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Abstract

Purpose

To identify the prevalence of rhodopsin (RHO) mutations in French patients with autosomal dominant rod-cone dystrophies (adRP).

Methods

Detailed phenotypic characterization was performed including precise family history, best corrected visual acuity using 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 seventy-nine 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

Among this French adRP cohort, 16.5% revealed a RHO mutation. While three unrelated families showed each a novel missense mutation (p.Leu88Pro, p.Met207Lys and p.Gln344Pro), ten unrelated families showed recurrent previously published mutations (p.Asn15Ser, p.Leu131Pro, p.Arg135Trp, p.Ser334GlyfsX20 and p.Pro347Leu). All mutations co-segregated with the phenotype within a family and the novel mutations were not identified in a control population.

Conclusion

Our studies revealed that the prevalence of RHO mutations in French adRP patients is in accordance with other studies from Europe. Most of the changes identified herein reflect recurrent mutations within which p.Pro347Leu substitution is the most prevalent. Nevertheless, almost a quarter 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 relevant to perform mutation analysis in the coding exons of RHO to detect novel changes. Our detailed phenotype-genotype analyses in all family members available deliver the basis for therapeutic approaches in those families.

MESH Keywords Adolescent ; Adult ; Child ; DNA Mutational Analysis ; Electroretinography ; European Continental Ancestry Group ; genetics ; Female ; Fluorescein Angiography ; France ; epidemiology ; Genes, Dominant ; Genotype ; Humans ; Male ; Middle Aged ; Mutation ; Pedigree ; Phenotype ; Photoreceptor Cells, Vertebrate ; pathology ; Polymerase Chain Reaction ; Prevalence ; Retinitis Pigmentosa ; diagnosis ; genetics ; Rhodopsin ; genetics ; Tomography, Optical Coherence ; Visual Acuity ; Young Adult

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 . RP patients initially often complain of night blindness. This is attributed to the primarily affected rods and clinical sign of the impaired rod function. Later on, when the secondary cone dysfunctions manifests, 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 about 1 in 4000 births and more than 1 million affected individuals over 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 of which the inheritance trait can not be established 1 .

To date, 20 autosomal dominant RP (adRP) genes have been reported (http://www.sph.uth.tmc.edu/Retnet/). 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% cases of adRP in cohorts from various geographical 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 also specifically reflected in adRP with RHO mutations: Over 120 mutations have been identified in different sites of the gene including specific hot spots (http://www.sph.uth.tmc.edu/Retnet/ , http://www.hgmd.cf.ac.uk/ac/all.php , http://www.retina-international.org/sci-news/rhomut.htm) 19 .

Certain mutations in RHO lead to diffuse rod-cone dysfunction whereas other cases 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 and co-workers have distinguished two classes of disease expression with allele-specificity 21 : class A mutants show severely generalized abnormal rod function early in life with a constant rate of cone disease progression across the retina with time. Class B mutants show more restricted disease and absent or lateonset night blindness.

Other classifications have been proposed based on the underlying pathogenic mechanism involved in adRP due to RHO mutations. Mendes and co-workers classified the different types of mutations in 6 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 endopasmic reticulum (ER) and cannot easily reconstitute with 11-cis -retinal. Class III refers to mutations that affect endocytosis. Class IV mutations do not affect folding per se but might 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 presented herein aim to investigate in detail a French adRP cohort coming from 2 different clinical centers, namely Quinze-Vingts hospital in Paris and the Centre Hospitalier Régional in Montpellier located in the south of France. We will present the prevalence of rhodopsin mutations in this cohort and show precise phenotype-genotype correlations. Novel mutations will be analyzed on its predicted pathogenic mechanism as well as frequently mutated sites will be 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 of the Quinze-Vingts hospital, Paris (67 families) and in Montpellier (12 families). Informed consent was obtained from each patient and normal individual controls 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 using ETDRS chart, kinetic and static perimetry and colour vision using the desaturated Farnsworth Panel D-15. Fullfield and multifocal electroretinography (ERG and mfERG) were performed with DTL recording electrodes and incorporated the ISCEV Standards (Espion2 Diagnosys® for full field ERG and Veris II for Multifocal ERG) 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) (HRAII® and Spectralis® OCT, Heidelberg Engineering, Dossenheim, Germany). At the end of clinical evaluation, patients and family members were asked to donate a blood sample for further genetic studies.

Mutation analysis

Total genomic DNA was extracted from peripheral leucocytes in blood samples by standard salting out procedures 24 or according to manufacturer recommendation (Puregen Kit, Qiagen, Courtaboeuf, France). Subsequently, either genotyping or direct sequencing of RHO was performed. For genotyping 2 to 3 polymorphic microsatellite markers within or contiguous to known adRP genes (RHO, RDS, PRPF31, RP1, PRPF8, IMPDH1, PRPF3, NRL, CA4, CRX, TOPORS, PAP1, NR2E3) was used. Results were analysed with GeneMapper software (version 4.0, Applied Biosystems). The coding 5 exons of rhodopsin (RHO RefSeq NM000539.2) and the flanking intronic regions were amplified with oligonucleotides previously described 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, United Kingdom).

RESULTS

Mutation analysis

Thirteen index patients of the investigated 79 French autosomal dominant RP patients revealed a RHO mutation (Table 1). These mutations co-segregated with the phenotype when tested in family members available. Three index patients showed each a novel missense mutation, while ten index patients revealed previously described mutations in RHO (Table 1 and Figure 2a).

- 1

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 (Figures 1A and 2A)

- 2

Patient CIC00716 of family 475, from Northern France revealed a novel mutation c.620T>A in exon 3 leading to a p.Met207Arg substitution, which segregates with an unusual restricted chorioretinal atrophy phenotype (Figure 1b) (Audo et al., 2010, in press).

- 3

Patient CIC00590 from family 394, with Sephardim Jewish origins, revealed a novel mutation, c.1031A>C in exon 5, leading to a p.Gln344Pro substitution (Figure 1c and 2a).

- 4 and 5

Two patients from two unrelated families (PB41 and 42) from a similar region in France revealed the known c.44A>G mutation in exon 1 leading to a p.Asn15Ser exchange (Figure 1d).

- 6

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 (Figure 1e).

- 7 and 8

Two index patients CIC00364 and CIC00974 from two unrelated families 247 and 610 respectively revealed the known mutation c.403C>T in exon 2 leading to a p.Arg135Trp substitution, which co-segregated with the disease (Figure 1f).

- 9

Index patient 2296 from family RP827 revealed the earlier described c.998_999insAGGC insertion leading to a predicted frameshift mutation (p.Ser334GlyfsX20), which is assumed to change the open reading frame and elongates the altered protein (Figure 1g).

- 10–13

Four index patients CIC00161, CIC00841, CIC00944 and CIC01125 from 4 unrelated families with origins in 4 distinct regions of France (family 119, family 546, family 598 and family 681, respectively) revealed the c.1040C>T mutation in exon 5 leading to the p.Pro347Leu substitution, which co-segregated in family members available for genetic testing (Figure 1h).

Prevalence of different RHO mutations in France

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

Phenotypic characteristics of patients with RHO mutation

Thirty affected subjects, between age 8 and 62, from the 13 families found with RHO mutation underwent complete clinical examination. Their phenotypic details are summarized in Table 2 . The group of patients reported here shows 3 distinct phenotypes and resemble either class A or B mutants from the classification proposed by Cideciyan and co-workers 21 :

- **a generalized rod-cone dysfunction** observed in patients carrying mutations (p.Leu88Pro, p.Leu131Pro, p.Arg135Trp, p.Ser334GlyfsX20, p.Gln344Pro, p.Pro347Leu), which resemble the class A mutants.

- **a sector RP** associated with the p. Asn15Ser mutation and

- **a restricted chorioretinal dystrophy predominant at the posterior pole** associated with the p.Met207Lys substitution. Due to the more restricted phenotype, we classified the two latter mutations as class B mutations.

In **generalized forms** , symptoms are classical for RP with no obvious phenotype/genotype differences and are dominated by night blindness, from early childhood, progressive peripheral visual field constriction and late photophobia. Age at time of diagnosis varies from 8 to 49 with a majority within the teenage years, earlier than restricted diseases. Central vision ranges from 20/20 to 20/400. It decreases with age, after peripheral visual field impairment, and is usually relatively conserved up to the 5th decade. However, in 8/21 patients, atrophic changes within the macula occur after the mid-twenties and compromised further central vision. Some degree of cataract or intraocular lens is present as early as 34 in 11/21 patients. Fundus examination, shows in most patients classical RPE changes in the periphery with intraretinal pigment migrations, sign of photoreceptor cell death, increasing with age. White dots are present in 5 patients who are 43 or younger associated with three genotypes are in our series: p.Leu131Pro, p.Gln344Pro and p.Pro347Leu. OCT findings are summarized in Table 2 . There was no correlation between OCT abormalities and genotype. Cystoid Macular Edema (CME) is present in 4/30 patients in association with 4 different genotypes. A perifoveal ring of hyper-autofluorescence is present 13/18 patients for whom fundus autofluorescence imaging has been performed. Absence of this ring is associated with irregular loss of autofluorescence within the macula in relation with atrophic changes (Figure 3). ERG responses are usually undetectable for both scotopic and photopic recordings after 30 or show only residual photopic Flicker responses. When ERGs are detectable, in younger patients, they usually show more decreased amplitudes for scotopic than photopic responses with implicit time shift, consistent with generalized rod-cone dysfunction.

Sector RP was seen in 2 families (PB41 and PB42) carrying the same p.Asn15Ser change. Five patients, from age 28 to 60, underwent full ophthalmic examination. Night blindness is an inconstant sign in these subjects who all retain a normal central vision with inferior peripheral field defect correlated with fundus abnormalities. ERG responses show decreased scotopic responses with additional photopic abnormalities in some patients. There is however no implicit time shift consistent with a restricted rod-cone dysfunction.

One additional family, F475 with a novel p.Met207Arg, shows also restricted chorioretinal degeneration. Phenotype-genotype correlations are described in more details elsewhere (Audo et al., 2010, in press). Briefly, onset of symptoms appears in the fourth decade in this family with moderate night blindness and asymmetric visual loss. Affected family members show patchy areas of chorioretinal atrophy within the posterior pole (Figure 3) with decreased ERG response amplitudes for both scotopic and photopic responses and no implicit time shift consistent with restricted disease.

DISCUSSION

The current study reports mutation spectrum on the rhodopsin gene in a cohort of patients from 2 major French centres and further outlines phenotypic variability associated with rhodopsin mutation showing both, generalized or sectorial retinal degeneration. To the best of our knowledge to date only two studies on RHO mutations in a French cohort were published: One describing the prevalence of RHO mutations in Southern France and the other reported on the identification of 5 new mutations with no information on prevalence and ethnic origin 13 , 26 .

The overall prevalence of RHO mutations in our cohort is 16.5%. This is consistent with previous reports on European cohorts including Spain (20%)10 , Germany (16%)11 , Italy (16%)12 and Southern France (10%).13 This is higher than reports from China (2–7%) 14 , 15 , 27 , Japan (0–6%) 16 India (0 – 2%) 17 and South-Africa (7%) 18 . Studies from the UK and Norway revealed higher numbers with 30–50% 8 , 9 , 28 . However, the studied cohorts were small (12–20 families) and thus these results must be validated in larger cohorts. In the US population RHO mutations were shown to account for up to 30% of adRP 3 –7 . The prevalence of the p.Pro23His mutation in the US 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 30 , including the current report, nor in Asian cohorts 31 , 32 , 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 current study: p.Leu88Pro, p.Met207Lys and Gln344Pro.

The p.Leu88Pro substitution leads 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 hypothesised as a pathogenic mechanism 4 , 33 . The Leucine in 88 is located within the alpha helix of the second transmembrane domain of rhodopsin. The residue at this position is not invariant among Metazoan organism (Figure 2b), but shows always hydrophobic characteristics, necessary for the maintenance of this alpha 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 after Mendes and colleagues 19 .

The novel p.Met207Arg substitution was associated with unusual chorioretinal atrophy. Mutation consequences are discussed elsewhere (Audo et al., 2010, in press) and would suggest a change in sterical 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 evolutionary highly conserved (Figure 2b). It is located in the C-term external loop and it is unlikely that mutations in this residue would induce a misfolding. This would classify our novel change p.Gln344Pro in class I after Mendes and colleagues 19 . Previously a c.1030C>T change leading to a p.Gln344Stop was associated with normal phototransduction function but with mislocalization 34 . Furthermore, Tai and co-workers 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 35 . 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 represents probably a founder effect. Asn15 represent one of the important N-glycosylation sites of RHO. Thus the underlying pathogenic mechanism of the p.Asn15Ser was proposed to be trafficking defect 36 .

The p.Leu131Pro mutation was identified in a large family from Martinique with typical diffuse rod-cone dystrophy, type A from Cideciyan and co-workers 21 . This amino-acid substitution is assumed to lead to misfolding 37 . Since this exchange has also been previously reported in another study from France 26 , 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 from Cideciyan and co-workers 21 as previously reported 38 39 . Of notes, none of the examined patients in these 2 families demonstrated the white dots previously described in association with this genotype 39 , 40 . An explanation would be that the examined patients were either too young or too old to exhibit this distinct feature since, Oh and co-worker have reported the transient nature of these white dots appearing in the second decades of life then fading to leave place to RPE atrophy and bone spicules. It is also noteworthy that these white dots, which are located at the level of the RPE, are not specific of the p.Arg135Trp mutation since it was also seen in association with other RHO mutations in our series and may represent a non-specific sign of photoreceptor degeneration (see table 2 on clinical data).

The p.Pro347Leu mutation was the most prevalent, found in 4 families which would represent 5% of our adRP families. This mutation has also been reported in other populations 8 , 10 , 29 , 32 , 41 . Although the 4 families studied herein were unrelated and from different geographical origin, a founder effect cannot be excluded. Haplotype analysis was not performed for this study. However, the gene location is a known hotspot through a higher probability of C>T transition due to a CpG sequence 3 and 6 disease causing amino-acid substitutions have reported at this location (see http://www.retina-international.org/sci-news/rhomut.htm). Again, it was suggested that for these substitutions a trafficking defect represent the pathogenic mechanism. Patients carrying the p.Pro347Leu mutation have a comparable phenotype as patients carrying the p.Gln344Pro and p.Ser334GlnfsX20 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 5th decades. The severity of the disease associated with C-terminal changes within the cytoplasmic domain is well documented in the literature 42 –44 showing a worse prognosis compared in particular to the p.Pro23His mutation located in the N-terminal intradiscal/extracellular portion of the protein 43 , 44 . Our cohort in whom genotype-phenotype correlation was performed is still too small to judge the severity associated with a specific mutation but recurrent follow-up will further address this question.

One additional criterion that will need to be further precisely evaluated is the course of macular involvement: perifoveal and foveal atrophy is not uncommon in our series (see table 2 with clinical details) as well as cystoid macular edema which was present in 4/31 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 precise the course of the disease for each genotype and will help identifying suitable markers and therapeutic windows for photoreceptor rescue, gene replacement or cell based therapies.

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Footnotes:

Data regarding family 475 have been presented as a poster at the ARVO 2009 meeting and are also published elsewhere (Audo et al. 2010, in press).

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Figure 1

Pedigrees of adRP patients with RHO mutations and co-segregation in available family members. Filled symbols represent affected and unfilled unaffected persons. Squares indicate males, circles females. Arrows reflect the index patients.

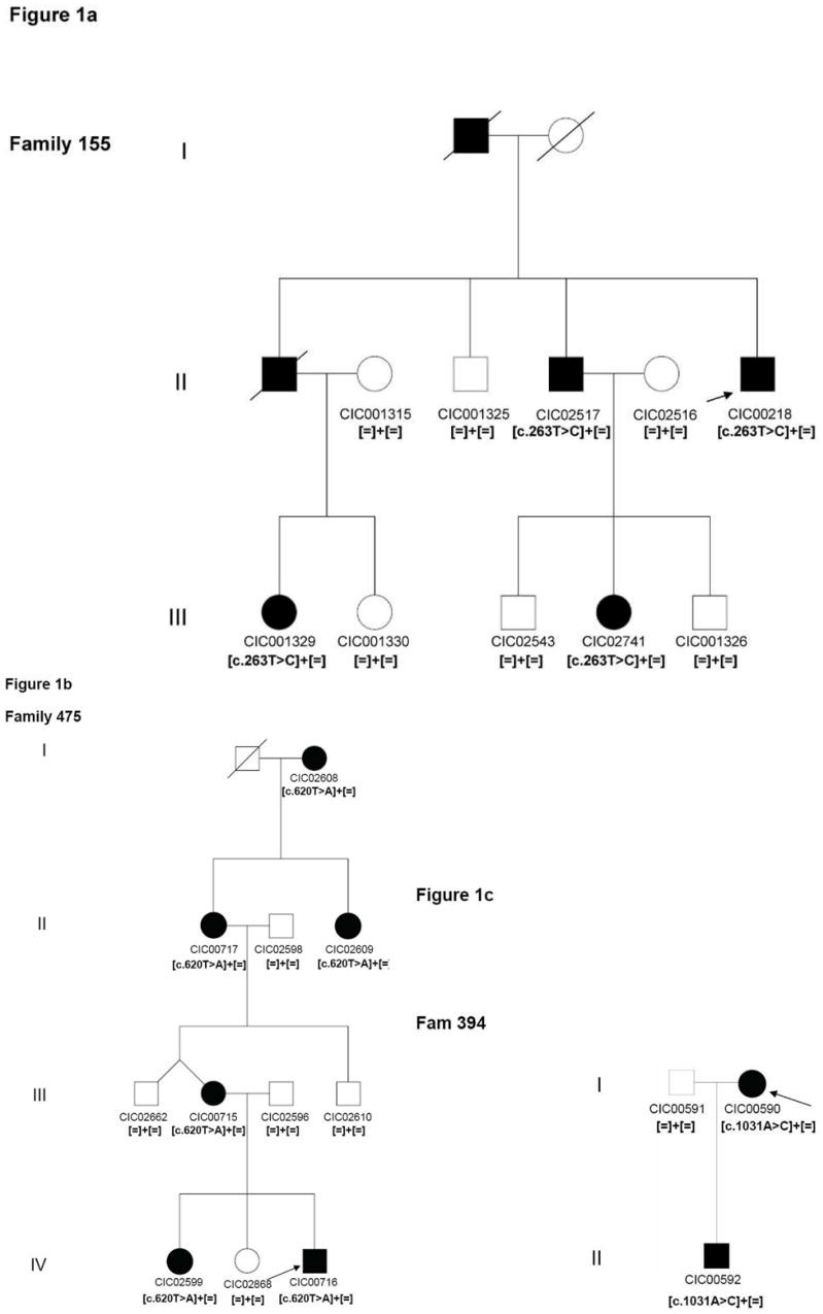


Figure 1d

Fam PB41

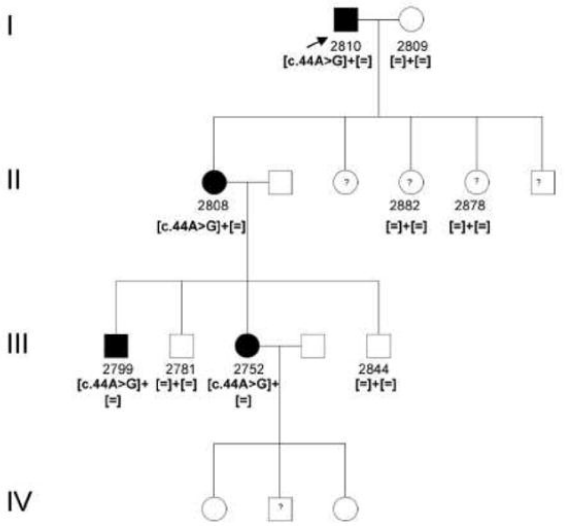


Figure 1d

Fam PB42

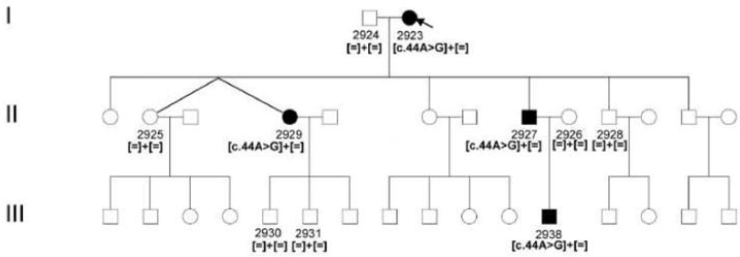


Figure 1e

Fam 172/96

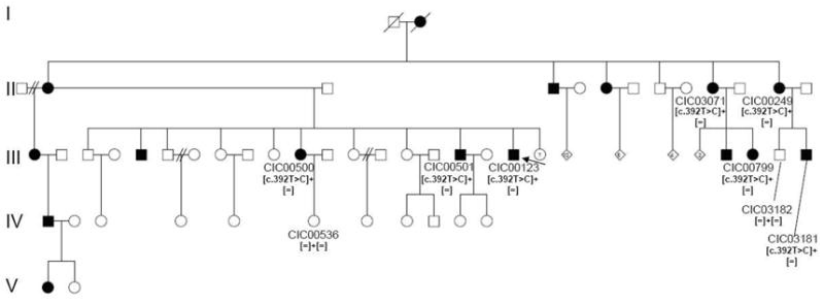


Figure 1f

Fam 247

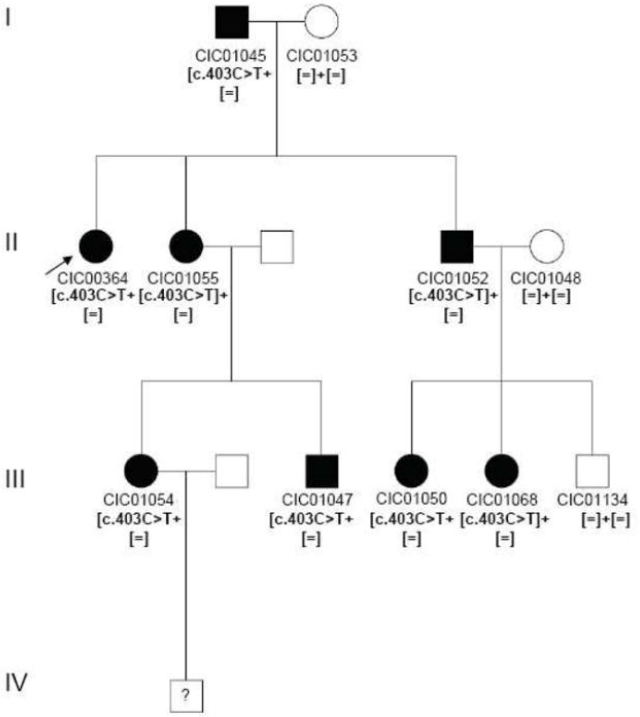


Figure 1f

Fam 610

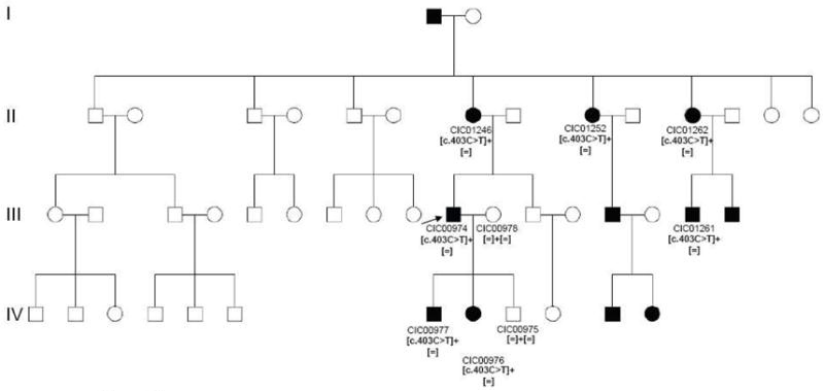


Figure 1g

Fam RP827

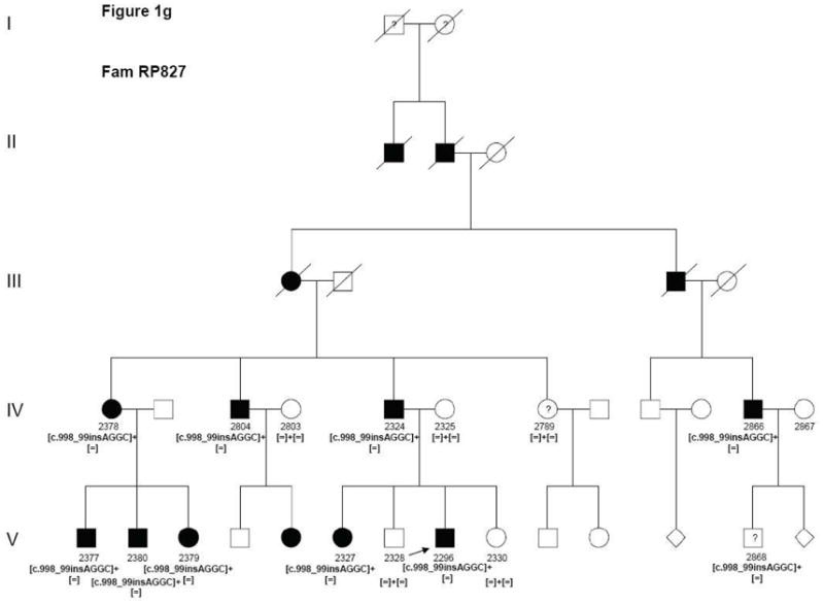


Figure 1h

Fam 119

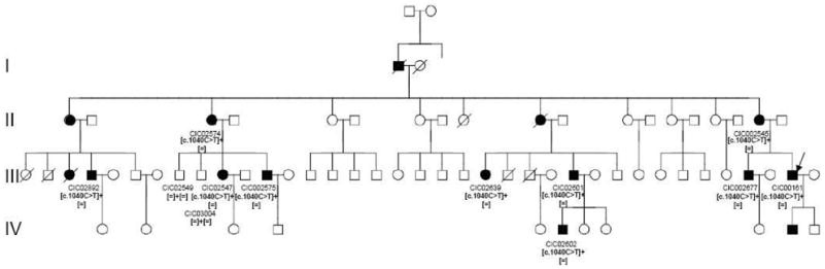
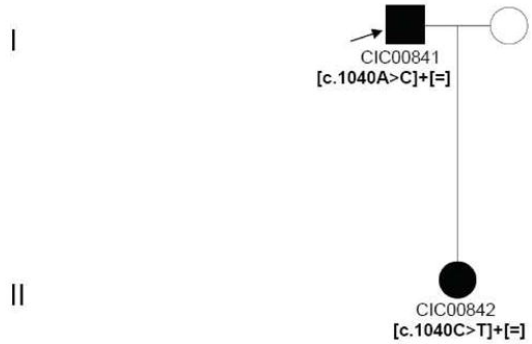


Figure 1h

Fam 546



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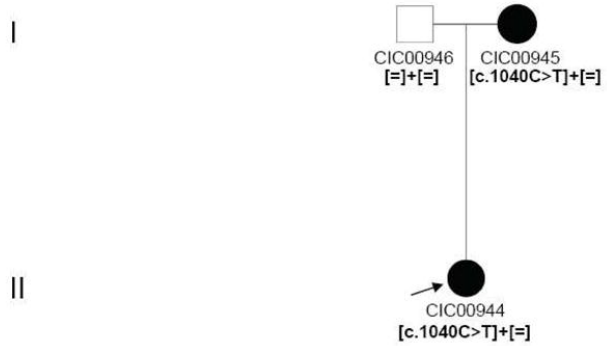

Figure 1h

Fam 681

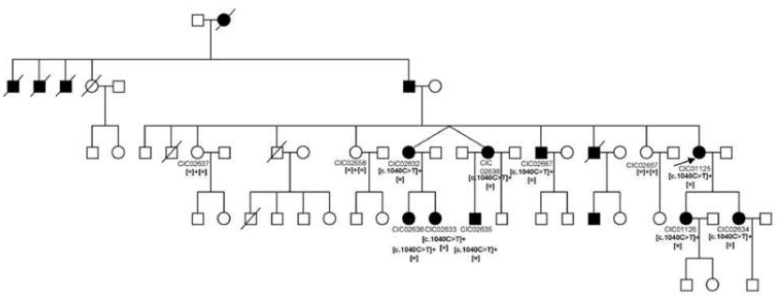

Figure 2

(a). Electropherograms of novel RHO mutations highlighted by an arrow. (b) Multiple amino acid sequence alignments of different species of novel mutated residues (depicted in green). Amino acid substitutions are highlighted in red. The position of the respective amino acids is shown in black numbers.

Figure 2a

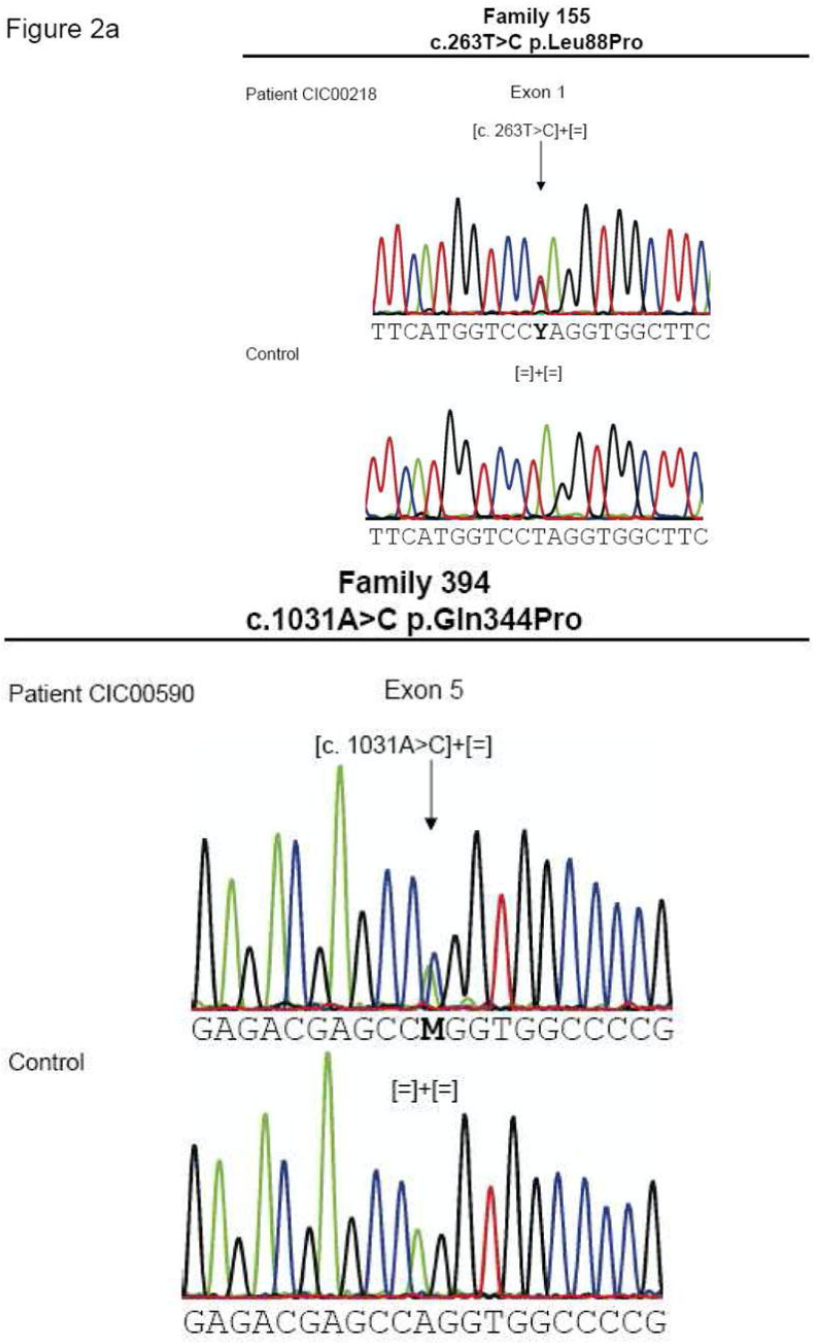

Figure 2b

	88	344
	P	P
Human	DLFMVLGGFTS	KTETSQVAPA*
Rhesus	DLFMVFGGFTT	KTETSQVAPA*
Tarsier	DLFMVFGGFTS	KTETSQVAPA*
Mouse	DLFMVFGGFTT	KTETSQVAPA*
Dog	DLFMVFGGFTT	KTETSQVAPA*
Elephant	NHFMVFGGFTT	----------
Opossum	DLFMVFGGFTM	KTETSQVAPA*
Platypus	NHFMVLGGFTT	KTETSQVSPA*
Chicken	DLFMVFGRFTT	KTETSQVSPA*
Lizard	NLFMVLMGFTT	KTETSQVSPA*
X_tropicalis	NHFMVLCGFTV	KTESSQVSPA*
Stickleback	DLFMVLGGFTT	KTQSSQVAPA*

Figure 3

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

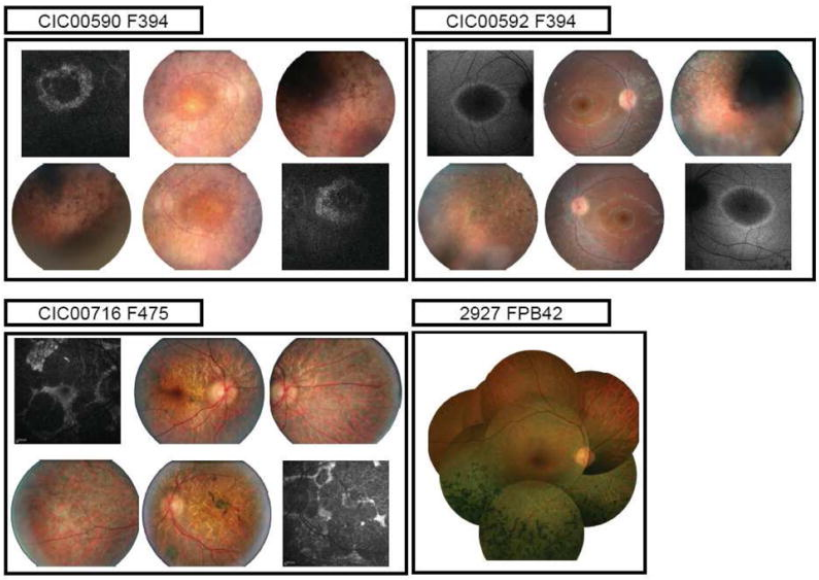

Table 1

Novel and known RHO mutations in the French cohort.

Index (family)	Exon	Nucleotide Exchange	Protein Effect	Publication
2810 (PB41) 2923 (PB42)	1	c.44A>G	p.Asn15Ser	36
CIC00218 (F155)	1	c.263T>C	p.Leu88Pro	novel
CIC00123 (F172/96)	2	c.392T>C	p.Leu131Pro	26
CIC00364 (F247) CIC00974 (F610)	2	c.403C>T	p.Arg135Trp	4
CIC00716 (F475)	3	c. 620T>A	p.Met207Lys	novel (phenotypegenotype correlation published Audo et al., in press)
2296 (RP827)	5	c.998_999insAGGC	p.Ser334GlyfsX20	13
CIC00590 (F394)	5	c.1031A>C	p.Gln344Pro	novel
CIC00161 (F119) CIC00841 (F546) CIC00944 (F598) CIC01125 (F681)	5	c.1040C>T	p.Pro347Leu	29

Table 2

Clinical features of affected members from familiies with ausosomal dominant retinitis pigmentosa (adRP) due to RHO mutations.

Family and RHO mutation	Patient	Sex	Age of diagnos	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°) Isoptre 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	Isoptre V4 65 °N, 70°T	20% of normal value for scotopic responses 35% of normal value for photopic rresponses No implicit time shift
	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	Isoptre V4 80 °N, 70°T	30% of normal value for scotopic responses Photopic 30Hz ERG slightly reduced No implicit time shift
Family PB42 c.44A>G	2927 II.8	M	30	PVFI at 30 No night blindness	52	20/20 + 2.50 (− 2.75; 20°)	-	Bone spicules in lower sector Epiretinal membrane OD/OS	Normal foveal lamination	Isoptre V4 80 °N, 90°T	30% of normal value for scotopic responses

p.15Asn>Ser						20/20 + 2.75 (− 2.50; 170 °)					80% of normal value for photopic 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 30Hz 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	Isoptre V4 20 ° central ODS	Not detectable
	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, few white dots	Normal foveal lamination	Isoptre 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	Isoptre III4 20° central ODS	Not detectable
Family 96/172 c.392T>C p.Leu131Pro	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 hyperAF	Normal foveal lamination	Isoptre II4 110° horizontally and 90° vertically	No responses detectable in scotopic conditions, some residual flicker responses
	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 hyperAF	Normal foveal lamination	Isoptre 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	Isoptre 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	Isoptre III4 30°	Not detectable

	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 hyperAF	Foveal thinning	Isoptre III4 170° horizontal x100° vertical	Not detectable
Family 610 c.403C>T p.Arg135Trp	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 hyperAF	Normal foveal lamination	Isoptre 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 hyperAF	CME	Isoptre III4 140° horizontal X120° vertical	Scotopic responses 10% of normal Photopic responses 50% normal Both amplitude reduction and implicit time shift
	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 isoptre	65% of normal for scotopic response amplitudes and 90% for scotopic responses; No implicit time shift
Family 475 c.620T>A p.Met207Lys	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 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 isoptre	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
	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°)	+	Bones 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	+	Bones spicules 360° Foveal photoreceptor loss	Foveal thinning	15°	Not detectable except for residual 30 Hz flicker ERG

Family RP827 c.998_999insAGGC p. Ser333GlyfsX22						20/400 + 6.00 (− 0.75; 60°)					
	2379 V.3	F	childhood	Night blindness since early childhood Photophobia at 5	45	20/30 (− 2.00; 90°) 20/40 (− 1.25; 105 °)	+	Bones spicules 360° small Perifoveal ring of hyperAF	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	Bones spicules 360°	Foveal thinning	10°	Not detectable except for residual 30 Hz flicker ERG
	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
Family 394 c.1031A>C p.Gln344Pro, novel	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 hyperAF	Normal foveal lamination	normal	Scotopic responses 10% of normal Photopic responses 80% normal Both amplitude reduction and implicit time shift
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 hyperAF	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 hyperAF	CME	Isoptre III4 20°	Not detectable
	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 hyperAF	CME	normal	Scotopic responses 10% of normal Photopic responses 80% normal Both amplitude reduction and implicit time shift
Family 598 c.1040C>T p.Pro347Leu	CIC 00945	F	9	Night blindness	43	20/32 + 3.5(− 1.25)10°	-	bone spicules 360°, no ring on AF	Foveal thinning	20°	Not detectable

						20/32 + 3.75(− 0.75)5°					
	CIC 01125	F	49	Night blindness	56	20/400 + 1.50(− 0.75)20°	OD IOL OS +	Few bone spicules 360° some areas of central atrophy, incomplete perifoveal ring of hyperAF	Foveal thinning	Isoptre III4 40°	Not detectable
Family 681 c.1040C>T p.Pro347Leu						20/200 + 3.25(− 0.75)10°					
	CIC 01126	F	29	Night blindness	36	20/20 20/20	-	Few bone spicules 360° Perifoveal ring of hyperAF	Normal foveal lamination	Isoptre III4: 150° horizontally X 60° vertically	No detectable scotopic responses Some residual flicker responses

PVFI = Peripheral Visual Field Impairment, VF = Visual Field, IOL = Intraocular lens, BCVA: Best Corrected Visual Acuity, OD/OS: Right eye/Left eye, RPE: Retinal pigment epithelium, HM: Hand Motion; CME: Cystoid Macular Edema;

* ERG performed with skin electrodes which precluded us to have a precise quantification of abnormalities, AF: autofluoresce., OCT: Optical Coherence Tomography.