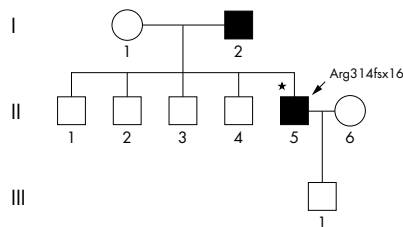
Clinical studies
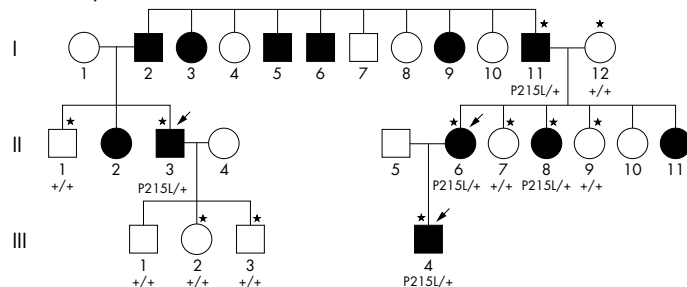
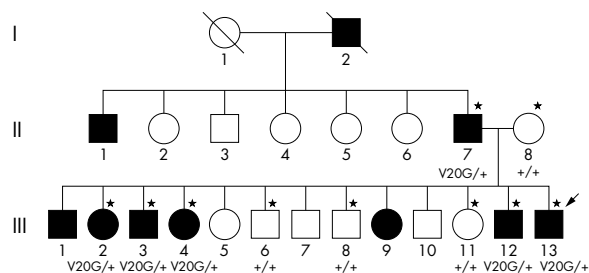Figure 1 (A to E) shows the pedigree of the patients/families investigated.
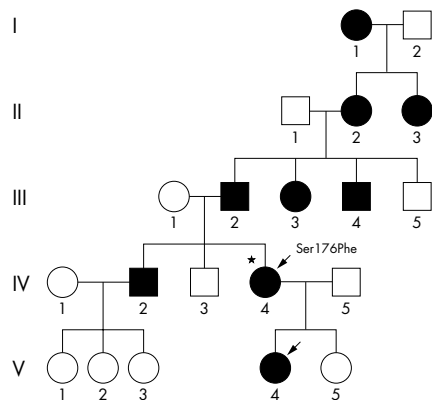
Figure 1 (A to E) Pedigrees of adRP families. Circles, females; squares, males; solid symbols, affected members; open symbols, unaffected members; slashed symbols, deceased members; arrows, persons examined ophthalmologically; asterisks, DNA analysis performed.
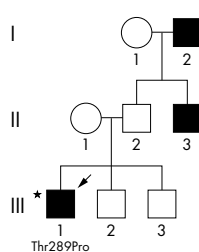
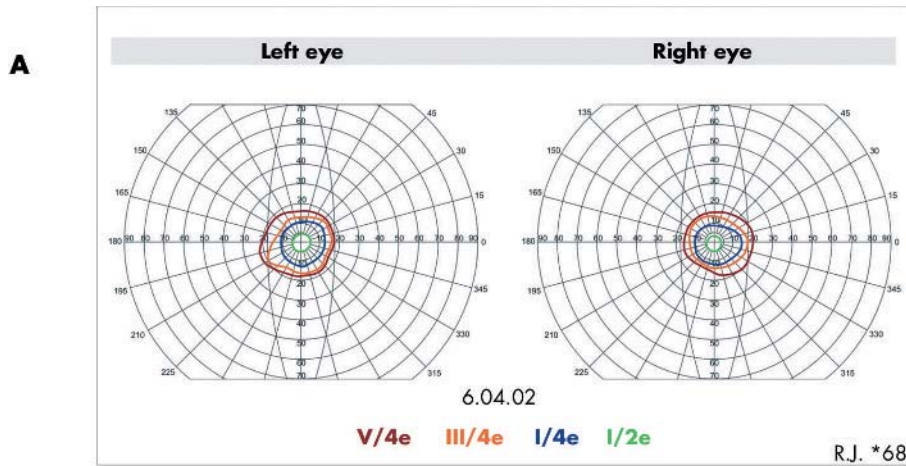Figure 2 Patient IV:4 (family 1; Ser176Phe). (A) Perimetry (targets V/4e, III/4e, I/4e, and I/2e). (B) MfERG responses showing 61 single traces of right eye (RE) (right lane) and left eye (LE) (left lane). (C) Ganzfeld-ERG. Left column: waveforms (top to bottom) are isolated rod b-wave of RE (1-R, 3-R, 5-R) and LE (2-L, 4-L, 6-L), scotopic mixed rod/cone response of RE (7-R) and LE (8-L). Right column: photopic 30 Hz flicker response of RE (1-R) and LE (2-L). Traces 3-R and 4-L show 30 Hz repetition. "1": peaks of the 30 Hz flicker response. Left column: calibrations: 5 microV/div;5 ms/div (traces 1-R; 2-L) and 50 microV/div;5 ms/div for other traces. Right column: calibrations 20 microV/div;3 ms/div.
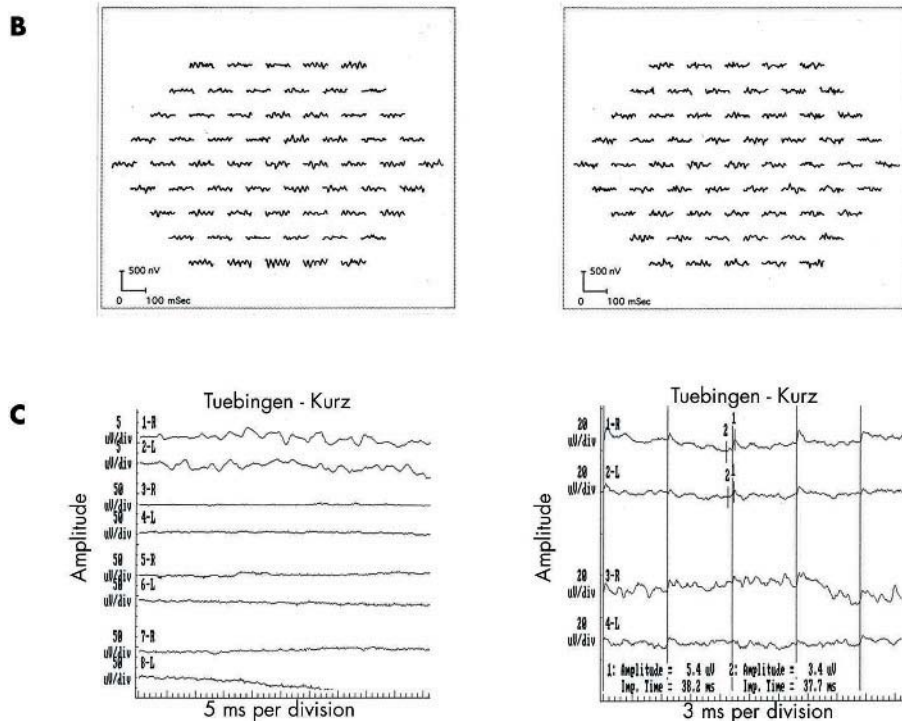
The clinical characteristics of affected patients are summarised in table 2. Original phenotype data is indicated in figures 2–4.

DISCUSSION

We studied patients from five independent families showing typical clinical features of autosomal dominant RP. Two of the novel mutations were missense mutations, and one was a 1bp insertion (c.942insG) that results in a frameshift and subsequent translation termination.

We found considerable relation between the individual mutation and disease expression. Cideciyan and colleagues distinguished two patterns of rod disease expression in a variety of rhodopsin mutations.[24] Other widely accepted classification systems have been developed by Massof & Finkelstein,[25] Lyness et al,[26] and Fishman et al.[27] According to the latter systems the phenotypes of our study can be subdivided into distinct groups.

Patients with the novel mutations Arg314fs16 (family 2) and Val20Gly (family 4) express a remarkably mild "mostly type 2" phenotype[25] with late onset of symptoms and a more favourable visual prognosis or "R"-type[26] and the Ser176Phe phenotype (family 1) discloses an intermediate "mostly type 1" or "D-" phenotype. In contrast, the Pro215Leu (family 3) and Thr289Pro (family 5) mutations result in a severe type 1- or D-phenotype with early onset of symptoms and rapid loss of visual field area, corresponding with a diffuse and progressive loss of rod and cone function.

Based on current models of rhodopsin (fig 5), two of the novel mutations (Val20Gly and Ser176Phe) involve amino acids on the intradiscal/extracellular side and one occurs at the cytoplasmic side (Arg314fs16). Mutations Pro215Leu and Thr289Pro involve transmembrane domains.

Considering intradiscal/extracellular mutations, it has been reasoned that missense mutations affecting residues 2–4[28] or 15–17[29] interfere with N-glycosylation or that the
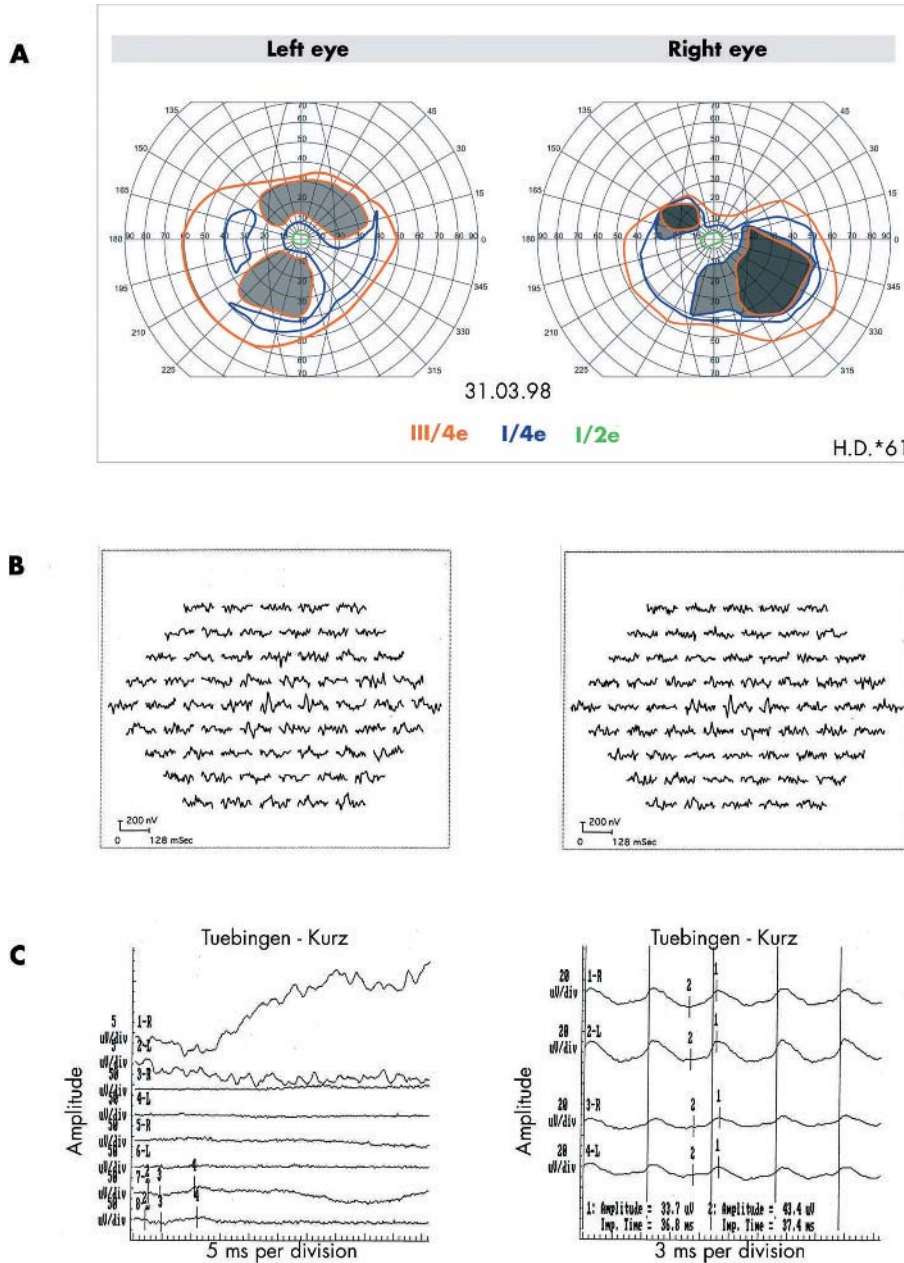
Figure 3 Data of patient II:5 (family 2; Arg314fs16). (A) Perimetry (targets III/4e, I/4e, and I/2e). (B) See figure 2B. (C) Ganzfeld-ERG: waveforms are indicated as in figure 2. ''3'' indicates a-wave, ''4'' indicates b-wave peak. Peaks of 30 Hz flicker signals are indicated by ''1''. Calibrations: see figure 2.

replacement of cysteines 110 and 187 prevent the formation of the disulfide bridge.[15]

It could be speculated that the Ser176Phe mutation may induce a structural alteration of the cysteine 187 neighbourhood.

The Val20Gly missense mutation is close to previously described mutations in the N-terminal region of the polypeptide. The phenotype of patient III:13 carrying this novel mutation was extremely mild with moderately affected rod and cone function even at age 34.

The c.942insG mutation causes a frameshift mutation with premature stop codon that results in an alteration and shortening of the cytoplasmatic domain, the first rhodopsin mutation to be described in a Bosnian family. The phenotype of the single available patient is relatively mild with late onset symptoms and relatively well preserved central cone function. Cideciyan and colleagues described the phenotype of other C-terminal truncated mutations (Gln312X and Gln344X) and classified them as class B mutants.[24]

The Pro215Leu and Thr289Pro mutations involve amino acid exchanges within transmembrane domains, and both mutations eliminate or introduce proline residues. Recent publications described mutations perturbing critical interhelical interactions between TM III and TM V, namely the Glu122, His211 salt bridge, resulting in a severe type of adRP in vivo.[30–33]

The Thr289Pro phenotype (family 5, individual III:1) may serve as another example of severe type adRP induced by a missense mutation in TMVII. Within TM VII, 11-cis-retinal covalently binds to opsin at the epsilon amino group of Lys296.[34 35] The phenotype presented in our study parallels many features of the unusually severe Lys296Glu phenotype.[36 37]

Both intrafamilial and interfamilial phenotype differences among carriers of identical rhodopsin mutations have been described.[38 39] On the other hand, a disease course fairly constant in all affected persons within the same large pedigree has also been documented.[40]

Table 2 RHO Mutations and Phenotype

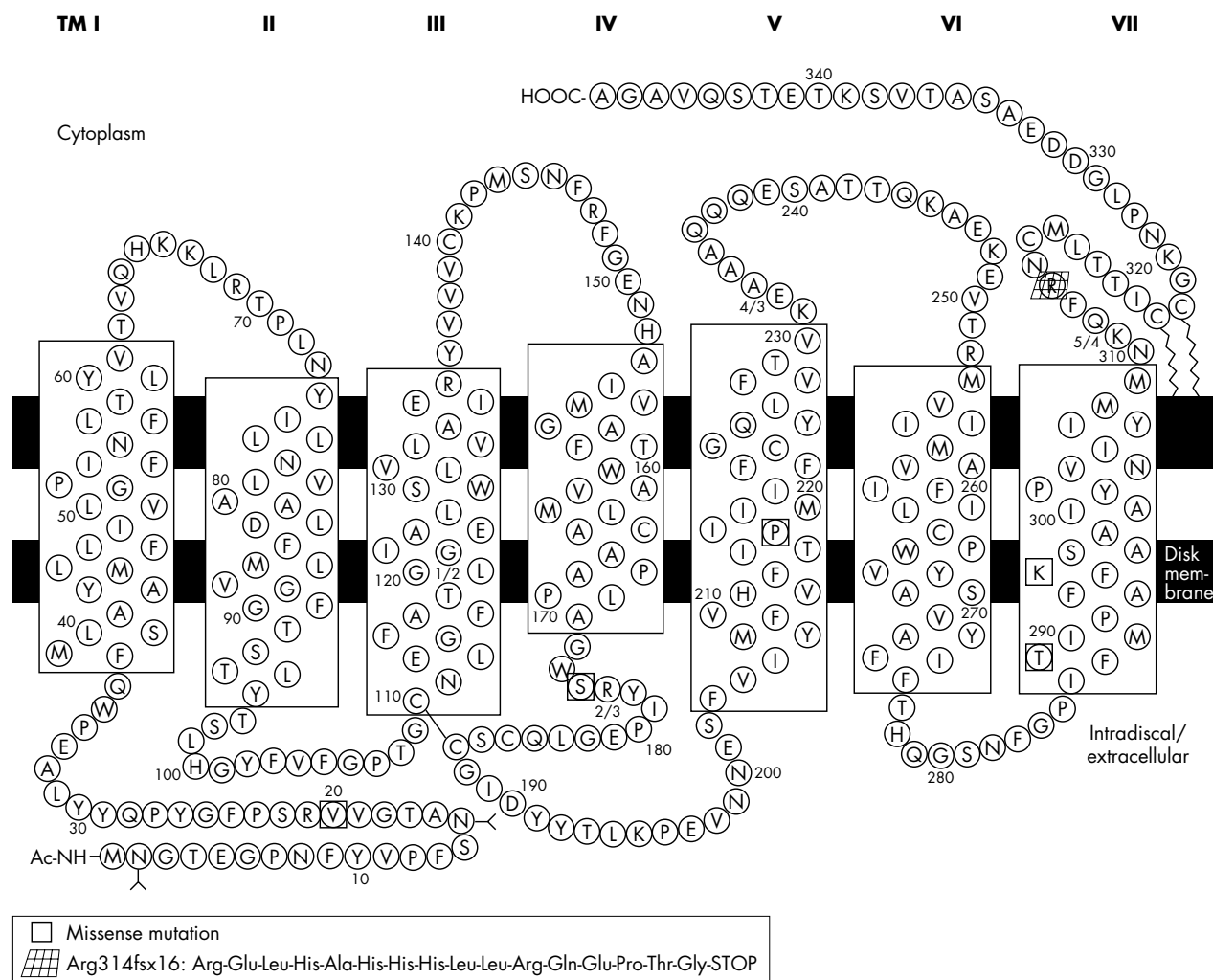| Family number/mutation	Age of onset/mode of onset (age)	VA: RE/LE initial presentation (age)	Refractive error: RE/LE	Visual field: RE/LE (age)	ERG	Fundus	DA/panel D15
1/Ser176Phe (German)	IV:4 Night blindness; field constriction (13) Loss visual acuity (30)	60/100; 60/100 (33)	−1.75/−1.75	Constriction to 15° (III/4e 90°) (33)	Scotopic: noise level Cone 30Hz-flicker: residual responses (33)	Slight optic atrophy, vessel narrowing, absent macular reflexes, peripheral pigment mottling, slight peripheral hyperpigmentation	Significant elevation/desaturated: normal
	V:4 Night blindness, glare sensitivity (2)	80/100; 100/100 (6)	+1.5/+1.5	Near normal (III/4e), constriction to 10–20° (I/4e 90°) (6)	not performed	Normal optic discs, peripheral RPE atrophy without bone spicules	Desaturated: normal
2/Arg314fs16 (Bosnian)	II:5 Night blindness, glare sensitivity, field constriction (33)	100/100; 120/100 (36)	Emmetropic	Constriction to 50–60° with large scotomas 15–40° (III/4e 90°) (36)	Scotopic: amplitude reduction to 10% normal range Photopic: amplitude reduction to 50% normal range MfERG: preserved foveal cone responses	Mild optic atrophy, moderately narrowed vessels, atypical macular ILM reflexes; bone-spicules in lower sector, diffuse RPE atrophy	Significant elevation/desaturated: normal
3/Pro215Leu (German)	II:3 Night blindness (birth), visual acuity loss, field constriction (33), glare sensitivity (45)	10/200; 10/200 (48)	+1.25/+1.25	Constriction to 5–7° (III/4e 90°) (48)	Noise level (48)	Waxy optic nerve atrophy, vessel attenuation, RPE atrophy, and hyperpigmentation	Significant elevation/saturated: disturbances all axes
	II:6 Night blindness (birth)	No details	No details	Constriction to 5–10° (III/4e 90°) (35)	Noise level (35)	No details	No details
	III:4 Night blindness (birth), not aware of other symptoms	120/100; 80/100 (16)	+2.5/+2.75	Constriction to 10–25° (right eye), 7–15° (left eye) (III/4e 90°) (16)	Rod responses: noise level; cone 30 Hz: residual responses (16)	Normal optic disc, slightly narrowed vessels, cystoid macula oedema, peripheral RPE atrophy with hyperpigmentation	Significant elevation/desaturated: slight disturbances all axes
4/Val20Gly (Austrian)	III:13 No symptoms	100/100; 80/100 (34)	0; +3.5 RE: cataract surgery at age 27	Not performed	Scotopic and photopic amplitude reduction with rest function; Electro-oculogram: no light peak, pathological Arden ratio	Absent macular reflexes, narrowed vessels, mid-peripheral hyperpigmentation	No details
5/Thr289Pro (Austrian)	III:1 Night blindness, field constriction (3), color vision (9), glare sensitivity (12), visual acuity (18), cataract extraction (27)	10/250; 10/250 (27)	Emmetropic	Constriction to <5° (III/4e 90°)	Scotopic/photopic noise level; MfERG noise level	Temporal optic disc pallor, no macular reflexes, mid-peripheral hyperpigmentation	Significant elevation

In summary, the phenotype description of Ser176Phe gives at least consistent examples of mother (IV:4) and daughter (V:4) expressing an intermediate type of adRP with early onset of symptoms, but possibly long time preserved visual acuity. For the Arg314fsX16 and Val20Gly mutations, we could describe an unusually mild phenotype. The alteration of transmembrane helices TM V or TM VII by the Pro215Leu and Thr289Pro missense mutation lead to severe adRP. However, more interfamilial and intrafamilial clinical data will be necessary to draw conclusions on a constant genotype-phenotype correlation in these mutations.

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Authors' affiliations
A Schuster, H Jägle, D Besch, E Zrenner, University Eye Hospital, Department of Neuroophthalmology, Tuebingen, Germany
N Weisschuh, S Tippmann, B Wissinger, Molecular Genetics Laboratory, Tuebingen, Germany
A R Janecke, Department of Medical Genetics, and Molecular and Clinical Pharmacology, Innsbruck Medical University, Austria

Figure 4 (A) Fundus LE (patient II:3; family 3; Pro215Leu). Nasal periphery (top) and posterior pole (bottom). (B) Fundus LE (patient III:4; family 3; Pro215Leu). Nasal periphery (top) and posterior pole (bottom).

Figure 5 Two dimensional model of rhodopsin (mutations boxed). The TM helices are labelled I-VII. Arg314fs16 mutation: Arg-Glu-Leu-His-Ala-His-His-His-Leu-Leu-Arg-Gln-Glu-Pro-Thr-Gly-STOP.

H Zierler, Institute of Medical Biology and Human Genetics, University of Graz, Austria

Correspondence to: Dr A Schuster, University Eye Hospital, Department of Neuroophthalmology, Schleichstr 12–16, D-72076 Tuebingen, Germany; andreas.schuster@med.uni-tuebingen.de

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