Spectrum of Rhodopsin Mutations in French Autosomal Dominant Rod–Cone Dystrophy Patients

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Purpose. To identify the prevalence of rhodopsin (*RHO*) mutations in French patients with autosomal dominant rod–cone dystrophies (adRPs).

Methods. Detailed phenotypic characterization was performed, including precise family history, best corrected visual acuity with the ETDRS chart, slit lamp examination, kinetic and static perimetry, full-field and multifocal electroretinography (ERG), fundus autofluorescence imaging (FAF), and optical coherence tomography (OCT). For genetic diagnosis, genomic DNA of 79 families was isolated by standard methods. The coding exons and flanking intronic regions of *RHO* were PCR amplified, purified, and sequenced in the index patient.

Results. Of this French adRP sample, 16.5% carried an *RHO* mutation. Three different families showed a novel mutation (p.Leu88Pro, p.Met207Lys and p.Gln344Pro), while ten unrelated families showed recurrent, previously published mutations (p.Asn15Ser, p.Leu131Pro, p.Arg135Trp, p.Ser334GlyfsX21 and p.Pro347Leu). All mutations co-segregated with the phenotype within a family, and the novel mutations were not identified in control samples.

Conclusions. This study revealed that the prevalence of *RHO* mutations in French adRP patients is in accordance with that in other studies from Europe. Most of the changes identified herein reflect recurrent mutations, within which p.Pro347Leu substitution is the most prevalent. Nevertheless, almost one fourth of the changes are novel, indicating that, although *RHO* is the first gene implicated and probably the most studied gene in RP, it is still important performing mutation analysis in *RHO* to detect novel changes. The detailed phenotype–genotype analyses in all available family members deliver the basis for therapeutic approaches in those families. (*Invest Ophthalmol Vis Sci.* 2010;51:3687–3700) DOI:10.1167/iovs.09-4766

Rod–cone dystrophies, also called retinitis pigmentosa (RP), are a clinically and genetically heterogeneous group of inherited retinal disorders primarily affecting rods with secondary cone degeneration.[1] Often, the initial symptom that RP patients report is night blindness, which is attributable to the primarily affected rods and is a clinical sign of the impaired rod function. Later on, when the secondary cone dysfunctions manifest, progressive visual field constriction, abnormal color vision, and loss of central vision can be observed—signs of decreasing cone function. As the disease progresses and retinal dysfunction decreases, visual impairment increases: in some cases the disease may eventually result in very severe visual impairment or even blindness. RP is the most common inherited form of severe retinal degeneration, with a frequency of approximately 1 in 4000 births and more than 1 million affected individuals throughout the world. The mode of inheritance can be X-linked (5%–15%), autosomal dominant (30%–40%), or autosomal recessive (50%–60%). The remaining patients represent isolated cases in which the inheritance trait could not be established.[1]

To date, 20 autosomal dominant (ad)RP genes have been reported (http://www.sph.uth.tmc.edu/Retnet/ RetNet/ provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX). One of the major genes underlying this disorder is rhodopsin (*RHO*) coding for the light-absorbing molecule that initiates the signal transmission cascade in rod photoreceptors. According to the literature, *RHO* mutation prevalence ranges from 0% to 50% of cases of adRP in cohorts from various geographic origins, with higher numbers reported in the United States.[2–18]

The genetic and phenotypic heterogeneity is not only found in RP in general but is also specifically reflected in adRP with *RHO* mutations. More than 120 mutations have been identified in different sites on the gene, including specific hot spots (http://www.sph.uth.tmc.edu/Retnet/; http://www.hgmd.cf.ac.uk/ac/all.php, provided in the public domain by the University of Cardiff, Cardiff, Wales, UK; http://www.retina-international.org/sci-news/rhomut.htm; provided in the public domain by

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TABLE 1. Novel and Known *RHO* Mutations in the French Cohort

Index (Family)	Exon	Nucleotide Exchange	Protein Effect	Publication
2810 (PB41)	1	c.44A>G	p.Asn15Ser	26
2923 (PB42)				
CIC00218 (F155)	1	c.263T>C	p.Leu88Pro	Novel
CIC00123 (F172/96)	2	c.392T>C	p.Leu131Pro	27
CIC00364 (F247)	2	c.403C>T	p.Arg135Trp	4
CIC00974 (F610)				
CIC00716 (F475)	3	c.620T>A	p.Met207Lys	Novel (phenotype-genotype correlation, described in 28)
2296 (RP827)	5	c.995_998dup	p.Ser334GlyfsX21	13
CIC00590 (F394)	5	c.1031A>C	p.Gln344Pro	Novel
CIC00161 (F119)	5	c.1040C>T	p.Pro347Leu	29
CIC00841 (F546)				
CIC00944 (F598)				
CIC01125 (F681)				

Retina International and hosted by the University of Regensburg, Regensburg, Germany).[19]

Certain mutations in *RHO* lead to diffuse rod–cone dysfunction, whereas others are implicated in a more restricted disease that may predominate in the inferior part of the retina such as in sector RP.[20] Phenotypic classifications have been proposed to reflect this variability. In particular, Cideciyan et al.[21] have distinguished two classes of disease expression with allele specificity: Those with the Class A mutation show severely generalized abnormal rod function early in life, with a constant rate of cone disease progression across the retina with time. Those with the class B mutation show more restricted disease and absent or late-onset night blindness.

Other classifications have been proposed based on the underlying pathogenic mechanism involved in adRP due to *RHO* mutations. Mendes et al.[19] classified the different types of mutations into six groups. Class I refers exclusively to rhodopsin mutations that fold correctly but are not transported to the outer segment. Class II refers to mutations that misfold, are retained in the endoplasmic reticulum (ER), and cannot be easily reconstituted with 11-*cis*-retinal. Class III refers to mutations that affect endocytosis. Class IV mutations do not affect folding per se, but may affect rhodopsin stability and posttranslational modification. Similarly, Class V mutations have no obvious folding defect but show an increased activation rate for transducin. Mutants that appear to fold correctly but lead to the constitutive activation of opsin in the absence of the chromophore and in the dark constitute class VI. Other mutations with unclear biochemical or cellular defect or uninvestigated defect were not classified.[19]

Our comprehensive study was conducted to investigate in detail a French adRP cohort coming from two different clinical centers: Quinze-Vingts hospital in Paris and the Centre Hospitalier Régional in Montpellier located in the south of France. We present the prevalence of rhodopsin mutations in this cohort and show precise phenotype–genotype correlations. Novel mutations are analyzed on their predicted pathogenic mechanism, and frequently mutated sites are presented as putative candidates for therapeutic approaches.

METHODS

Clinic

Seventy-nine families with a provisional diagnosis of autosomal dominant rod–cone dystrophy (adRP) were ascertained in the CIC (Center for Clinical Investigation) of the Quinze-Vingts hospital, Paris (67 families), and in Montpellier (12 families). Informed consent was obtained from each patient and normal individual control subjects after explanation of the study and its potential outcome. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committees. Each patient underwent full ophthalmic examination with assessment of best corrected visual acuity with the ETDRS chart, kinetic and static perimetry, and color vision with the desaturated Farnsworth Panel D-15. Full-field and multifocal electroretinography (ERG and mfERG) were performed with DTL recording electrodes and incorporated the ISCEV Standards (Espion E2; for full field ERG; Diagnosys, Lowell, MA; and Veris II for multifocal ERG; EDI, Redwood City, CA).[22,23] Severe rod–cone dysfunction was considered when no detectable responses where recorded. Clinical assessment was completed with fundus autofluorescence imaging (FAF) and optical coherence tomography (OCT; HRT II and Spectralis OCT; Heidelberg Engineering, Dossenheim, Germany). At the end of the clinical evaluation, the patients and family members were asked to donate a blood sample for further genetic studies.

Mutation Analysis

Total genomic DNA was extracted from peripheral leukocytes in blood samples by standard salting out procedures[24] or according to the manufacturer's recommendation (Puregen Kit; Qiagen, Courtaboeuf, France). Subsequently, either genotyping or direct sequencing of *RHO* was performed. For genotyping, two to three polymorphic microsatellite markers within or contiguous to known adRP genes (*RHO*, *RDS*, *PRPF31*, *RP1*, *PRPF8*, *IMPDH1*, *PRPF3*, *NRL*, *CA4*, *CRX*, *TOPORS*, *PAP1*, and *NR2E3*) was used, and the results were analyzed (GeneMapper software; ver. 4.0; Applied Biosystems, Inc. [ABI], Foster City, CA). The coding five exons of rhodopsin (*RHO* RefSeq NM000539.2; http://www.ncbi.nlm.nih.gov/refseq/ provided in the public domain by National Center for Biotechnology Information, Bethesda, MD) and the flanking intronic regions were amplified with oligonucleotides described elsewhere.[25] At least 125 commercially available control samples were used to validate the pathogenicity of the novel sequence variants (human random control panel 1-3; Health Protection Agency Culture Collections, Salisbury, UK).

RESULTS

Mutation Analysis

Thirteen index patients of the investigated 79 French adRP patients carried an *RHO* mutation (Table 1). These mutations co-segregated with the phenotype when tested in the family members available (Fig. 1). Three index patients showed a novel missense mutation, whereas 10 index patients had previously described mutations in *RHO* (see Table 1, Figs. 1, 2A, and 2B).

Patient CIC00218, from family 155, originating from the Southwest of France, had a novel c.263T>C mutation on exon 1 leading to a p.Leu88Pro substitution (Figs. 1A, 2A).

Patient CIC00716, from family 475, from Northern France had the novel mutation c.620T>A in exon 3, leading to a p.Met207Arg substitution, which segregates with an unusual restricted chorioretinal atrophy phenotype (Fig. 1B).[28]

Patient CIC00590, from family 394, with Sephardic Jewish origins, carried a novel mutation, c.1031A>C in exon 5, leading to a p.Gln344Pro substitution (Figs. 1C, 2B).

Patients PB41 and 42, from two unrelated families from a similar region in France, had the known c.44A>G mutation in exon 1, leading to a p.Asn15Ser exchange (Fig. 1D).

Patient CIC00123, from family 172/96 originating from Martinique, within the French West Indies, showed a previously described heterozygous c.392T>C mutation in exon 2, leading to a p.Leu131Pro substitution (Fig. 1E).

Two index patients, CIC00364 and CIC00974, from the two unrelated families 247 and 610, respectively, carried the known mutation c.403C>T in exon 2, leading to a p.Arg135Trp substitution (Fig. 1F).

Index patient 2296, from family RP827, had the earlier described c.995_998dup insertion, leading to a predicted frameshift mutation (p.Ser334GlyfsX21) that is assumed to change the open reading frame and elongates the altered protein (Fig. 1G).

Four index patients, CIC00161, CIC00841, CIC00944, and CIC01125, from four unrelated families with origins in four distinct regions of France (families 119, 546, 598, and 681, respectively), bore the c.1040C>T mutation in exon 5 leading to the p.Pro347Leu substitution, which co-segregated in the family members available for genetic testing (Fig. 1H).

Prevalence of Different *RHO* Mutations in France

Altogether, our study of adRP patients from France showed that 16.5% had novel or known *RHO* mutations. Mutation locations showed no specific hot spots, since they involved all exons. However, three mutations occurred in at least two families, indicating that the p.Asn15Ser, p.Arg135Trp, and p.Pro347Leu substitutions in *RHO* are frequent causes of RP in this population.

Phenotypic Characteristics of Patients with *RHO* Mutation

Thirty affected subjects, between ages 8 and 62 years, from the 13 families found to have *RHO* mutation(s) underwent complete clinical examination. Their phenotypic details are summarized in Table 2. The group of patients reported herein showed three distinct phenotypes and resembled either class A or B mutants from the classification proposed by Cideciyan et al.[21]:

A generalized rod-cone dysfunction observed in patients carrying mutations (p.Leu88Pro, p.Leu131Pro, p.Arg135Trp, p.Ser334GlyfsX21, p.Gln344Pro, and p.Pro347Leu) that resemble the class A mutations.

A sector RP associated with the p. Asn15Ser mutation.

A restricted chorioretinal dystrophy predominant at the posterior pole associated with the p.Met207Lys substitution. Because of the more restricted phenotype, we classified the two latter mutations as class B.

In generalized forms, symptoms were classic for RP with no obvious phenotype–genotype differences and were dominated by night blindness from early childhood, progressive peripheral visual field constriction, and late photophobia. Age at time of diagnosis varied from 8 to 49 years, with most in the teenage years, earlier than in the restricted diseases. Central vision ranged from 20/20 to 20/400. It decreased with age, after peripheral visual field impairment and was usually relatively conserved up to the fifth decade. However, in 8 of 21 patients, atrophic changes within the macula occurred after the mid-20s and compromised further central vision. Some degree of cataract or intraocular lens was present as early as 34 years in 11 of 21 patients. In most patients, fundus examination, showed classic RPE changes in the periphery with intraretinal pigment migrations, sign of photoreceptor cell death, increasing with age. White dots were present in five patients who were 43 years of age or younger, associated with three genotypes in our series: p.Leu131Pro, p.Gln344Pro, and p.Pro347Leu. OCT findings are summarized in Table 2. There was no correlation between OCT abnormalities and genotype. Cystoid macular edema (CME) was present in 4 of 30 patients in association with four different genotypes. A perifoveal ring of hyper-AF was present in 13 of 18 patients for whom fundus autofluorescence imaging was performed. Absence of this ring is associated with irregular loss of autofluorescence within the macula in relation with atrophic changes (Fig. 3). ERG responses were usually undetectable for both scotopic and photopic recordings after 30 or showed only residual photopic flicker responses. In younger patients, when ERGs were detectable, they usually showed more decreased amplitudes for scotopic than photopic responses, with implicit time shift, consistent with generalized rod–cone dysfunction.

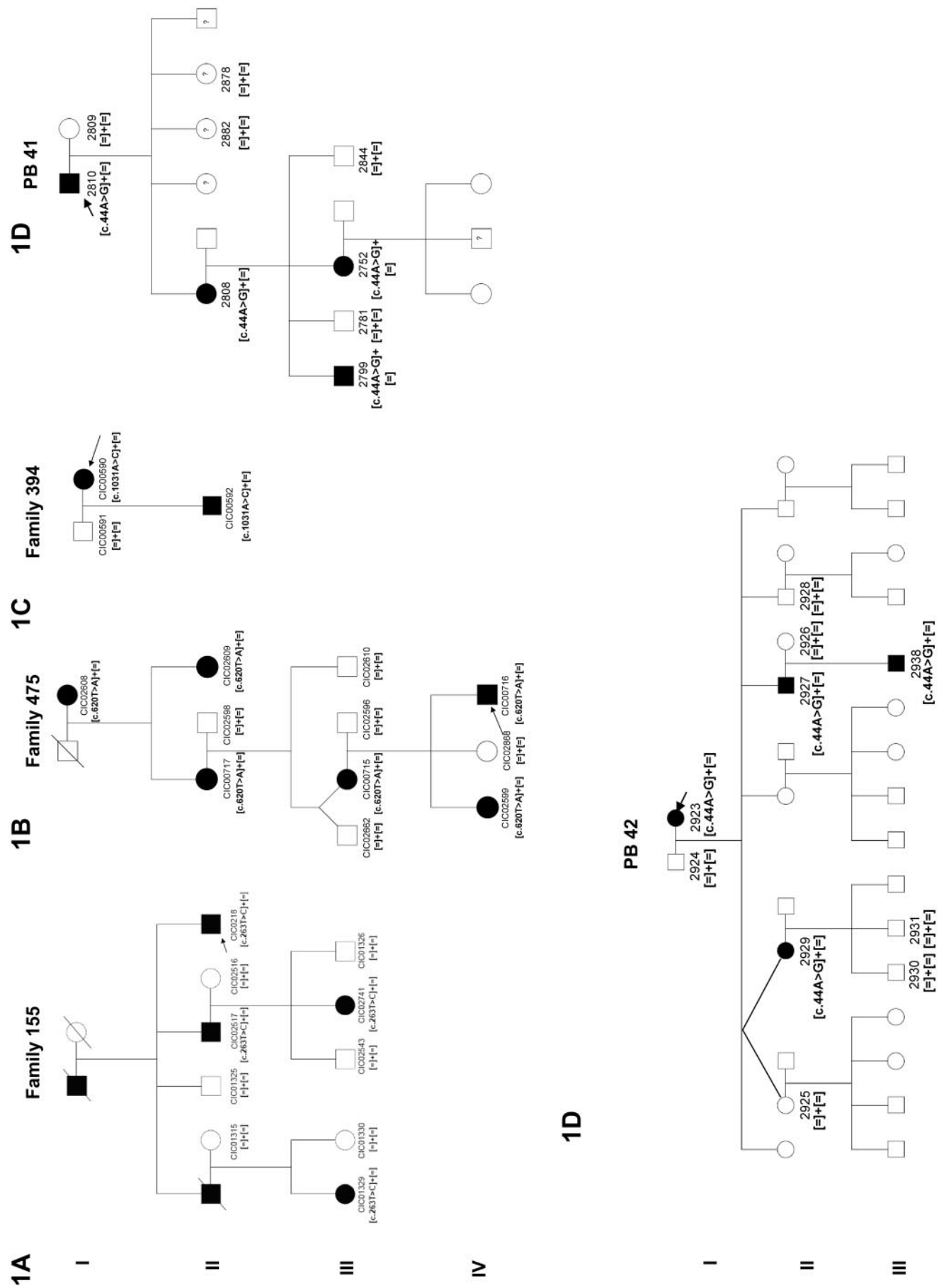
Sector RP was seen in two families (PB41 and PB42) carrying the same p.Asn15Ser change. Five patients, from ages 28 to 60 years, underwent a full ophthalmic examination. Night blindness was an inconstant sign in these subjects, all of whom retained a normal central vision with inferior peripheral field defect correlated with fundus abnormalities. ERG responses showed decreased scotopic responses with additional photopic abnormalities in some patients. There were, however, no implicit time shifts consistent with a restricted rod–cone dysfunction.

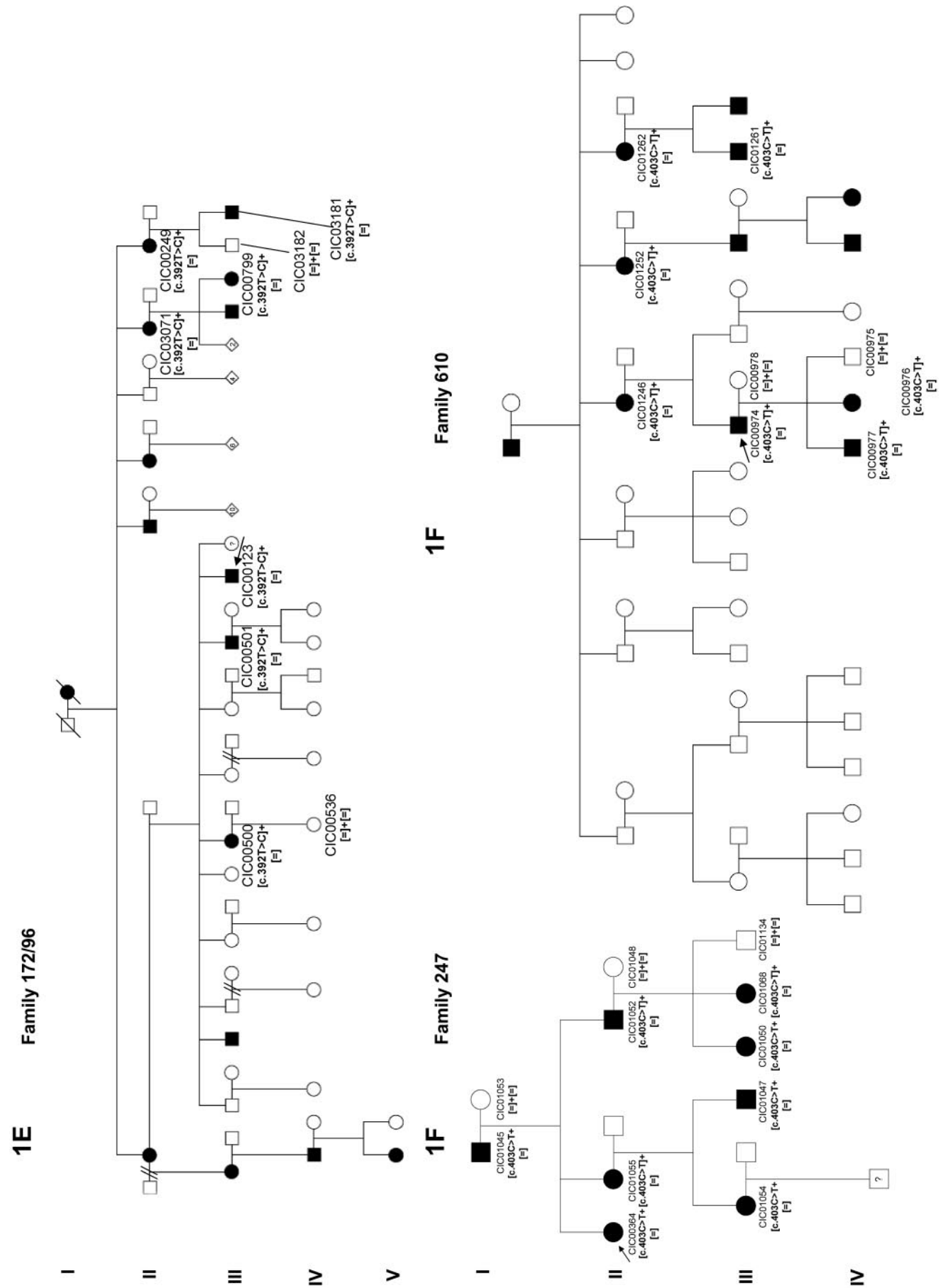
One additional family, F475 with a novel p.Met207Arg, showed restricted chorioretinal degeneration. Phenotype–genotype correlations are described in more detail elsewhere.[28] Briefly, onset of symptoms occurred in the fourth decade in this family, with moderate night blindness and asymmetric visual loss. Affected family members showed patchy areas of chorioretinal atrophy within the posterior pole (Fig. 3), with decreased ERG response amplitudes for both scotopic and photopic responses and no implicit time shift consistent with restricted disease.

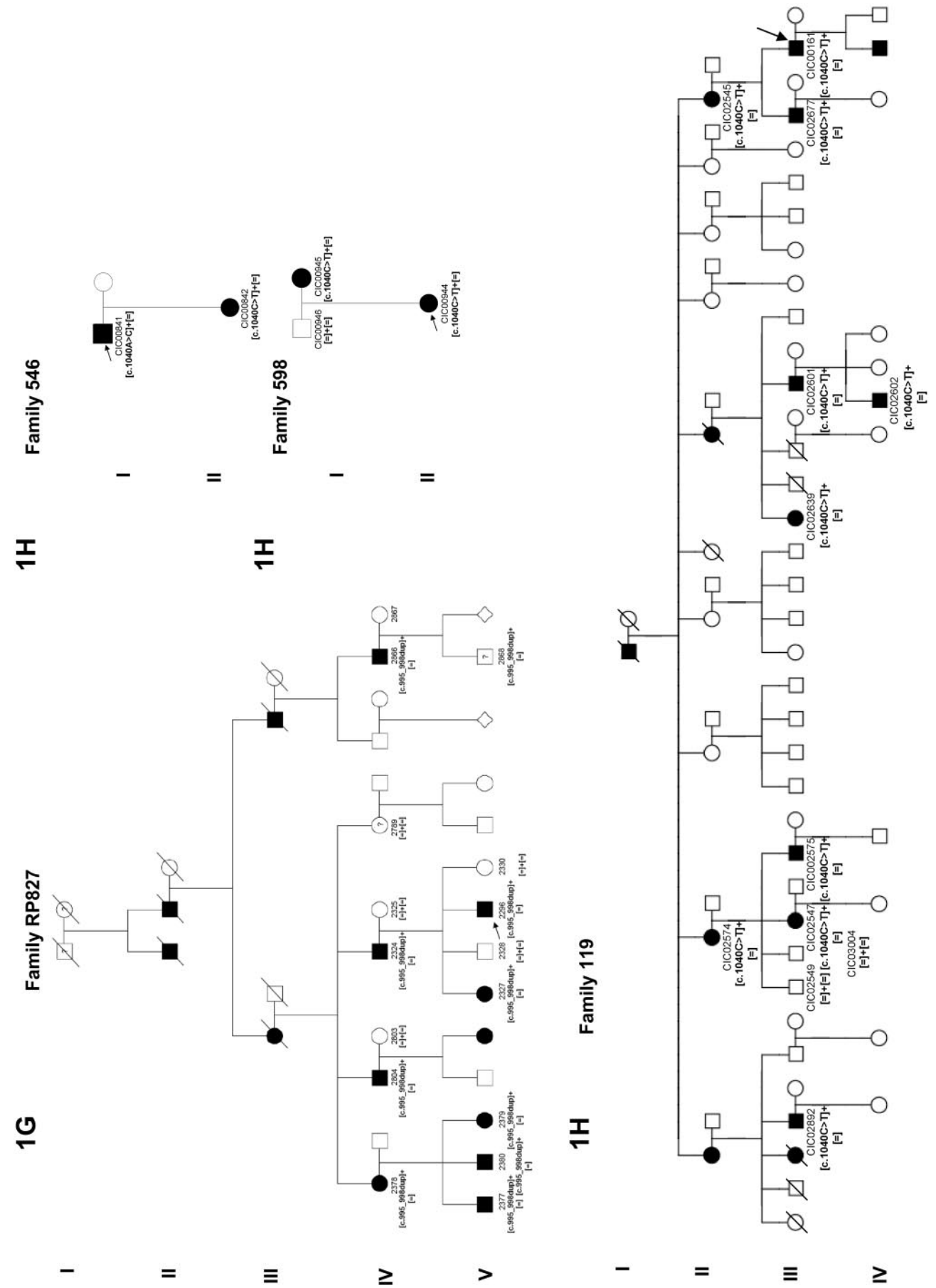
Discussion

The present study reports the mutation spectrum in the rhodopsin gene in a cohort of patients from two major French centers and further outlines the phenotypic variability associated with rhodopsin mutations, showing both generalized and sectorial retinal degeneration. To the best of our knowledge, to date only two studies on *RHO* mutations in a French cohort have been published: one describing the prevalence of *RHO* mutations in Southern France[13] and the other reported on the identification of five new mutations with no information on prevalence and ethnic origin.[13,27]

The overall prevalence of *RHO* mutations in our cohort was 16.5%. This prevalence is consistent with that in reports on European cohorts, including Spain (20%),[10] Germany (16%),[11] Italy (16%),[12] and Southern France (10%).[13] These rates are higher than those in reports from China (2%–7%),[14,15,30] Japan (0%–6%),[16] India (0%–2%),[17] and South-Africa (7%).[18] Studies from the United Kingdom and Norway revealed higher numbers (30%–50%).[8,9,31] However, the studied cohorts were small (12–20 families), and thus these results must be validated in







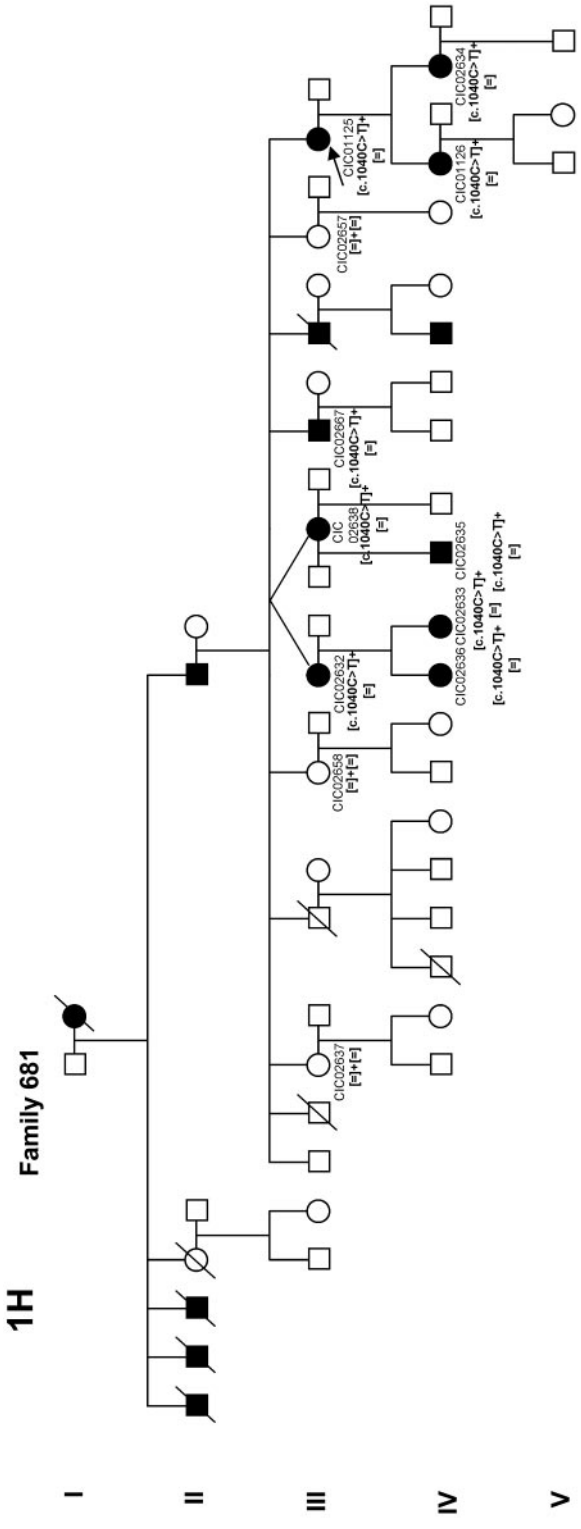


FIGURE 1. Pedigrees of adRP patients with *RHO* mutations and cosegregation in available family members. *Filled symbols*: affected and unfilled unaffected persons. *Squares*: males; *circles*: females; *arrows*: index patients.

larger cohorts. In the U.S. population, *RHO* mutations have been shown to account for up to 30% of adRP.[3–7] The prevalence of the p.Pro23His mutation in the United States has been reported as high as 12% of adRP due to a founder effect from a common British ancestor.[29] This mutation has never been found in European cohorts of adRP,[32] including the current report, nor in Asian cohorts,[33,34] which would account for differences in the overall *RHO* mutation prevalence between the American population and reports from other populations.

Three novel changes were identified in the present study: p.Leu88Pro, p.Met207Lys, and Gln344Pro. The p.Leu88Pro substitution led to a severe generalized rod–cone dystrophy phenotype in the patients. Disease-causing mutations have already been reported for the surrounding residues (namely p.Val87Asp and p.Gly89Asp) and misfolding has been hypothesized as a pathogenic mechanism.[4,35] The leucine in 88 is located within the α helix of the second transmembrane domain of rhodopsin. The residue at this position is not invariant among Metazoan organisms (Fig. 2C), but always shows the hydrophobic characteristics necessary for the maintenance of this α helix. The substitution of the leucine by a proline would induce a kink in the helix and destabilize the protein through rhodopsin misfolding. This would classify the p.Leu88Pro within class II, according to Mendes et al.[19]

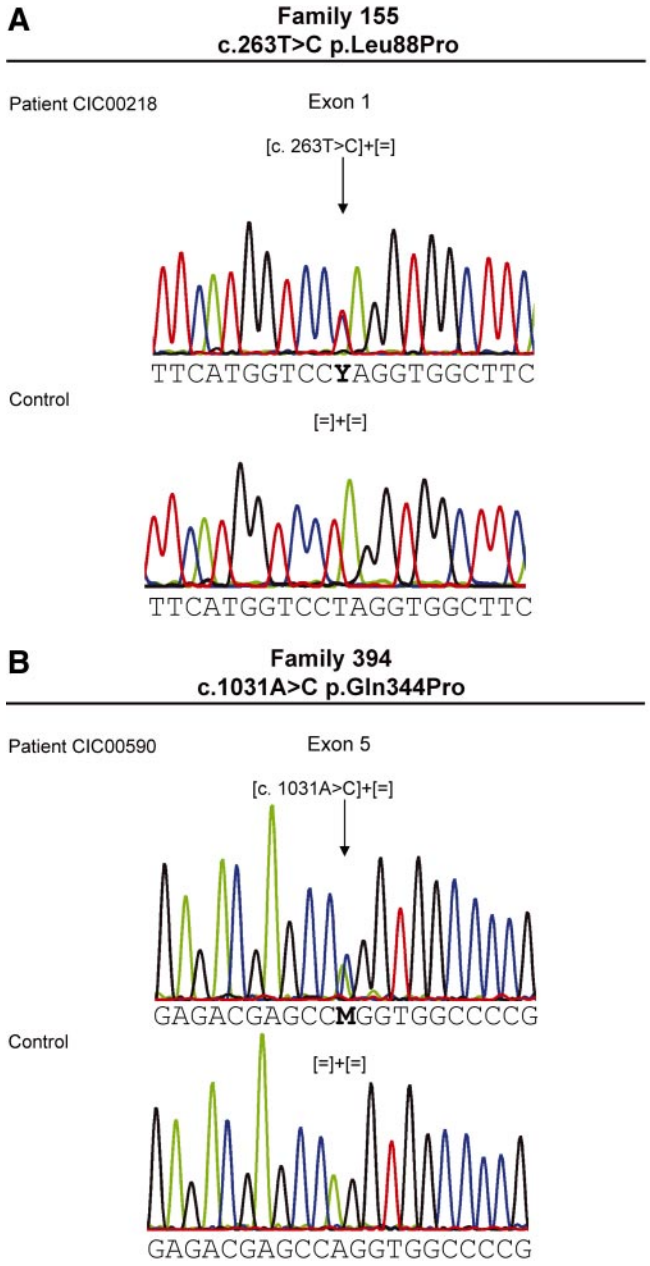
The novel p.Met207Arg substitution was associated with an unusual chorioretinal atrophy. Mutation consequences are discussed elsewhere[28] and suggest a change in steric constraints within the retinal binding pocket.

The c.1031A>C change in exon 5 leading to a p.Gln344Pro substitution was associated with a severe generalized rod–cone dystrophy. Gln at this position is evolutionarily highly conserved (Fig. 2C). It is located in the C-term external loop and it is unlikely that mutations in this residue would induce a misfolding, classifying our novel change p.Gln344Pro in class I, according to Mendes et al.[19] A c.1030C>T change leading to a p.Gln344Stop has been associated with normal phototransduction function, but with mislocalization.[36] Furthermore, Tai et al.[37] identified the direct interaction between a dynein light-chain subunit and the C terminus of rhodopsin, which is important for the correct protein transport of post-Golgi rhodopsin-containing vesicles along the microtubules up to the outer segment. Different C-terminal mutations were unable to interact with this domain and thus led to a trafficking defect. A similar mechanism can be advocated for the novel reported change p.Gln344Pro.

The 10 other families identified with *RHO* mutation showed already described changes. The p.Asn15Ser mutation was identified in two different families from a similar region of France and thus probably represents a founder effect. Asn15 represents one of the important N-glycosylation sites of RHO. Thus, the underlying pathogenic mechanism of the p.Asn15Ser was proposed to be a trafficking defect.[26]

The p.Leu131Pro mutation was identified in a large family from Martinique with typical diffuse rod–cone dystrophy type A according to Cideciyan et al.[21] This amino acid substitution is assumed to lead to misfolding.[38] Since this exchange has also been reported in another study from France,[27] it may represent a major mutation in the affected French population.

The p.Arg135Trp was found in two unrelated families and was associated with typical severe, diffuse rod–cone dystrophy type A according to Cideciyan et al.,[21] as has been reported.[39,40] Of note, none of the examined patients in these



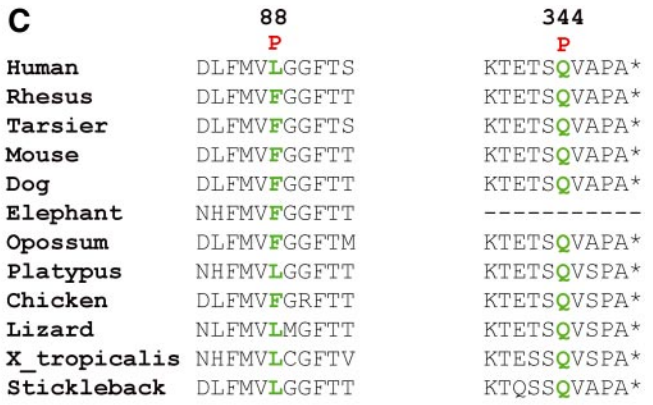


FIGURE 2. (**A**, **B**) Electropherograms of novel *RHO* mutations (*arrows*). (**C**) *Green*: multiple amino acid sequence alignments of different species of novel mutated residues. *Red*: amino acid substitutions. Numbers at *top*: the position of the respective amino acids.

two families demonstrated the white dots that have been described in association with this genotype.[40,41] An explanation for the absence of the dots could be that the examined patients were either too young or too old to exhibit this distinct feature. Oh et al.[40] have reported the transient nature of these dots, appearing in the second decade of life and then fading to give way to RPE atrophy and bone spicules. It is also noteworthy that the dots, which are located at the level of the RPE, are not specific to the p.Arg135Trp mutation, since they have also been seen in association with other *RHO* mutations in our series and may represent a nonspecific sign of photoreceptor degeneration (see Table 2 on clinical data).

The p.Pro347Leu mutation was the most prevalent, found in four families that would represent 5% of our adRP families. This mutation has also been reported in other populations.[8,10,29,34,42] Although the four families studied herein were unrelated and had different geographic origins, a founder effect cannot be excluded. Haplotype analysis was not performed in this study. However, the gene location is a known hot spot because of the higher probability of C>T transition due to a CpG sequence,[3] and six disease-causing amino acid substitutions have been reported at this location (see http://www.retina-international.org/sci-news/rhomut.htm). Again, it has been suggested that for these substitutions, a trafficking defect represents the pathogenic mechanism. Patients carrying the p.Pro347Leu mutation have a phenotype comparable to that of patients carrying the p.Gln344Pro and p.Ser334GlyfsX21 changes, all being located at the C terminus, with early-onset night blindness and generalized severe rod–cone dystrophy with loss of central vision in the fifth decade. The severity of the disease associated with C-terminal changes within the cytoplasmic domain is well documented in the literature,[43–45] showing a worse prognosis, in particular when compared with the p.Pro23His mutation located in the N-terminal intradiscal–

TABLE 2. Clinical Features of Affected Members in Families with adRP, Due to *RHO* Mutations

Family and *RHO* Mutation	Patient	Sex	Age at Diagnosis	Symptoms	Age at Exam	BCVA OD/OS	Cataract	Fundus	OCT	VF	ERG ISCEV Standards
Family PB41 c.44A>G p.15Asn>Ser	2752 III.12	F		Mild night blindness	39	20/20 20/20	−	Bone spicules in lower sector	Normal foveal lamination	Central scotoma (15°) Isopter V4 60°N, 70°T	50% of normal value for scotopic responses; 60% of normal value for photopic responses: no implicit time shift
	2808 II.2	F		Night blindness	59	20/20 +2.50 (−1.25; 170°) 20/20 +2.00 (−0.50; 50°)	−	Bone spicules in lower sector	Normal foveal lamination	Isopter V4 65°N, 70°T	20% of normal value for scotopic responses; 35% of normal value for photopic responses; no implicit time shift; 30% of normal value for scotopic responses
Family PB42 c.44A>G p.15Asn>Ser	2929 II.4	F		No night blindness	60	20/25 +3.25 (−1.00; 144°) 20/25 +2.75 (−0.50; 10°)	−	Bone spicules in lower sector	Normal foveal lamination	Isopter V4 80°N, 70°T	Photopic 30-Hz ERG slightly reduced; no implicit time shift
	2927 II.8	M	30	PVFI at 30; no night blindness	52	20/20 +2.50 (−2.75; 20°) 20/20 +2.75 (−2.50; 170°)	−	Bone spicules in lower sector Epiretinal membrane OD/OS	Normal foveal lamination	Isopter V4 80°N, 90°T	30% of normal value for scotopic responses; 80% of normal value for photo pic responses; no implicit time shift
	2938 III.12	M		Mild photophobia; no night blindness	28	20/20 −0.25 (−0.50: 15°) 20/20 −0.5	−	Bone spicules in lower sector	Normal foveal lamination	Normal	Normal scotopic responses Photopic 30-Hz ERG slightly reduced; no implicit time shift
Family 155 c.263T>C p.Leu88Pro novel	CIC 00218	M	15	Night blindness since childhood; photophobia at age 59, followed by progressive loss of central vision	62	20/63 20/80	IOL at age 48	Bone spicules 360°; some areas of central atrophy	Foveal thinning	Isopter V4 20° central ODS	Not detectable
Family 96/172 c.392T>C p.Leu131Pro	CIC 00123	M	10	Night blindness since childhood	27	20/32 +1.25(−1.25)85° 20/32 +1(−1)90°	−	Bone spicules 360°; some areas of central atrophy; a few white dots	Normal foveal lamination	Isopter V4 OD 15° central OS<10°	Not detectable
	CIC 00249	F	30	Night blindness since childhood Progressive decreased vision and photophobia	56	20/400 +3.505(−1.75)90° 20/500 +3(−1.50)90°	+	Bone spicules 360°; some areas of central atrophy; no ring on AF	Foveal thinning	Isopter III4 20° central ODS	Not detectable
	CIC 00799	F	Teens	Night blindness since childhood	31	20/32 −1(−1)50° 20/32 −1(−1.25)135°	−	Few peripheral RPE changes 360°; white dots; small perifoveal ring of hyper-AF	Normal foveal lamination	Isopter II4 110° horizontally and 90° vertically	No responses detectable in scotopic conditions, some residual flicker responses

(*continues*)

TABLE 2 (*continued*). Clinical Features of Affected Members in Families with adRP, Due to *RHO* Mutations

Family and *RHO* Mutation	Patient	Sex	Age at Diagnosis	Symptoms	Age at Exam	BCVA OD/OS	Cataract	Fundus	OCT	VF	ERG ISCEV Standards
	CIC 00500	F	Teens	Night blindness since childhood	43	20/40 +0.25(−0.75)120° 20/40 +0(−1)60°	+	Bone spicules 360°; some areas of central atrophy; white dots; small perifoveal ring of hyper-AF	Normal foveal lamination	Isopter III4 60° horizontally and 30° vertically	Not detectable
	CIC 00501	M	28	Night blindness since childhood	51	20/40 +0.75(−0.25)95 20/40 pl(−1.25)90°	−	Bone spicules 360°; Some areas of central atrophy; no ring on AF	Foveal thinning	Isopter V4 20°	Not detectable
Family 247 c.403C>T p.Arg135Trp	CIC 00364	F		Night blindness since childhood	52	20/40 +3.75(−0.25)35° 20/63 +5.75(−1)130°	+	Bone spicules 360°; Some areas of central atrophy; no ring on AF	Foveal thinning	Isopter III4 30°	Not detectable
Family 610 c.403C>T p.Arg135Trp	CIC 00974	M	10	Night blindness	37	20/40 −8(−2.75)0° 20/40 −8(−2.25)175°	−	Peripheral RPE/choroidal atrophy with bone spicules 360°, small perifoveal ring of hyper-AF	Foveal thinning	Isopter III4 170° horizontal × 100° vertical	Not detectable
	CIC 00976	F	8	Night blindness	8	20/63 −2(−3.25)0° 20/40 +0.75(−2.25)180°	−	Nearly normal fundus, Perifoveal ring of hyper-AF	Normal foveal lamination	Isopter III4 140° horizontal; 100° vertical	Both scotopic and photopic amplitudes reduction*
	CIC 00977	M	10	Night blindness	11	20/20 +0.25(−2.75)5° 20/20 +0.25(−2.50)170°	−	Few RPE changes with no bone spicules Bilateral CME; perifoveal ring of hyper-AF	CME	Isopter III4 140° horizontal × 120° vertical	Scotopic responses 10% of normal; photopic responses 50% normal; both amplitude reduction and implicit time shift
Family 475 c.620T>A p.Met207Lys	CIC 00716	M	23	None	23	20/13 0(−1)160° 20/15 0(−1.50)15°	−	Preserved macula besides some perifoveolar RPE clumps; moderate salt-and-pepper appearance of retinal periphery	Normal foveal lamination	Normal	Scotopic response amplitudes 80% of normal, normal photopic responses, no implicit time shift
	CIC 00715	F	38	Decreased VA; some degree of night vision disturbances	46	20/25 +1(−0.50)160° HM	−	Patchy chorioretinal atrophy with some RPE clumps in posterior pole and mid periphery; no pale disc and no narrowing of blood vessels; salt-and-pepper aspect in retinal periphery; no bone spicules	OD normal foveal lamination OS foveal thinning	OD normal OS normal peripheral isopter	65% of normal for scotopic response amplitudes and 90% for scotopic responses; no implicit time shift

(*continues*)

Table 2 (*continued*). Clinical Features of Affected Members in Families with adRP, Due to *RHO* Mutations

Family and *RHO* Mutation	Patient	Sex	Age at Diagnosis	Symptoms	Age at Exam	BCVA OD/OS	Cataract	Fundus	OCT	VF	ERG ISCEV Standards
	CIC 00717	F	40	Night blindness since age 40; decreased VA	58	20/200 +1.75(−0.50)20° 20/25 +2.25(−0.50)140°	+	Patchy chorioretinal atrophy with some RPE clumps in the posterior pole and mid periphery; no pale disc and no narrowing of blood vessels, salt and pepper aspect in retinal periphery; no bone spicules	OD foveal thinning OS normal foveal lamination	Normal peripheral isopter	Not performed
	CIC 02599	F	26	None	26	20/15 ODS with no correction	−	Normal aspect of posterior poles besides some perifoveolar RPE clumps and one small area of atrophy; moderate salt and pepper; appearance of retinal periphery	Normal foveal lamination	Normal	Not performed
Family RP827 c.995_998dup p.Ser334GlyfsX21	2296 V.8	M	13	Night blindness at early childhood; PVFI at 13; intense photophobia	34	20/40 +5.50 (−0.50; 165°) 20/30 +5.00 (−0.50; 170°)	+	Bone spicules 360°; CME	CME	15°	Not detectable
	2327 V.6	F	11	Night blindness at 11; PVFI at 20; photophobia at 25	38	20/100 +5.00 20/400 +6.00 (−0.75; 60°)	+	Bone spicules 360°; foveal photoreceptor loss	Foveal thinning	15°	Not detectable except for residual 30-Hz flicker ERG
	2379 V.3	F	Childhood	Night blindness since early childhood; photophobia at 5	45	20/30 (−2.00; 90°) 20/40 (−1.25; 105°)	+	Bone spicules 360°; small perifoveal ring of hyper-AF	Foveal thinning	20°	Not detectable
	2324 IV.5	M	50	Night blindness at early childhood; PVFI at 25; photophobia	60	20/400 +1.50 (−1.00; 95°) 20/200 +2.00 (−1.00; 85°)	IOL	Bone spicules 360°	Foveal thinning	10°	Not detectable except for residual 30-Hz flicker ERG
Family 394 c.1031A>C p.Gln344Pro, novel	CIC 00590	F	32	Night blindness since age 4; PVFI since age 19	53	20/125 −1.50(−1)35° 20/200 −1.25(−1)150°	IOL at 48	Bone spicules 360°, no ring on AF; perifoveal atrophy	Foveal thinning	20°	Not detectable
	CIC 00592	H	13	Moderate night blindness	13	20/25 +1.5(−1.75)170° 20/32 +2(−2.75)175°	−	Peripheral RPE changes; 360° with white dots; perifoveal ring of hyper-AF	Normal foveal lamination	Normal	Scotopic responses 10% of normal; photopic responses 80% normal; both amplitude reduction and implicit time shift

(*continues*)

TABLE 2 (*continued*). Clinical Features of Affected Members in Families with adRP, Due to *RHO* Mutations

Family and *RHO* Mutation	Patient	Sex	Age at Diagnosis	Symptoms	Age at Exam	BCVA OD/OS	Cataract	Fundus	OCT	VF	ERG ISCEV Standards
Family 119 c.1040C>T p.Pro347Leu	CIC 00161	H	11	Night blindness	42	20/32 plano(−1)90° 20/25 plano(−0.50)80°	−	Bone spicules 360°; perifoveal ring of hyper-AF	Normal foveal lamination	20°	Not detectable
Family 546 c.1040C>T p.Pro347Leu	CIC 00841	H	Teens	Night blindness since early childhood, PVFI at 25, recent photophobia	42	20/40 0(−1)115° 20/63 −1(−0.25)15°	+	Bone spicules 360°; white dots; bilateral CME, small perifoveal ring of hyper-AF	CME	Isopter III4 20°	Not detectable
Family 598 c.1040C>T p.Pro347Leu	CIC 00944	F	10	Night blindness	14	20/20 +2(−1.25)5° 20/20+1.5(−05)10°	−	Some peripheral RPE changes over 360°; CME; perifoveal ring of hyper-AF	CME	Normal	Scotopic responses 10% of normal; photopic responses 80% normal; both amplitude reduction and implicit time shift
	CIC 00945	F	9	Night blindness	43	20/32 +3.5(−1.25)10° 20/32 +3.75(−0.75)5°	−	Bone spicules 360°; no ring on AF	Foveal thinning	20°	Not detectable
Family 681 c.1040C>T p.Pro347Leu	CIC 01125	F	49	Night blindness	56	20/400 +1.50(−0.75)20° 20/200 +3.25(−0.75)10°	OD IOL OS +	A few bone spicules 360°; some areas of central atrophy, incomplete perifoveal ring of hyper-AF	Foveal thinning	Isopter III4 40°	Not detectable
	CIC 01126	F	29	Night blindness	36	20/20 20/20	−	Few bone spicules 360°; perifoveal ring of hyper-AF	Normal foveal lamination	Isopter III4; 150° horizontally × 60° vertically	Not detectable scotopic responses; some residual flicker responses

PVFI, peripheral visual field impairment; VF, visual field; IOL, intraocular lens; BCVA, best corrected visual acuity; HM, hand motion.

* ERG performed with skin electrodes which precluded us to have a precise quantification of abnormalities:

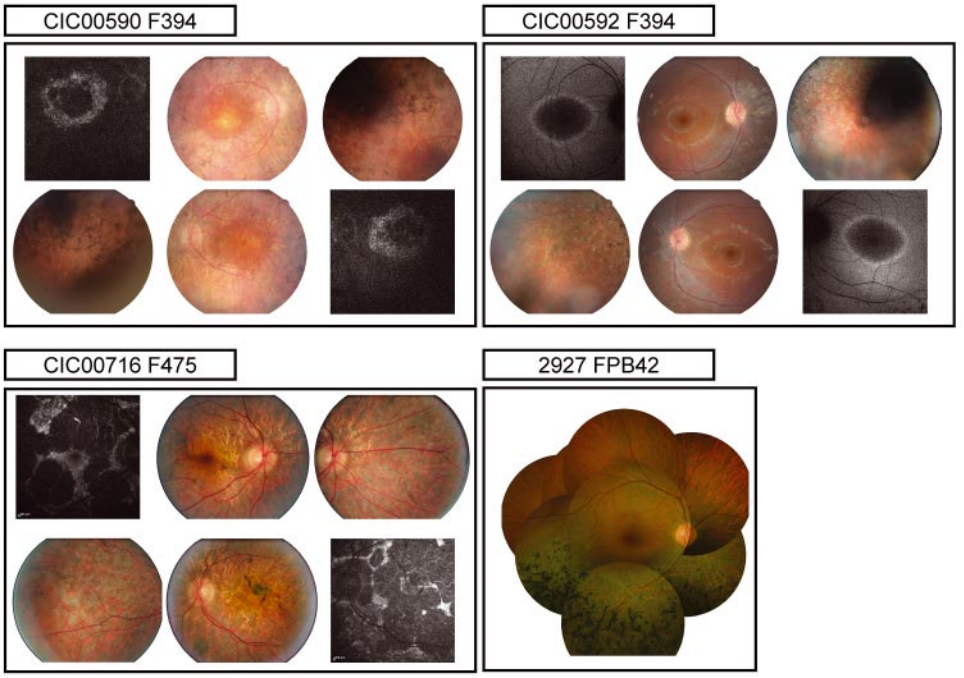


FIGURE 3. Fundus and autofluorescence photographs of three index patients with distinct adRP phenotypes (diffuse, sector RP, and restricted chorioretinal atrophy).

extracellular portion of the protein.[44,45] Our sample of subjects in whom genotype–phenotype correlation was determined is too small to judge the severity associated with a specific mutation, but recurrent follow-up will further address this question.

An additional criterion that should be evaluated further is the course of macular involvement. Perifoveal and foveal atrophy was not uncommon in our series (see Table 2 with clinical details) nor was CME, which was present in 4 of 31 patients with no genotype specificity. These macular changes are responsible for decreased central vision, and their prevention should be the major target of future therapeutic interventions.

Further longitudinal studies will determine the precise course of the disease for each genotype and will help in identifying suitable markers and therapeutic windows for photoreceptor rescue, gene replacement, or cell-based therapies.

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