EYS is a major gene for rod-cone dystrophies in France

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EYS is a major gene for rod-cone dystrophies in France

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Short Title: *EYS* is a major gene for arRP

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ABSTRACT: Autosomal-recessive retinitis pigmentosa (arRP) was recently associated with mutations in a novel gene *EYS*, spanning over 2 Mb, making it the largest known gene expressed in the human eye. The purpose of this study was to establish the prevalence and nature of *EYS* mutations in a clinically well-characterized cohort of 239 sporadic and arRP French cases. Direct sequencing of *EYS* was performed in 186 subjects for whom known mutations had previously been excluded by applying microarray technology. We mostly identified novel mutations in *EYS* in a total of 29 patients: Fifteen of the mutations were predicted to create premature stop codons and two represent exonic deletions. In addition, twenty missense, silent or splice-site mutations were detected. Patients revealed homozygous or compound heterozygous mutations and in some cases, only a single mutation. Most patients showed classical signs of RP with relatively preserved central vision and visual field until late in the course of the disorder. One patient showed predominance of the disease in the inferior part of the retina suggesting potential phenotypic variability. With a prevalence of 12% or more we provide evidence that *EYS* is a major gene for RP in France and probably elsewhere. ©2010 Wiley-Liss, Inc.

KEY WORDS: Rod-cone dystroyphy, RP, EYS, major gene

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INTRODUCTION

Rod-cone dystrophy also known as Retinitis Pigmentosa (RP) is a heterogeneous group of inherited disorders primarily affecting rods with secondary cone degeneration. It is the most common inherited form of potentially severe retinal degeneration and blindness, with a frequency of about 1 in 4000 births and more than 1 million individuals affected worldwide. (Hartong et al. 2006). Patients initially complain of night blindness due to rod dysfunction followed by progressive visual field constriction, abnormal colour vision and eventually loss of central vision. Inheritance of RP can be either autosomal recessive (ar), autosomal dominant (ad) or X chromosomal (xl) with also rare cases of mitochondrial transmission. Recessive and isolated cases account for 50-60% of all forms of RP. (Hartong et al. 2006). So far, 26 genes have been implicated in arRP and 4 additional loci have been mapped (http://www.sph.uth.tmc.edu/Retnet). Until recently, most of the identified genes accounted for only 1-2% of cases each, besides *USH2A* (OMIM:608400) which was found to be mutated in more than 5% of arRP without hearing loss (Rivolta et al. 2000; Bernal et al. 2003). Linkage analysis, initially performed in Spanish families (Ruiz et al. 1998), mapped the RP25 locus on chromosome 6q, which was subsequently reproduced in families from Pakistani (Khaliq et al. 1999) and Chinese origins (Abd El-Aziz et al. 2007). Additional Spanish families were also mapped to the same locus (Barragan et al. 2008). The high frequency of linkage to RP25 found in the Spanish population (13.7 to 27.7%) and the reproduction of this linkage in families from other parts of the world, suggested RP25 to be a major locus for arRP (Barragan et al. 2008). Refinement of the genetic interval followed by a bioinformatics based systematic positional cloning approach led to the identification of *EYS* (NCBI Reference Sequence: NM_001142800.1, OMIM: 612424) as the gene responsible for RP25 (Abd El-Aziz et al. 2008; Barragan et al. 2008). Authors reported 6 different mutations in 5 unrelated Spanish families including 4 deletions and 2 nonsense substitutions. All these changes were predicted to result in a premature stop codon leading to nonsense-mediated decay of the resulting mRNA with complete absence of a functional protein (Abd El-Aziz et al. 2008). Independently, Collin and co-workers, using homozygosity mapping, reported 3 consanguineous families linked to the RP25 locus with 2 of them showing the same frameshift mutation in *EYS* indicative of a founder effect and one with a homozygous nonsense mutation, both changes predicted to result in a premature termination codon and protein truncation (Collin et al. 2008).

Abd El –Aziz and co-workers described *EYS* as a large gene spanning approximately 2Mb of genomic DNA. The gene contains 43 exons, of which exons 4-43 encode a protein with 3144 amino acids (Abd El-Aziz et al. 2008). The study by Collin and co-workers revealed a similar gene structure with an additional exon between exon 41 and 42, making the predicted protein slightly larger with a size of 3165 amino acids (Collin et al. 2008). Both groups reported *EYS* as the human ortholog of the Drosophila 'eyes shut' (eys) gene, also known as spacemaker or spam, which plays an important role in photoreceptor morphogenesis in the insect eye with an open system such as in fruitflies and houseflies (Zelhof et al. 2006). The predicted human SPAM/EYS protein, consists of multiple epidermal growth factor (EGF)-like domains in its N-terminus followed by several Laminin G-like domains, interspersed by further EGF-like repeats, at the C-terminus (Abd El-Aziz et al. 2008). It was shown to be specifically expressed in the human retina and Y79 retinoblastoma cell lines, with a subcellular localization in the photoreceptor outer segment in porcine retina (Abd El-Aziz et al. 2008; Collin et al. 2008). The additional exon identified by Collins and co-workers belongs to a retina-specific alternative spliced variant, which can be present or not (Collin et al. 2008). Based on the known function of the drosophila ortholog, EYS is expected to play an important role in retinal morphogenesis and maintenance of its integrity. To investigate if *EYS* mutations are a major cause for RP in autosomal recessive and simplex cases, we evaluated a French cohort comprising 239 individuals including patients of different ethnic origins.

PATIENTS AND MATERIALS

Two hundred and thirty nine index patients with a presumed diagnosis of simplex or arRP were ascertained. 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 committee. Each patient underwent full ophthalmic examination with assessment of best corrected

visual acuity, using the EDTRS chart, kinetic and static perimetry and colour vision using 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 (Espion² Diagnosys® for full field ERG and Veris II for Multifocal ERG) (Marmor et al. 2003; Marmor et al. 2004). 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 blood samples for further genetic studies. Total genomic DNA was extracted from peripheral blood leucocytes blood samples according to manufacturer recommendation (Puregen Kit, Qiagen, Courtaboeuf, France). Prior to *EYS* mutation analysis, the DNA of all index patients was analyzed for known mutations by microarray analysis on a commercially chip (ASPER Ophthalmics, Tartu, Estonia). In total the DNA of 186 index patients were excluded for a likely disease causing mutation by this approach and were further investigated for mutations in the coding exons and flanking intronic regions of *EYS* by PCR. The reaction mix contained 1.5 mM (2.5mM for exon 16) $MgCl_2$ and a commercially available polymerase (HOT FIREPol DNA Polymerase, Solis BioDyne, Tartu, Estonia) at an annealing temperature of 60°C using primers previously described (Abd El-Aziz et al. 2008). PCR products were enzymatically purified (ExoSAP-IT, USB Corporation, Cleveland, Ohio, USA purchased from GE Healthcare, Orsay, France) and sequenced with a commercially available sequencing mix (BigDyeTerm v1.1 CycleSeq kit, Applied Biosystems, Courtaboeuf, France). For exon 12, two additional sequencing primers were used (EYS_Ex12seqF: 5'-GACTATTGCTTAGGGAACCAC-3' and EYS_Ex12seqR 5'-TCCTGGGACACACTTGCG-3'). The sequenced products were purified on a presoaked Sephadex G-50 (GE Healthcare) 96-well multiscreen filter plate (Millipore, Molsheim, France), the purified product analyzed on an automated 48-capillary sequencer (ABI 3730 Genetic analyzer, Applied Biosystems) and the results interpreted by applying a software (SeqScape, Applied Biosystems). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Seven synthetic MLPA (Multiplex Ligation dependent Probe Amplification) probes for several exons of *EYS* including exons 12, 32 and 33 of *EYS* were designed according to the MRC Holland guidelines. Each probe sequence was checked for localization and putative polymorphisms using SNP databases (http://www.ncbi.nlm.nih.gov./, and http://genome.ucsc.edu/cgi-bin/hgBlat). A set of control probes was included in each synthetic probe mix in order to provide constant values. The reactions were carried out as previously described (Schouten et al. 2002). The MLPA interpretation and analysis criteria were as follows: i) normal if the individual dosage quotient values were within a range of 0.8-1.0; ii) deletions or duplications if the dosage quotient values were around 0.5 or 1.5, respectively; and iii) the mean standard deviation of all samples for each peak should be below 10%.

RESULTS AND DISCUSSION

Prior to the mutation screening of *EYS,* the DNA of all index patients was analyzed for known mutations using a commercially available microarray chip (ASPER Ophthalmics, Tartu, Estonia) In total DNA samples from 186 index patients were excluded for a likely disease causing mutation using this approach. Mutation screening of *EYS* by direct sequencing of all coding exons revealed for 29 of the 186 patients one or two likely pathogenic nonsense, frameshift, missense, splice-site or silent mutations, which co-segregated with the phenotype when tested in family members available (Tables 1, 2 and 3). A sequence variant was considered to be pathogenic if the same nucleotide exchange was not found in another species, or if it represents a nonsense or frameshift mutation, or affects a conserved amino acid residue, which may localize in a predicted important domain of EYS, appeared only 1-3 times in 186 patients and did not appear in control samples investigated (total number of alleles studied ≥ 400, see Table 1 and 2). Furthermore, splice site (http://www.fruitfly.org/seq_tools/splice.html) and exonic splicing enhancer prediction programs (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home) were applied to estimate the pathogenic nature of a sequence variant.

In total 12 of the 186 patients screened for *EYS* revealed 15 different new mutations, which are predicted to result in a truncated protein or lead to nonsense mediated mRNA decay (Table 1 and 2). They represent 8 different deletions in exons 4, 23, 26, 27, 34 and 43, and 7 nonsense mutations in exons 11, 20, 26 and 43. For two other

patients' exon12 and exons 32-33 could not be amplified, respectively indicating the presence of large homozygous deletions covering these exons and the flanking intronic regions. MLPA studies performed herein confirmed that exon 12 in one patient (CIC01222) and exons 32 and 33 in another patient (CIC00157) are homozygously deleted. Interestingly, also Abd El-Aziz and co-workers described an exon 12 deletion (Abd El-Aziz et al. 2008). Due to the different origins of both patients (Egyptian Table 4 reported here versus Spanish published by Abd El-Aziz 2008), we hypothesize that this exonic deletion most probably represents a recurrent mutation rather than a founder effect in *EYS*.

Furthermore, we report 14 likely pathogenic missense mutations in exons 12, 14, 22, 23, 25, 26, 29, 31, 32 and 43 (Tables 1 and 2). They either affect evolutionary well or highly conserved nucleotide and amino acid residues (Figure 1) or may have an effect on predicted functional regions of EYS like Laminin G, EGF/EGF-like or EGF/Laminin domains (Table 1).

Additionally, 4 putative pathogenic mutations at or close to splice sites in 4 different patients were identified (Tables 1 and 2).

1. The c.748+6A>T mutation in intron 4 identified in 1 patient affects a nucleotide, which is present in all but one case as an A (Figure 2). The pathogenicity of this exchange remains unclear since splicing was not predicted to be significantly changed compared to the normal sequence.
2. The c.2023+1G>C mutation in intron 12 identified in 1 patient affects a donor site, which is evolutionary and functionally highly conserved (Figure 2) and may thus lead to exon skipping of exon 12:
3. The c.2847-1G>T mutation in intron 18 identified in 1 patient affects a predicted acceptor splice site, which is evolutionary and functionally conserved (Figure 2) and is predicted to lead to skipping of exon 19.
4. Another mutation, c.3164+15insT in intron 20 detected in 1 patient might influence the regulation of EYS. However further functional tests are pending to prove this hypothesis.

Also two putative pathogenic silent mutations were identified:

1. The c.5604A>T (p.Ser1868Ser) mutation in exon 26 detected in 1 patient affects a highly conserved nucleotide (Figure 2). Two additional exon splicing enhancer proteins were predicted to bind to the sequence variant and might thus influence splicing (data not shown).
2. The c. 5886T>C (p.Thr1962Thr) mutation in exon 28 detected in 1 patient affects a conserved nucleotide. Only in two species an A and G occurs instead of the T (Figure 2). Furthermore the mutation is predicted to delete a binding site for an exon splicing enhancerprotein (data not shown).

Thus our study revealed 29 patients with 15 nonsense mutations, 2 exonic deletions, 14 missense mutations, 4 splice site and 2 silent mutations, all predicted to be disease causing. In the two original articles describing the gene identification of *EYS*, frameshift and nonsense mutations were identified in exons 12, 15-19, 17, 28, 33, 41 and 43. Together with our findings describing the prevalence in a large RP cohort, exons 4, 11, 12, 14, 15-19, 17, 20, 22, 23, 25, 26, 27, 28, 29, 31, 32, 33, 34, 41 and 43 harbor a likely pathogenic mutation and these exons might represent a common target for other cohort studies. However, all mutations identified in this study were novel and thus it is likely that other studies will find other exons of *EYS* to be frequently mutated in RP patients.

Of the 29 patients with a mutation in *EYS*, 13 were apparently homozygous or compound heterozygous for the mutations, while for the remaining patients the second mutation could not be identified (Table 2). This may be due to relatively large heterozygous deletions, which are not detectable by direct sequencing methods. When the DNA of proband's family members were available, we performed co-segregation studies which are summarized in Table 2. See below some examples of such investigations in families reported here:

1. Patient CIC00513 (II-5) was apparently homozygous for a deletion, which is predicted to lead to a protein missing almost all predicted Laminin G domains (p.Lys1945SerfsX42). The mutation co-segregated with the phenotype: The mother (I-2) and the two unaffected sisters (II-1 and II2) were heterozygous and the affected brother (II-3) homozygous for the mutation (Figure 3, Fam 347).
2. Interestingly patient CIC00951 (II-3) and his affected brother CIC03294 (II-1) carried 3 nonsense mutations: two in exon 26 (p.Gln1751X and p.Tyr1753X) and one in exon 43 (p.Tyr3059X). Co-segregation analysis revealed that the mother (I-2) carried the p.Gln1751X and p.Tyr3059X on the same allele, while the father (I-1) carried the p.Tyr1753X mutation (Figure 3, Fam 602). Since the affected brother showed the same genotype as the index patient it is not possible to say which of the two changes inherited from the mother may

be pathogenic. However, if all mutations in this family would lead to a premature stop codon or nonsense mediated mRNA decay, it is then likely that all three mutations can be disease causing.

3. Index patient CIC01265 (II-1) was compound heterozygous for two different alterations (one deletion and one insertion), both predicted to lead to a truncated protein (p.Thr308IlefsX4 and Asn3123LysfsX4), which co-segregated with the disease. The affected sister also showed both mutations, the father was heterozygous for the p.Thr308IlefsX4, while the mother carried the other Asn3123LysfsX4 mutation (Figure 3, Fam 760).

Thus, although we could show for some families co-segregation of the mutations with the phenotype and our criteria to classify a mutation as pathogenic was quite stringent, functional analysis of the mutations are needed to further prove the pathogenic character of these changes.

Genotype-Phenotypic characterization

Clinical and functional findings are summarized in table 4 and table 5, respectively. Patients' age ranges from 3 to 58 years at the time of diagnosis, with an average age of 25 years and from 16 to 73 at time of testing with an average age of 39 years. The patients came from diverse geographical origins including Europe, North Africa, West Africa and Egypt. They show typical signs of progressive rod-cone dystrophy with relatively preserved central vision and visual field until late in the course of the disorder, except for 2 patients (CIC01223 and CIC00492), for whom loss of central vision appeared early in the course of the disease. For most patients, symptoms include early onset night blindness, followed by progressive visual field constriction and a certain degree of photophobia later in the course of the disease. Three patients did not report night blindness at onset but instead one reported photophobia (CIC00167) and two others reported constricted visual fields at time of diagnosis (CIC00139 and CIC01066). Strabismus was present in 2/29 patients (CIC00081 and CIC00656). All patients were ametropic except one showing various degree of myopia, hyperopia or astigmatism. Regarding visual acuity, only two patients, aged 30 and 48 years, show vision limited to hand motion perception and this low vision is associated with atrophic changes within the macula. The other patients have 20/100 vision or better at least in one eye and, when measurable, Best Corrected Visual Acuity (BCVA) ranges between 20/16 and 20/100 and tend to be worse in older patients. Anterior segment examination show small pigmented opacities on posterior capsule for one 19 year-old patient. Ten other patients, age 34 and older had subcapsular cataract or had undergone cataract surgery. Fundus examination shows typical sign of RP for most patients with pale optic nerve disc, narrowed blood vessels, pigment changes with intraretinal migration over 360° of the fundus (Figure 4, patient CIC00656), which are more prominent in older patients. Two patients show small white dots in the peripheral retina with limited pigment migration. No white dots were visible when RPE changes and pigment migration were prominent. Two patients had more severe involvement with retinal atrophy involving the posterior pole (Figure 4, CIC00656). One patient (CIC01222) showed distinct fundus abnormalities with predominance of pigmentary changes in the inferior part of both retinas resembling sector RP (Figure 4, patient CIC01222). This represents a distinct phenotype, which is usually associated with autosomal dominant RP linked to mutations in the rhodopsin gene (*RHO*, OMIM: 180380) (Heckenlively et al. 1991) (Audo et al. 2010). However, ERG responses (Table 5) were not detectable for this patient, consistent with severe generalized rod-cone dysfunction which is unusual for typical sector RP associated with rhodopsin mutation. The role of light exposure is usually suggested to explain the preferred location of retinal involvement. Since the precise function of EYS in photoreceptor biology is unknown, there is no accurate explanation for mutations leading to this peculiar sectorial phenotype and it can be speculated that EYS may be interacting or influencing rhodopsin expression. In addition, one patient had a history of Coats disease treated by cryo-application, a feature often associated with mutations in the *CRB1* gene (OMIM: 604210) (den Hollander et al. 2001). Twelve patients showed macular atrophic changes. Nine patients had a history of cystoid macular edema (CMO) (31%) which is consistent with previous reports on CMO frequency in RP (Hajali et al. 2008). The two later macular changes were confirmed with Optic Coherence Tomography. On Fundus Autofluorescence imaging, 14/29 patients showed a perifoveal ring of increased autofluorescence (Figure 4, patients CIC00589 and CIC01265) and its absence was correlated with the presence of atrophic changes and loss of autofluorescence within the macula (Figure 4, patient CIC00656).

Tritanopia was the most common color abnormality in this cohort: color vision was normal in 6/29 patients, showed a dyschormatopsia with tritan axis in 16/29 patients in at least one eye, a dyschromatopsia with no major axis in 8/29 and could not be tested due to low vision in 2/29 patient. There was a correlation between the presence of a dyschromatopsia and visual acuity or between the presence of a dyschromatopsia and atrophic changes within the macula.

Based on kinetic visual field testing, all patients showed constriction of their visual field with some level of correlation between the degree of constriction and age, younger patients having a more conserved visual field than older patients.
Patients had undetectable responses on the full field ERG in both scotopic and photopic conditions except one with residual photopic responses. On multifocal ERG, 19/28 patients showed some degree of preserved responses to central hexagons. These preserved responses are poorly correlated with visual acuity, preservation of macular region on fundus examination, or presence of CMO.

Overall, in 12% of this French cohort with autosomal recessive or simplex RP we have identified at least one very likely pathogenic mutation providing evidence that *EYS* is a major gene for RP in France and probably elsewhere. Most patients showed classical signs of RP with relatively preserved central vision and visual field until late in the course of the disease. One patient with predominance of the disease in the inferior part of the retina would suggest potential phenotypic variability, a hypothesis which needs to be confirmed from studies analyzing other cohorts.

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Tables and Figure Legends

Figure 1-2 Evolutionary conservation of the altered amino acid residues in other orthologs. Multiple amino acid sequence alignments show evolutionary conservation of 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 3 *EYS* mutations and co-segregation analysis in families with RP.
Corresponding pedigrees of selected arRP patients with *EYS* 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 4 Colour fundus photograph and autofluorescence imaging examples of patients carrying *EYS* pathogenic mutations: CIC01222 has predominance of retinal pigment epithelium changes in the inferior part of the retina (a), well documented by autofluorescence (b), resembling sector RP; CIC00589 has little pigmentary changes in the periphery (a) with loss of autofluorescence in these areas (b). She also presents a perifoveal ring of hyperautofluorescence and a bilateral cystoïd macular edema documented by OCT (c); CIC01265 shows also little pigmentary changes in peripheral retina (a) with a perifoveal ring of hyperautofluorescence (b). The diameter of this ring appears larger than of CIC00589 who is also older, consistent with a less advanced retinopathy; CIC00656 shows atrophic changes in the periphery and within the macula (a) manifested by a loss of autofluorescence in the posterior pole (b).

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Table 1: Mutations and sequence variants identified in a Fench arRP cohort

Table 1-3: Mutation analysis has been performed in the exons and intronic sequence of the coding region of *EYS*. Mutations, sequence variants and their putative pathogenic characters identified in the different exons are depicted. New truncating mutations are highlighted in red and bold letters, new missense, splice site and silent mutations are highlighted in red. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Exon	Sequence variation	Consequence	Frequency	SNP ID, Conclusions, Extra Control Alleles (mut/normal)
1				
2				
3				
4	c.281C>A	p.Pro94Gln	1	Not conserved, SNP
	c.334G>A	p.Val112Ile	4	Not conserved, SNP
	c.359C>T	p.Thr120Met	200	rs12193967
	c.403AC>CT	p.Thr135Leu	1	Not conserved, SNP
	c.406G>T	p.Val136Phe	1	Not conserved, SNP
	c.408_423delTAATTCTAAGTGGCTG	**p.Asn137ValfsX24**	**1 family**	**New mutation**
	c.544delT	**p.Cys183AlafsX74**	**1 family**	**New mutation**
	c.748+6A>T	unknown	1	0/136, conserved, new mutation
5	c.777G>A	p.Gln259Gln	1	0/178, but not conserved, SNP
6	c.977G>A	p.Ser326Asn	2	1/200, SNP
7	c.1146T>C	p.Asn382Asn	145	rs974110
8	---	---	---	---
9	c.1300-3C>T	---	161	rs1936439
10	c.1596A>C	p.Lys532Asn	1	rs61753611
11	c.1652G>T	p.Arg551Leu	1	0/180, but Leu als also in Orangutan, SNP
	c.1642C>T	**p.Gln548X**	**1**	**New mutation**
	c.1674G>A	**p.Trp558X**	**1**	**New mutation**

	c.1712A>G	p.Gln571Arg	43	rs61753610
12	c.1809C>T	p.Val603Val	140	rs9345601
	c.1852G>A	p.Gly618Ser	1	0/180, unknown domain, conserved, new mutation
	c.1891G>A	p.Gly631Ser	140	rs9342464
	c.1922A>T	p.Glu641Val	100	rs17411795
	c.2023+1G>C	Splice defect?	1	0/180, conserved, new mutation
	deletion exon 12	p.Cys590TyrfsX4	1	**Probably same mutation as (Abd El-Aziz et al. 2008)**
13	---			
	c.2157C>T	p.Cys719Cys	3	rs9453148
14	c.2234A>G	p.Asn745Ser	1	0/328, EGF/LAMININ, conserved, new mutation
15	---	---	---	---
16	c.2500G>A	p.Val834Ile	1	0/190, also a in mouse, Ile in Drosophila, SNP
	c.2555T>C	p.Leu852Pro	271	rs9294631
	c.2598C>T	p.Cys866Cys	21	T also in opossum, platypus, CN_1202103, SNP
17	---	---	---	---
18	c.2739G>A	p.Arg913Arg	5	0/158, but A also found tarsier, dog, elephant, opossum, SNP
	c.2813A>G	p.Lys938Arg	1	also found in lizard, SNP
19	c.2847-1G>T	Splice defect?	1	0/134, conserved, new mutation
20	**c.3003T>A**	**p.Cys1001X**	**1**	**New mutation**
	c.3164+15insT	Splice defect?	1	new mutation
21	---	---	---	---
22	c.3329C>G	p.Thr1110Ser	1	0/178, EGF/EGF-like domain, new mutation
23	c.3444-5C>T	---	196	rs9445051chimp
	c.3489T>A	p.Asn1163Lys	1	Co-segregation excluded this variant to be pathogenic
	c.3526T>C	p.Cys1176Arg	1	0/190, EGF/EGF-like domain, new mutation
	c.3567delA	**p.Gly1190AspfsX39**	**1**	**New mutation**
24	---	---	---	---
25	c.3694A>T	p.Ile1232Phe	1	0/200, EGF/EGF-like domain, new mutation
	c.3787A>G	p.Ile1263V	84	rs17404123
26	c.3906C>T	p.His1302His	40	rs12663916
	c.3936A>G	p.Thr1312Thr	40	rs12662610

	c.3973C>G	p.Gln1325Glu	40	rs12663622
	c.4026C>T	Ser1342Ser	40	rs12663619
	c.4081A>G	p.Ile1361Val	40	rs17403955
	c.4093A>G	p.Lys1365Glu	2	rs16895519
	c.4256T>C	p.Leu1419Ser	183	rs623851
	c.4352T>C	p.Ile1451Thr	40	rs62415828
	c.4543C>T	p.Arg1515Trp	39	rs62415827
	c.4549A>G	p.Ser1517Gly	39	rs62415826
	c.4593G>A	p.Glu1531Glu	38	rs62415825
	c.4827_4830delTTCA	**p.Ser1610PhefsX7**	**1**	**New mutation**
	c.4985A>T	p.Asp1662Val	1	1/174, conserved, unknown domain, SNP
	c.4991C>T	p.Thr1664Ile	1	0/176, T also found Opossum and Platypus, SNP
	c.5044G>T	p.Asp1682Tyr	3	0/248, unknown domain, conserved, new mutation
	c.5216C>T	p.Pro1739Leu	1	0/156, T also found in Opossum and Platypus, SNP
	c.5240A>G	p.Glu1747Gly	1	0/158, conserved, new mutation
	c.5244A>C	p.Leu1748Phe	27	rs57312007
	c.5251C>T	**p.Gln1751X**	**1**	**New mutation**
	c.5259T>A	**p.Tyr1753X**	**1**	**New mutation**
	c.5376A>G	p.Ala1792Ala	1	0/158, not conserved, SNP
	c.5510G>C	p.Trp1837Ser	1	20/158, conserved, SNP
	c.5604A>T	p.Ser1868Ser, splicing ?	1	0/158, conserved, new mutation
	c.5605C>A	p.Leu1869Met	1	0/148, unknown domain, conserved, new mutation
	c.5617C>G	p.Leu1873Val	40	rs16895517
27	c.5743A>G	p.Ser1915Gly	1	0/180, but G also in Tarsier and Lizard, SNP
	c.5705A>T	p.Asn1902Ile	74	rs9353806
	c.5834delA	**pLys1945SerfsX42**	**1**	**New mutation**
28	c.5886T>C	p.Thr1962Thr	1	0/200, conserved, new mutation
29	c.5928-2A>G	---	1	rs36133910
	c.5959A>C	p.Thr1987Pro	1	0/362,conserved, G-Laminin domain, new mutation
	c.5977A>G	p.Thr1993Ala	3	other species also a G instead of A, SNP
	c.5995A>G	p.Ile1999Val	1	1/362, SNP
30	c.6078-2delCT	---	175	rs35395170
	c.6119T>A	p.Val2040Asp	3	2/380, SNP

31	c.6416G>A	p.Cys2139Tyr	2	0/182, conserved, EGF/EGF-like domain, new mutation
32	c.6452A>G	p.Asn2151Ser	2	Present in other species, SNP
	c.6566T>C	p.Leu2189Pro	1	0/174, Laminin G domain, new mutation
32-33	deletion exon 32 -33	p.Asp2142AlsfsX14		**New mutation**
33	c.6645G>T	p.Gly2215Gly	1	0/180, not conserved, SNP
	c.6632C>T	p.Ser2211Leu	3	0/280,Laminin G, not conserved, SNP
34	**c.6794delC**	**p.Pro2265GlnfsX46**	**1**	**New mutation**
35	c.6855C>T	p.Phe2285Phe	1	0/160, not conserved, SNP
	c.6977G>A	p.Arg2326Gln	210	rs4710457
36	c.7083T>C	p.Cys2361Cys	1	0/380, not conserved, SNP
	c.7182T>C	p.Ile2394Ile	1	0/380, not conserved, SNP
37	---	---	---	---
38	---	---	---	---
39	c.7608C>T	p.Ile2536Ile	1	0/170, not conserved, SNP
	c.7666A>T	p.Ser2556Cys	25	rs66462731
40	c.7737T>C	p.Thr2579Thr	2	4/192 control alleles also, SNP
	c.7796A>G	p.His2599Arg	4	2/192 control alleles also, SNP
41	---	---	---	---
42	c.8206G>C	p.Ala2736Pro	1	0/90, Laminin G, not conserved, SNP
43	c.8422G>A	p.Ala2808Thr	1	0/58, Laminin G, conserved, Laminin G, new mutation
	c.8429C>T	p.Thr2810Ile	1	0/58, Laminin G, not conserved, SNP
	c.8712T>C	p.Asp2904Asp	1	0/360, not conserved, SNP
	c.8349G>A	**p.Trp2783X**	**1**	**New mutation**
	c.8669G>A	p.Cys2890Tyr	1	0/58, EGF-EGF-like domain, conserved, new mutation
	c.8720G>A	p.Gly2907Glu	1	0/360, EGF7EGF-like domain conserved, new mutation
	c.9030A>G	p.Ala3010Ala	5	rs61754905
	c.9111_9112delCA	**p.Thr3038IlefsX4**	**1**	**New mutation**
	c.9368insA	**p.Asn3123LysfsX3**	**1**	**New mutation**
	c.9177C>G	**p.Tyr3059X**	**1**	**New mutation**

Table 1-3: Mutation analysis has been performed in the exons and intronic sequence of the coding region of EYS. Mutations, sequence variants and their putative pathogenic characters identified in the different exons are depicted. New truncating mutations are highlighted in red and bold letters, new missense, splice site and silent mutations are highlighted in red. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Table2: arRP patients and family members with likely pathogenic EYS mutations

Index Patient, Family Members	Family	Exon	Nucleotide Exchange	Allele State Human Mutation	Protein Effect	Extra Control Alleles (Mut/Normal), Conclusion
CIC00081	58	11	c.1674G>A	het	p.Trp558X	Only this patient
CIC00338	228	20	c.3003T>A	het	p.Cys1001X	Only this mutation patient,
CIC00513 (II-5)	347	27	c.5834delA	Homo	p.Lys1945SerfsX42	Only this patient, new mutation, co-segregates, presumably homo
unaff. mother CIC00514 (I-2)		27	c.5834delA	het	p.Lys1945SerfsX42	
unaff. sister CIC03285 (II-1)		27	c.5834delA	het	p.Lys1945SerfsX42	
aff. brother CIC01885 (II-3)		27	c.5834delA	homo	p.Lys1945SerfsX42	
unaff. sister CIC03299 (II-2)		27	c.5834delA	het	p.Lys1945SerfsX42	
CIC00006	6	23	c.3567delA	het	p.Gly1190AspfsX39	only this patient
unaff. wife CIC03273		23	no	-	no	
unaff. daughter CIC03274		23	c.3567delA	het	p.G1190AspfsX39	
unaff. son CIC03275		23	c.3567delA	het	p.G1190AspfsX39	
CIC00264	181	11	c.1642C>T	het	p.Gln548X	Only this patient
		12	c.1852G>A	het	p.Gly618Ser	Only this patient
CIC00656	444	43	c.8349G>A	het	p.Trp2783X	Only this patient
Unaff. father CIC03288		43	c.8349G>A	het	p.Trp2783X	
Unaff. mother CIC03289		43	no	-	no	
Unaff. brother CIC03287		43	c.8349G>A	het	p.Trp2783X	
Unaff. sister CIC03340		43	no	-	no	
CIC00951 (II-3)	602	26	c.5251C>T	het	p.Gln1751X	Only this patient
		26	c.5259T>A	het	p.Tyr1753X	
		43	c.9177C>G	het	p.Tyr3059X	
unaff.mother CIC03295 (I-2)		26	c.5251C>T	het	p.Gln1751X	
		43	c.9177C>G	het	p.Tyr1753X	
unaff. father CIC03296 (I-1)		26	c.5259T>A	het	c.Tyr1753X	
aff. brother CIC03296 (II-1)		26	c.5251C>T	het	p.Gln1751X	
		26	c.5259T>A	het	p.Tyr1753X	
		43	c.9177C>G	het	p.Tyr3059X	
CIC01001	618	4	c.547delT	het	p.Cys183AlafsX74	Only this patient
unaff. wife		4	no			

CIC03487						
aff. sister		4	c.547delT	het	p.Cys183AlafsX74	
CIC03499						
unaff. mother		4	c.547delT	het	p.Cys183AlafsX74	
CIC03605						
unaff. brother		4	c.547delT	het	p.Cys183AlafsX74	
CIC03639						
CIC01223	736	4	c.408_423del	homo	p.Asn137ValfsX24	Only this patient
Unaff. sister		4	c.408_423del	het	p.Asn137ValfsX24	
CIC03643						
CIC01265 (II-1)	760	43	c.9111_9112delCA	het	p.Thr3038IlefsX4	Only this patient
		43	c.9368insA	het	p.Asn3123LysfsX3	
Unaff. father		43	c.9111_9112delCA	het	p.Thr3038IlefsX4	
CIC01268 (I-1)						
Unaff. mother		43	c.9368insA	het	p.Asn3123LysfsX3	
CIC01266 (I-2)						
Aff.sister CIC01267 (II-2)		43	c.9111_9112delCA	het	p.Thr3038IlefsX4	
		43	c.9368insA	het	p.Asn3123LysfsX3	
CIC00456	311	34	c.6794delC	het	p.Pro2265GlnfsX46	only this patient
CIC00529	360	26	c.4827_4830delTTCA	het	p.Ser1610PhefsX7	only this patient
unaff. father CIC00794		26	c.4827_4830delTTCA	het	p.Ser1610PhefsX7	
unaff. mother		26	no	-	no	
CIC00528						
unaff. sister CIC00710			no	-	no	
unaff. siser		26	c.4827_4830delTTCA	het	p.Ser1610PhefsX7	
CIC00766						
CIC01222	735	12	Deletion	homo	p.Cys590TyrfsX4	only this patient
unaff. brother 3588		12	no	-	no	
CIC00157	115	32-33	Deletion	homo	p.Asp2142AlafsX14	only this patient
aff. brother		32-33	Deletion	homo	p.Asp2142AlafsX14	
CIC00152						
unaff. mother		32-33	Deletion	het	p.Asp2142AlafsX14	
CIC00158						
unaff. father CIC00883		32-33	Deletion	het	p.Asp2142AlafsX14	
CIC00139	109	20	c.3164+15insT	het	splice defect?	Only this patient
		26	c.5044G>T	het	p.Asp1682Tyr	3 patients, 0/248, unknown domain, conserved
CIC00492	336	25	c.3694A>T	het	p.Ile1232Phe	Only this patient, 0/200, EGF/EGF-like domain,

						conserved
		26	c.5604A>T	het	p.Ser1868Ser	Only this patient, 0/158 conserved
		26	c.5605C>A	het	p.Leu1869Met	Only this patient, 0/148,unknown domain, conserved
Unaff, mother		25	c.3694A>T	het	p.Ile1232Phe	
CIC00493		26	none of the 2	-	none of the 2	
CIC00630	422	26	c.5044G>T	het	p.Asp1682Tyr	3 patients, 0/248 unknown domain, conserved,
CIC00589	393	32	c.6566T>C	het	p.Leu2189Pro	Only this patient, 0/174, Laminin G domain, conserved,
unaff. paternal uncle CIC01179		32	no	-	no	
CIC00714	474	29	c.5959A>C	het	p.Thr1987Pro	only this patient, 0/362, Laminin G, conserved
		43	c.8669G>A	het	p.Cys2890Tyr	only this patient, 0/138, EGF/EGF-like domain, conserved
CIC00729	481	43	c.8720 G>A	het	p.Gly2907Glu	only this patient, 0/380, EGF/EGF-like domain, conserved
Unaff mother CIC00730		43	c.8720 G>A	het	p.Gly2907Glu	
Unaff. husband CIC00779		43	no	-	no	
Unaff.daughter CIC00780		43	c.8720 G>A	het	p.Gly2907Glu	
Unaff. daughter CIC00781		43	c.8720 G>A	het	p.Gly2907Glu	
Unaff daughter CIC00782		43	c.8720 G>A	het	p.Gly2907Glu	
Unaffected son CIC00783		43	c.8720 G>A	het	p.Gly2907Glu	
CIC00153	116	23	c.3526T>C	het	p.Cys1176Arg	Only this family, 0/190
Unaff. father CIC00156		23	c.3526T>C	het	p.Cys1176Arg	
Unaff. mother CIC00155		23	no	-	no	
Unaff. sister CIC00154		23	c.3526T>C	het	p.Cys1176Arg	
CIC00100	76	4	c.748+6A>T	het	unclear	Only this patient, 0/136, conserved
		26	c.5240A>G	het	p.Glu1747Gly	only this patient, 0/158, conserved, unknown domain
CIC01066	649	31	c.6416G>A	het	p.Cy2139Tyr	In 2 patients, 0/182, conserved
CIC01159	695	43	c.8422G>A	het	p.Ala2808Thr	Only this patient, 0/68, Laminin G, conserved
		22	c.3329C>G	het	p.Thr1110Ser	Only this patient,0/178, EFG/EGF-like domain, conserved

CIC01193	715	26	c.5044G>T	het	p.Asp1682Tyr	3 patients, 0/248, unknown domain, conserved,
CIC00677	454	31	c.6416G>A	het	p.Cys2139Tyr	In 2 patients, 0/182, conserved
		int18	c.2847-1G>T	het	Splice defect?	Only this patient, 0/134, conserved
Unaff. mother CIC00678		31	c.6416G>A	het	p.Cys2139Tyr	
		int 18	no	.	no	
CIC00111	85	Int12	c.2023+1G>C	het	Splice defect?	Only this patient, 0/180, conserved
unaff. father 00930		Int12	No	-	no	
unaff. mother CIC00929		Int12	c.2023+1G>C	het	Splice defect	
unaff. sister CIC00906		Int12	c.2023+1G>C	het	Splice defect	
unaff. sister CIC00907		In12	No	-	no	
CIC00167	123	14	c.2234A>G	het	p.Asn745Ser	0/328,EGF/LAMININ, conserved
CIC00113	87	28	c.5886T>C	het	p.Thr1962Thr, splicing?	0/200, conserved

Table 1-3: Mutation analysis has been performed in the exons and intronic sequence of the coding region of EYS. Mutations, sequence variants and their putative pathogenic characters identified in the different exons are depicted. New truncating mutations are highlighted in red and bold letters, new missense, splice site and silent mutations are highlighted in red. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Table 3:Patients with unknown but unlikely pathogenic EYS variants

Index Patient, Family Members	Family	Exon	Nucleotide Exchange	Allele State	Protein Effect	Conclusion, Control Alleles (Mut/WT)
CIC00103	79	23	c.3489T>A	het	p.Asn1163Lys	
CIC00165		23	no		no	Since affected CIC00165 not carrier, SNP
CIC00104		23	c.3489T>A	het	p.Asn1163Lys	
CIC00858	554	40	c.7737T>C	het	p.Thr2579Thr	2 patients, 4/192, SNP
CIC00393	269					
CIC00682	457	40	c.7796A>G	het	p.His2599Arg	4 patients, 1/192, SNP
CIC00266	182					
unaff. father CIC00267						
unaff. mother CIC00265			no	-	no	
CIC00410	282		c.7796A>G	het	p.His2599Arg	
CIC00234	162					
CIC00492	336	6	c.977G>A	het	p.Ser326Asn	2, patients, 1/200, SNP
CIC00264	181					
		4	c.281C>A	het	p.Pro94Gln	Only this patient, but Gln also in mouse, SNP
CIC01240	748	30	c.6119T>A	het	p.Val2040Asp	3 patients, 2/380, SNP
CIC00834	539					
CIC01101	672					

CIC00081 **CIC00502** **CIC00470**	58 341 322	29	c.5977A> G	het	p.Thr1993Ala	3 patients, 0/382, but other species also a G instead of A, SNP
CIC01111	678	29	c.5995A>G	het	p.Ile1999Val	Only this patient, 1/382, SNP
CIC00468	320	11	c.1652G>T	het	p.Arg551Leu	Only this patient, 0/180, but Leu also in Orang Utan
CIC00089	66	16	c.2500G>A	het	p.Val834Ile	Only this patient, 0/190, a also found mouse, Ile in Drosophila, SNP
CIC00089 **CIC00630** **CIC00100** **CIC00413** **CIC0189**	66 422 76 284 141	18	c.2739G>A	het	p.Arg913Arg/splice site	In 5 patients, 0/158, also found tarsier, dog, elephant, opossum, SNP
CIC00328 unaff. mother CIC00329	221	26 26	c.5216C>T c.5216C>T	het het	p.Pro1739Leu p.Pro1739Leu	Only this patient, not conserved, SNP
CIC01225	737	27	c.5743A>T	het	p.Ser1915Gly	Only this patient, 0/180, but not conserved, SNP
CIC00866	557	43	8429	het	p.Thr2810Ile	Only this patient, 0/68, LAMININ G, but not conserved
CIC00030	26	26	c.4985A>T	het	p.Asp1662Val	Only this patient, 1/174, unknown domain, conserved
CIC00881 Unaff. mother CIC00882	567	26 26	c.5510G>C c.5510G>C	het het	p.Trp1837Ser p.Trp1837Ser	Only 1 patient, 20/158 conserved, SNP
CIC00081 **CIC00380** **CIC00714**	58 260 474	33	c.6632C>T	het	p.Ser2211Leu	3 patients, 0/280, Laminin G, but not conserved
CIC00589	393	42	c.8206G>C	het	p.Ala2736Pro	only this patient, 0/90, LaminiG, not conserved
CIC00413	284	5	c.777G>A	het	p.Gln259Gln	Only this patient, 0/178, not conserved

CIC00470	322	33	c.6645G>T	het	p.Gly2215Gly	0/180, not conserved, SNP
CIC00533	54	36	c.7182T>C	het	p.Ile2394Ile	only this patient 0/380, not conserved, SNP
Monozygotic twin CIC00077		36	c.7182T>C	het	p.Ile2394Ile	
CIC00519	350	26	c.5376A>G	het	p.Ala1792Ala	Only this patient, 0/158, not conserved, SNP
CIC00812	524	39	c.7608C>T	het	p.Ile2536Ile, splicing?	0/170, not conserved, SNP
CIC00020	17	43	c.8712T>C	het	p.Asp2904Asp, splicing?	0/380, not conserved, SNP
CIC00081	58	36	c.7083T>C	het	p.Cys2361Cys	Only this patient, 0/380, not conserved, SNP
CIC00630	422	35	c.6855C>T	het	p.Phe2285Phe	Only this patient, 0/158, but not conserved

Table 4: clinical data

Patient	Age at time of testing	Age at time of diagnosis	Sex	Relevant medical and ophthalmology history	Family history	Symptoms	BCVA OD/OS Refraction	Lens	Fundus examination	OCT	FAF
CIC00081	19	14	F	Surgery for convergent strabismus, no residual amblyopia Situs inversus	Adopted	Night blindness since early childhood	20/50 20/50 +2.50(-1.25)165° +2.75(-2.25)5°	Small pigmented opacities on posterior capsule	Pale optic disc; optic disc drusen; discrete narrowing of retinal vessels; little changes in the periphery with few bone spicules	Chronic CME	Patchy loss of AF outside the vascular arcade; perifoveal ring of increased AF; foveal changes due to CMO
CIC00338	30	26	F	None	None Greek descent	Night blindness since early childhood	20/25 20/20 -3.25 (-1.25)0° -1.75(-1.25)180°	Clear	No pale optic disc, narrowed retinal vessels, little changes in the periphery with white dots and few bone spicules, relatively preserved posterior pole	Relatively preserved foveal lamination	Patchy loss of AF outside the vascular arcade; perifoveol ring of AF
CIC00513	28	15	F	Unilateral hypocusis due to a vascular problem; cold agglutinin disease	One brother affected, parents are first cousin, from Senegalese descent (Dakar)	Night blindness since early childhood	20/25 20/32 -3.75(-3.50)0° -3.50(-3)180°	Clear	No pale optic disc, narrowed retinal vessels, little changes in the periphery with white dots and few bone spicules, relatively preserved posterior pole	Relatively preserved foveal lamination	Patchy loss of AF outside the vascular arcade; perifoveol ring of AF
CIC00006	73	35	M	None	none, from French-Italian-German descent	Night blindness since early childhood	20/40 20/32 -2.25 (-2)65° -2.5(-0.50)50° (before cataract surgery)	Bilateral IOL Since age 65	Pale optic disc; narrowed retinal vessels; bone spicules in periphery; some perifoveal RPE atrophy	Some degree of macular thickening; history of CME	loss of AF outside the vascular arcade also associated with perifoveal loss of AF
CIC00264	33	29	M	None	None, Consanguinity in the family including parents who are far cousins; from the Soninke people in Mali	Night blindness since age 13	20/40 20/40 -3.50(-0.50)20° -4(-0.75)165°	Clear	Mild pale optic disc and narrowed blood vessels, perifoveal atrophy	Relative preservation of foveal lamination	Loss of AF outside the vascular arcade associated with perifoveal loss of AF
CIC00656	38	13	M	Sugery for testicular	None, from	Night blindness	20/63	Clear	Waxy disc pallor;	Relatively	Loss of AF outside

				ectopia; strabismus with OS residual ambyopia Depression	French descent	since early childhood Photophobia	HM +1(-1.75)170 plano		narrowed retinal vessels; bone spicules in periphery; some perifoveal RPE atrophy	preserved foveal lamination with some areas of PR/IS/OS thining	the vascular arcade as well as in perifoveolar area
CIC00951	34	16	M	None	Older brother has RP; AMD for a cousin on father side, from French descent	Night blindness since early childhood	20/50 20/50 -4 -4	Clear	Optic disc pallor, narrowed retinal vessels; little changes in the periphery with few bone spicules	Relatively preserved foveal lamination	Patchy areas of loss of autofluorescence outside the vascular arcades with , perifoveal ring of increased AF
CIC01001	63	35	M	None	Sister has RP diagnosed at 45, from French descent	Night blindness since early childhood	20/63 20/100 -3(-0.75)170° -3.50(-0.50)110°	Some degree of bilateral posterior subcapsular cataract	waxy optic disc pallor narrowed retinal vessels RPE changes outside vascular arcades with relatively preserved central retina, peripappilary atrophy	CME	Widespread loss of peripheral AF with some preservation centrally
CIC01223	48	unknown	M	None	None, from Jewish Sephardim origin	Night blindness since early childhood	HM HM	Some degree of bilateral posterior subcapsular cataract	pale optic discs, optic disc drusen Narrowed blood vessels, perifoveal atrophy; RPE changes and bone spicules in periphery	Foveal thining	perifoveal loss of AF heretogeneous loss of AF outside and within the arcade
CIC01265	16	16	M	Autoimmune thyroiditis Septicaemia in childhood	None, from French descent	Night blindness since early childhood Photopsia	20/16 20/20 +0.50(-1.25)5° +0.75(-1.50)5°	Clear	normal optic disc and blood vessels; some RPE changes in periphery	Preserved foveal lamination	Relatively preserved outside vascular arcade, perifoveal ring of hyper AF
CIC00456	37	26	M	Pneumothorax at 34 Moderate deafness after trauma	Adopted	Night blindness since childhood, photopsia, visual field constriction	20/50 20/63 -1.50(-1)110° -1.50(-1)80°	Clear	Waxy optic disc pallor, narrowed retinal vessels, RPE atrophy and bone spicules in the periphery, perifoveal atrophy	Foveal thinning	Loss of AF outside the vascular arcade associated with perifoveal loss of AF
CIC00529	28	25	M	None	None, from French descent	Night blindness since childhood	20/20 20/20 0(-1.25)120° 0(-2.65)65°	Clear	Slight optic disc pallor and moderate narrowing of retinal blood vessels, some RPE changes in periphery	CME	Patchy loss of peripheral AF involving the posterior pole, perifoveal ring of hyper AF

CIC01222	42	Unknown	M	None	One brother affected, parents were first cousins, from Egyptian descent	Night blindness since childhood	20/25 20/20 -1(-1)30° -2.25(-0.75)95°	Clear	Normal optic disc and blood vessels, RPE atrophy and bone spicules predominant in the inferior part of the retina resembling sector RP	Preserved foveal lamination	Loss of autofluorescnec in the inferior part of the retina and within the temporal side outlined by an area of increased autofluorescence
CIC00157	25	10	F	None	One brother with more visual impairment, parents first cousin, from Turkish origin	Night blindness since early childhood, visual field constriction noticed at 10	20/32 20/32 +1(-1)145° +0.25(-0.75)40°	Clear	Normal optic disc and blood vessels; some RPE changes in periphery	Relative preservation of foveal lamination	Loss of AF outside the vascular arcade with perifoveal ring of increase AF
CIC00139	28	23	F	None	None, from North Africa	Visual field constriction	20/50 20/25	Clear	Waxy optic disc pallor, narrowed retinal vessels, few RPE changes in the periphery and within the posterior pole	Relative preservation of foveal lamination	Irregular AF with patchy atrophy within posterior pole and periphery
CIC00492	30	16	F	None	None, from Algeria	Night blindness since teenage years then decreased vision and nystagmus	HM HM	Clear	No optic disc pallor, narrowed retinal vessels, RPE changes in periphery and posterior pole	Foveal thinning	Irregular AF with patchy atrophy within posterior pole and periphery
CIC00630	39	22		None	None, from the French West Indies, Guadeloupe	Night blindness since teenage years	20/32 20/50 +1.50(-1)180° Plano	IOL OD at 38 IOL OS at 40	Waxy optic disc pallor, narrowed retinal vessels, few RPE changes in the periphery, perifoveal atrophy	Foveal thinning	Patchy loss of AF outside the vascular arcade and perifoveal irregular hypoAF
CIC00589	31	30	F	None	None, from French descent	Adaptation problem from light to dark	20/32 20/40 +0.25(-0.50)60° +0.75(-0.75)115°	Clear	Normal optic disc and blood vessels; some RPE changes in periphery, CMO	CME	Patchy loss of AF outside the vascular arcade with macular changes in relation with CMO; perifoveal ring of hyper AF
CIC00714	28	23	F	None	None, from French descent	Night blindness	20/20ODS -1.25 -2	Clear	Mild optic disc pallor, narrowed retinal vessels, RPE changes in the periphery	Preserved foveal lamination	Patchy loss of AF outside the vascular arcade and perifoveal ring of hyper AF of oval

											shape
CIC00729	51	18	F	Hypothyroidia Presbycousis	None, from French descent	No real symptoms at time of diagnosis, then night vision problem and photophobia	20/320 20/80 -1(-1.25)15° -4	IOL OD at 49 Posterior subcapsular cataract OS	Waxy optic disc pallor, narrowed retinal vessels, few RPE changes in the periphery, perifoveal atrophy	Foveal thinning	Irregular AF throughout the posterior pole, foveal hyperAF
CIC00153	34	3	M	Tachycardia Coats syndrome OD treated with cryoapplication	None, from French descent	Night blindness, photophobia+++	20/63 20/25 +3.25(-1.5)15° +3.25(-1.5)160°	Mild bilateral subcapsular cataract	Mild optic disc pallor, narrowed retinal vessels, RPE changes in the periphery and cryo scar in periphery of OD	Preserved foveal lamination	Loss of AF outside the vascular arcade with perifoveal loss of AF
CIC00100	48	48	M	None	None, from Ivory coast	Night blindness since age 38	20/50 20/50	Clear	Mild optic disc pallor, narrowed retinal vessels, RPE changes in the periphery and within the posterior pole	Relatively preserved foveal lamination	Patchy loss of AF outside the vascular arcade and within the posterior pole, small perifoveal ring of hyperAF
CIC01066	68	58	M	None	None, from Flemish Belgian descent	Visual field constriction, no night blindness initially	20/25 ODS +0.75(-3)20° +0.50(-2.25)170°	Moderate bilateral posterior subcapsular cataract	Waxy optic disc pallor, narrowed retinal blood vessels, RPE changes in the periphery, peripapillary atrophy and some degree of perifoveolar atrophy	Preserved foveal lamination	Irregular loss of AF outside and within vascular arcade, some perifoveaolar patchy loss of AF
CIC01159	50	Unknown	M	None	One brother and one half sister affected, From North Africa	Night blindness and visual field constriction	20/50 20/63 0(-2.50)15° 0(-3.75)155°	Moderate bilateral posterior subcapsular cataract	Mild disc pallor, narrowed retinal vessels, RPE changes in the periphery, relatively preserved posterior pole	Relatively preserved foveal lamination, CME OS	Loss of AF outside the vascular arcade, perifoveal ring of hyper AF with some patchy loss of AF within the posterior pole
CIC01193	52	36	M	Hepatitis C treated with in interferon, hyperuricemia	None, from Moroccan descent	Night blindness since childhood, visual field constriction	20/63 20/80 -9.25(-1.50)5° -11	Bilateral posterior subcapsular cataract++	Mild optic disc pallor, narrowed retinal vessels, RPE changes in the periphery and peripapilary atrophy	Relatively preserved foveal lamination	Loss of AF outside the vascular arcade, perifoveal, small perfioveal ring of AF
CIC00677	26	21	M	Asthma	None, from French descent	Night blindness, photopsia	20/20 20/25 -0.5(-2)5° -1.25(-1.75)175°	Clear	Mild optic disc pallor, no narrowing of retinal vessels, few RPE changes in the periphery	Preserved foveal lamination	Loss of AF outside the vascular arcade, perifoveal ring of hyper AF

CIC00111	37	Unknown	M	Mild neurosensory deafness from birth	None, from French descent	Night blindness since childhood	20/50 20/63 -3.75(-0.75)180° -3.50(-0.75)180°	Clear	Mild optic disc pallor, no narrowing of retinal vessels, few RPE changes in the periphery, CMO	CME	Patchy loss of AF outside the vascular arcade with macular changes in relation with CMO; perifoveal ring of hyper AF
CIC00167	66	49	F	Hypothyroidia	2 cousins on mother side affected, from French-Italian descent	No real night blindness, more photophobia	20/50 20/32 +0.50(-1)105° +0.25(-0.50)85°	IOL ODS at 62	Mild optic disc pallor and narrowed retinal vessels, few RPE changes in the periphery, CMO	CME	Patchy loss of AF outside the vascular arcade with macular changes in relation with CMO
CIC00113	36	15	F	None	None, from North Africa	Night blindness since childhood	20/400 20/640 +1.50(-1.75)50° +1.50(-1.50)130°	Clear	Normal optic disc color, narrowed retinal vessels, extensive RPE changes involving both the periphery and the posterior pole	CME	Patchy loss of autofluorescence involving both the periphery and the posterior pole

BCVA: best corrected visual acuity; CME: cystoid macular edema; ND: not detectable; AF: autofluorescence; OD: Oculis dextra (right eye); OS: Oculis Sinistra (left eye); IOL: intra ocular lens; HM: hand motion; LP: light perception; RPE: retinal pigment epithelium; AMD: age-related macular degeneration; RP: retinitis pigmentosa.

Table 5: Function data

Patient	Colour vision	Binocular Goldman visual field, III4 isopter	Full field ERG	Multifocal ERG
CIC00081	Normal at the 15 desaturated HUE	Central 70° horizontally; 60° vertically	ND	Some preserved responses to the central hexagons
CIC00338	Small tritan defect the Farnworth 28HUE	Central 60° horizontally and vertically	ND	Some preserved responses to the central hexagons
CIC00513	Mild dyschromatopsia without axis at the 15 desaturated HUE	Central 40° horizontally and vertically	ND	ND
CIC00006	Tritan defect at the Farnworth 28HUE	Central 15° horizontally and vertically	ND	Only residual responses to central hexagons
CIC00264	Dyschromatopsia without axis at the 15 desaturated HUE	Central 20° horizontally and vertically	ND	ND
CIC00656	Tritan defect at the Farnworth 28HUE	Central 10° horizontally and vertically	ND	ND
CIC00951	Tritan defect at the Farnworth 28HUE	Central 20° horizontally and vertically	ND	Only residual responses to central hexagons
CIC01001	Tritan defect at the Farnworth 28HUE	Central 15° horizontally and vertically	ND	preservation of responses to central hexagons with decreased responses to peripheral hexagons
CIC01223	Not tested due to low vision	Inferior to the fixation point preservation of an island of 25°x15°	ND	ND
CIC01265	Normal at the 15 desaturated HUE	Central 150° horizontally, 120° vertically	ND	preservation of responses to central hexagons with decreased responses to peripheral hexagons
CIC00456	Tritan defect at the Farnworth 28HUE	Central 20° horizontally and vertically associated with persistant crescent on both side	ND	ND
CIC00529	Small tritan defect at the Farnworth 28HUE	Central 180° horizontally, 120° vertically	ND	preservation of responses to central hexagons with decreased responses to peripheral hexagons
CIC01222	Mild dyschromatopsia without axis at the 15 desaturated HUE	Central 70° horizontally, 80° vertically with associated with a pericentral superior arciform scotoma	ND	preservation of responses to central hexagons with decreased responses to peripheral hexagons
CIC00157	Normal at the 15 desaturated HUE	Central 15° horizontally and vertically	ND	preservation of responses to central hexagons with decreased responses to peripheral hexagons
CIC00139	Dyschromatopsia without axis OD and tritan defect OS at the Farnworth 28HUE	Central 15° horizontally and vertically	ND	preservation of responses to central hexagons with decreased responses to peripheral hexagons
CIC00492	NP	NP	ND	NP
CIC00630	OD mild dyschromatopsia without axis, OS tritan defect with the 15 desaturated HUE	Central 15° horizontally and vertically	ND	ND
CIC00589	Tritan defect at the Farnworth 28HUE	Central 30° horizontally and vertically	ND	preservation of responses to central hexagons with decreased responses to peripheral hexagons
CIC00714	Normal at the 15 desaturated HUE	Central 130° horizontally and 110° vertically	ND	preservation of responses to central hexagons with decreased responses to peripheral hexagons
CIC00729	Dyschromatopsia without axis at the Farnworth 28HUE	Central 20° horizontally and vertically	ND	ND
CIC00153	Normal at the Farnworth 28 HUE	Central 80° horizontally and 50° vertically	ND	preservation of responses to central hexagons with loss of responses to peripheral hexagons
CIC00100	Tritan defect OD and mild dyschromatopsia without axis OS at the Farnworth 28HUE	Central 10° horizontally and vertically	ND	ND
CIC1066	Normal at the 15 desaturated HUE	Central 150° horizontally, 80° vertically	ND	Only residual responses to central hexagons
CIC01159	Mild dyschromatopsia without clear axis at the Farnworth 28HUE	Central 20° horizontally and vertically	ND	preservation of responses to central hexagons with decreased responses to peripheral hexagons
CIC01193	Tritan defect at the Farnworth 28HUE	Central 15° horizontally and vertically	UD	Only residual responses to central hexagons
CIC00677	Mild tritan defect at the 15 desaturated HUE	Central 20° horizontally and vertically	Only residual	preservation of responses to central hexagons with

			photopic responses	decreased responses to peripheral hexagons
CIC00111	Tritan defect at the Farnworth 28HUE	Central 15° horizontally and vertically	ND	Only residual responses to central hexagons
CIC00167	Mild tritan defect at the 15 desaturated HUE	Central 15° horizontally and vertically	ND	Only residual responses to central hexagons
CIC00113	Tritan defect at the Farnworth 28HUE	Central 15° horizontally and vertically with bilateral temporal island of perception	ND	ND

NP: not performed; ND: not detectable