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***RPI* and autosomal dominant rod-cone dystrophy: novel mutations, a review of published variants, and genotype-phenotype correlation**

Isabelle Audo^{1,2,3,4,5}, Saddek Mohand-Saïd^{1,2,3,4}, Claire-Marie Dhaenens⁶, Aurore Germain^{1,2,3}, Elise Orhan^{1,2,3}, Aline Antonio^{1,2,3,4}, Christian Hamel^{7,8}, José-Alain Sahel^{1,2,3,4,5,9}, Shomi S. Bhattacharya^{1,2,3,5,10} and Christina Zeitz^{1,2,3}.

¹INSERM, UMRS968, Paris, F-75012, France; ²UPMC Univ Paris 06, UMR_S 968, Institut de la Vision, Paris, F-75012, France; ³CNRS, UMR_7210, Paris, F-75012, France; ⁴Centre Hospitalier National d'Ophthalmologie des Quinze-Vingts, INSERM-DHOS CIC 503, Paris, F-75012, France; ⁵UCL-Institute of Ophthalmology, Bath Street, London, UK ; ⁶Centre Hospitalier Régional Universitaire de Lille, UF Génopathies, Laboratoire de Biochimie et Biologie Moléculaire - Université Lille Nord de France, Lille, France ; ⁷Centre de Référence Maladies Sensorielles Génétiques, Hôpital Guy de Chauliac, Montpellier, France; ⁸U583, INSERM, Institute for Neurosciences de Montpellier, Montpellier, France; ⁹Fondation Ophtalmologique Adolphe de Rothschild, Paris, France; ¹⁰Department of Cellular Therapy and Regenerative Medicine, Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Seville, Spain.

Running title: *RPI* mutations in French adRP patients

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Corresponding author: Isabelle Audo and Christina Zeitz

Institut de la Vision, Department of Genetics

17, Rue Moreau

75012 Paris

France

Email address: isabelle.audo@inserm.fr, christina.zeitz@inserm.fr

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ABSTRACT

Rod-cone dystrophies (RP) are a clinically and genetically heterogeneous group of inherited retinal disorders characterized by photoreceptor degeneration. *RPI* is a major gene underlying autosomal dominant (ad) RP though prevalence numbers vary depending on the origin of the cases from 0%-10% cases. Some mutations in *RPI* also lead to autosomal recessive RP. Herein we review all previously reported and several novel *RPI* mutations in relation to the associated phenotype in patients from a French adRP cohort. Prevalence studies from this cohort show that 5.3% of the cases have *RPI* mutations. This is in accordance with other studies reported from UK and USA. The majority of mutations represent truncating mutations which are located in a hot spot region. Similarly, we identified in total four novel deletions and nonsense mutations, of which two may represent recurrent mutations in a French population. In addition a novel missense mutation of uncertain pathogenicity was identified. With our findings, to date 43 *RPI* mutations are known to cause adRP. Variable penetrance of the disease was observed in our and other cohorts. Most patients with *RPI* mutations show classical signs of RP with relatively preserved central vision and visual field.

Background

Rod-cone dystrophies, also known as retinitis pigmentosa (RP), are a clinically and genetically heterogeneous group of progressive inherited retinal disorders, which often starts with night blindness and leads to visual field constriction and secondary macular involvement, which can eventually result in loss of central vision and complete blindness (Hartong et al. 2006). RP occurs in 1 of 4000 births and affects more than 1 million individuals worldwide. The mode of inheritance can be x-linked (xl), autosomal dominant (ad) or autosomal recessive (ar). In addition, many patients represent isolated cases, due to the absence of family history of RP. To date, mutations in 23 different genes are associated with autosomal dominant RP (adRP) (<http://www.sph.uth.tmc.edu/Retnet/>) and the majority of prevalence studies reveals rhodopsin (*RHO*: MIM#180380) being the most frequently mutated gene in adRP (Sullivan et al. 2006; Audo et al. 2010b). In addition, *PRPF31* (MIM#606419), *PRPH2* (MIM#179605) and *RPI* (MIM#603937) were proposed to represent major genes underlying this form of RP (Sullivan et al. 2006; Audo et al. 2010a). In some rare cases *RPI* mutations were also found in patients with arRP and unilateral RP (Khaliq et al. 2005; Riazuddin et al. 2005; Singh et al. 2009; Chen et al. 2010; Mukhopadhyay et al. 2011). *RPI* is located on chromosome 8q12.1, encompasses 4 exons of which 3 are coding. This work aims to review known mutations and to document novel mutations and genotype-phenotype correlations on a French cohort consisting of 114 cases.

Mutations in the *RPI* gene

So far 39 disease causing mutations in *RPI* have been identified of which most were found in the last exon, leading to a premature stop codon and are predicted to form a truncated protein (Table 1). The p.Arg677X has been described as the most commonly reported mutation (Bowne et al. 1999; Guillonneau et al. 1999; Pierce et al. 1999; Sullivan et al. 1999; Jacobson et al. 2000; Payne et al. 2000; Baum et al. 2001; Berson et al. 2001; Sohocki et al. 2001; Xiaoli et al. 2002; Schwartz et al. 2003; Ziviello et al. 2005; Chiang et al. 2006; Gamundi et al. 2006; Roberts et al. 2006; Sullivan et al. 2006).. Detailed phenotype-genotype correlations of patients with this mutation revealed incomplete penetrance and high variability of the disease expression in adRP suggesting modifiers to be involved (Jacobson et al. 2000). Similarly, incomplete penetrance was also noted for other mutations (Table 1). Mutation analysis in our cohort (85 of 114 adRP index patients screened for *RPI*) added two different deletions and two different nonsense mutations identified in 6 families to the previously described mutations, however none of the patients revealed the recurrent p.Arg677X mutation (Table 1, Supp. Table S1). With our findings, to date 43 *RPI* mutations are known to cause adRP. The prevalence of *RPI* mutations with 5,3% in our cohort is similar to studies from UK and USA, although the prevalence of *RPI* mutations varied from 0%-10% from different adRP cohorts (Bowne et al. 1999; Pierce et al. 1999; Payne et al. 2000; Baum et al. 2001; Berson et al. 2001; Sohocki et al. 2001; Kawamura et al. 2004; Ziviello et al. 2005; Gamundi et al. 2006; Roberts et al. 2006; Sullivan et al. 2006; Zhang et al. 2010)

Different pathogenic mechanisms of *RPI* truncating mutations have been proposed due to *in vitro* and *in vivo* studies leading to ar or adRP. Chen and co-workers proposed four classes of *RPI* mutations depending on their site and underlying pathogenicity (Chen et al. 2010). Class 1 mutations are rare, thought to be nonsense-mediated decay (NMD)-sensitive and locate in exons 2-3 (amino acids 1-236). Haploinsufficiency is not believed to be the disease causing mechanism since heterozygous carriers of those mutations are not affected. Thus it is more likely that the mutation creates a null allele due to NMD. The RPI protein made by the unaffected copy of the allele is completely functional and only if both alleles are affected, the patient will suffer from RP. Examples are p.Ser2ArgfsX16 (Chen et al. 2010) and p.Pro229GlnfsX35 (Pierce et al. 2010) (Table 1). Class II mutations occur frequently and are reported to be NMD-insensitive truncations, which are located approximately between amino acids 500 and 1053 in exon 4. The mutant proteins are expected to impose distinct dominant negative effect on photoreceptors, resulting in cell death and adRP (Chen et al. 2010) (Table 1). Similarly, the deletions and nonsense mutations identified in the French adRP cohort studied herein could also be classified as class II mutations. Two of them (p.Lys657AsnfsX7 and p.Ile725TyrfsX13) may represent re-current mutations in French adRP patients due to the appearance in two independent families (Table 1, Supp. Figure S1, Supp. Table S1). To our knowledge only one recessive truncating mutation lies in this region and leads to arRP (p.Asn949LysfsX32) (Table 1). Class III truncating mutations are thought to affect amino acids 264 to 499 and 1054 to 1751, which lead to loss-of-function in patients with arRP. Examples are p.Pro1648SerX13 (Chen et al. 2010) or p.Asn1751IlefsX4

(Riazuddin et al. 2005) (Table 1). Class IV truncating mutations are NMD-insensitive and thought to be located after amino acid 1816. The resulting truncated proteins are expected to be functional and thus not disease causing (Chen et al. 2010). A few missense mutations reported lead to ar or adRP (Table 1) (Khaliq et al. 2005; Chiang et al. 2006; Zhang et al. 2010). However for most of the missense mutations the pathogenic character remains to be elucidated or was considered to be benign (Supp. Tables S2 and S3). Similarly, we identified a missense mutation (c.4193C>G, p.Ser1398Cys, Supp. Figure S1, F745,) of which co-segregation analysis can not clearly predict if this variant lead to adRP (Supp. Table S4). Furthermore other variants in *RPI* have been identified, which are presumably due to predictions or co-segregation analysis not pathogenic (Supp. Table S5). All mutations identified by our extensive literature review and described herein, will be submitted to the existing Locus Specific Databases (LSDB) on *RPI*, which before the submission of our review cites only 4 pathogenic mutations.

Genotype-Phenotype correlation

Variable penetrance of the disease was observed in our and other cohorts. Most patients with *RPI* mutations show classical signs of RP with relatively preserved central vision and visual field. The detailed description about the phenotypic characteristics of the 7 index patients (3 females and 4 males) carrying different *RPI* mutations of the French adRP cohort can be used as key insights about *RPI* associated genotype-phenotype relationships (Table 2 and 3 with an illustration of retinal changes presented in figure 1). In brief, mean age of patients of the patients herein is 54.4. Except for one patient,

CIC01234 (carries the missense mutation of uncertain pathogenicity) examined at an asymptomatic young age during a family investigation, the majority of subjects were diagnosed in their twenties with an average age at time of diagnosis of about 23 years. This is older than reported in adRP associated with *RHO* and *PRPF31* mutations (Audo et al. 2010a; Audo et al. 2010b) and in accordance with previous reports for patients with *RP1* mutations (Berson et al. 2001). Family history for two families (F556 and F745) suggested incomplete penetrance and three patients reported variable age of onset in the family with diagnosis made as late as 55 years. This is in keeping with variable penetrance and expressivity associated with *RP1* mutations (Jacobson et al. 2000; Berson et al. 2001). None of the families in this study mentioned unilateral involvement so far (Mukhopadhyay et al. 2011). Symptoms at presentation were dominated by night vision disturbances. Visual acuity ranges from 20/25 to 20/800. All patients had some lens opacities or had undergone cataract surgery. In most cases, fundus changes were typical of RP with narrowed retinal vessels, pale discs and pigmentary changes in the periphery. All but one had some degree of atrophy with loss of autofluorescence in the perifoveal region and one patient also had cystoid macular oedema in one eye. Color vision abnormalities were correlated with worse visual acuities. All patients had severe constricted visual fields and generalized retinal dysfunction with four out of seven with some preservation of central macular function. Therefore, although phenotypic variability was suggested in certain families in this study, the index patients had a fairly homogeneous degree of severity within their retinal involvement.

Biological Relevance

RP1 consists of 2156 amino acids with a molecular weight of 240 kD, which is expressed specifically in photoreceptors in the retina (Bowne et al. 1999; Guillonneau et al. 1999; Pierce et al. 1999). The N-terminal 28-228 amino acids of RP1 contains a tandem repeat domain (Kim et al. 2003), which is known to interact with microtubules and was initially detected in the protein doublecortin (DCX). DCX is involved in directing neuronal migration during development of the central nervous system and mutations in this gene in patients lead to x-linked lissencephaly and double cortex syndrome (des Portes et al. 1998; Gleeson et al. 1998; Francis et al. 1999). Immunofluorescence analyses detected the human and mouse RP1 protein in the connecting cilium and microtubule-based axoneme of rod and cone photoreceptors. In addition, the amino acid sequences between codons p.486 and p.635 shares homology with the *Drosophila melanogaster* protein BIF, which is essential for normal photoreceptor morphogenesis (Pierce et al. 1999). Due to the specific localization of RP1 and the existence of the typical DCX and BIF domain, it was suggested that RP1 might participate in transport of proteins between the inner and outer segments of photoreceptors or in maintenance of the ciliary structure (Liu et al. 2002). Different mouse lines lacking Rp1 have been created to better understand the function and pathogenic mechanism of RP1 protein and mutations: One deletes the conserved N-terminal Dcx domain (Gao et al. 2002) and produces an abnormal Rp1 product that is lacking exon 2 and 3, while the other mimics the frequently identified mutation in RP patients: p.Arg677X, which contains the N-terminal Dcx domain and produces the shorter Rp1 protein (Liu et al. 2003). Both homozygous models experience

rapid retinal degeneration and show outer segment dysplasia and misalignment, suggesting a general role of RP1 in outer segment organization. Initially, only heterozygous mice lacking the Dcx domain and expressing about 50% of normal Rp1 protein revealed some retinal dysfunction despite normal-appearing retinal morphology (Gao et al. 2002), while heterozygous mice mimicking the human mutation p.Arg677X did not show any phenotype (Liu et al. 2003). However, recently it was shown that the severity of the retinal degeneration is dependent on the genetic background and only heterozygous mice for the A.129S(B6) background indeed developed RP suggesting that specific modifiers present in one or the other mouse strain are responsible for the severity of the phenotype in some heterozygous mouse lines (Liu et al. 2009). Furthermore the photoreceptor degeneration of a knock-in mouse line carrying the p.Gln662X mutation, analogous to the human p.Arg677X mutation, could be delayed or prevented by adding wild-type Rp1 protein (Pierce et al. 2010). Another mouse model, which was chemically induced (N-ethyl-N-nitrosourea = ENU) carries a p.Arg533X mutation and again only homozygous mice were affected by photoreceptor degeneration (Won et al. 2011). Since the retinal phenotype in knock-in mice carrying mutations in this region could be delayed or prevented by adding the wild-type gene, the underlying pathogenic mechanism was suggested to be dominant-negative and not gain-of-function.

Clinical and Diagnostic Relevance

In the study presented here we review all reported disease causing *RP1* mutations including 4 novel truncating mutations and 1 novel nucleotide change of uncertain

pathogenicity in 7 cases with a homogenous RP phenotype (Table 1, 2 and 3, Supp. Tables S1 and S2). Our study confirms that for adRP European cohorts, *RPI* represents a major gene defect. We propose that most of the *RPI* mutations leading to adRP represent truncation mutations and are located in a hot spot region (~nucleotides c.1490-c.3216). Therefore, to perform a time and cost-efficient mutations screening for adRP patients this region could be preferentially targeted. These truncating mutations can be classified as Class II mutations, which lead to a dominant negative effect of *RPI* resulting in photoreceptor cell degeneration. In addition, incomplete penetrance was observed for some of the mutations in this class (Dietrich et al. 2002; Roberts et al. 2006) (Table 1). The exact mechanism of this incomplete penetrance is not known. Another form of adRP associated with mutation in splicing factors is also associated with incomplete penetrance (Evans et al. 1995; Al-Magthteh et al. 1996; McGee et al. 1997; Vaclavik et al. 2010). In this case, haploinsufficiency is the admitted disease mechanism and an allelic imbalance with overexpression of the wild-type allele, compensating for the non-functional allele in asymptomatic carriers has been advocated (Vithana et al. 2003; Rivolta et al. 2006). In case of incomplete penetrance associated with *RPI* mutation, such mechanisms with the influence of the *trans* allele has been suggested in asymptomatic female carriers by Daiger and colleagues but warrants further study (Daiger et al. 2006). Influence of mouse strains of *Rp1* mutation upon severity in retinal degeneration advocate for modifying factors (Liu et al. 2009). A better understanding of the mechanisms underlying phenotypic variability should facilitate developing novel therapeutic approaches.

Clinically and genetically well-characterized patients such as those included here are critical in this regard.

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Table 1: Previously described and novel *RPI* mutations leading to ar or adRP.

| Disorder | Exon | Nucleotide Exchange | Protein Effect | Literature |
|---------------------|------|--|---|---|
| non syndromic RP | 2 | c.5_6delGT | p.Ser2ArgfsX16 | (Chen et al. 2010) |
| arRP | 3 | c.686delC | p.Pro229GlnX35 | (Pierce et al. 2010) |
| arRP but not adRP ! | 4 | c.1118C>T | p.Thr373Ile | (Payne et al. 2000; Berson et al. 2001; Khaliq et al. 2005) |
| arRP | 4 | c.1458_1461dup | p.Glu488X | (Khaliq et al. 2005; Riazuddin et al. 2005) |
| adRP | 4 | c.1498_1499insGT | p.Met500SerfsX33 | (Payne et al. 2000) |
| adRP | 4 | 1-bp insertion (A) not clear where exactly | p.Pro658HisfsX4 previously: frameshift in codon p.Pro 658 | (Jacobson et al. 2000) |
| RP sporadic case | 4 | c.2005G>A | p.Ala669Thr | (Khaliq et al. 2005) |
| adRP | 4 | c.2025delA | p.Lys675AsnfsX7 incomplete penetrance or later age of onset | This study: CIC00864, F556 and CIC03407, F1452 |

| | | | | |
|-------------------------------------|---|---|--|--|
| adRP sporadic case de novo | 4 | c.2029C>T | p.Arg677X incomplete penetrance | (Bowne et al. 1999; Guillonneau et al. 1999; Pierce et al. 1999; Sullivan et al. 1999; Jacobson et al. 2000; Payne et al. 2000; Baum et al. 2001; Berson et al. 2001; Sohocki et al. 2001; Xiaoli et al. 2002; Schwartz et al. 2003; Ziviello et al. 2005; Chiang et al. 2006; Gamundi et al. 2006; Roberts et al. 2006; Sullivan et al. 2006) |
| adRP | 4 | c.2029delC | p.Arg677AspfsX5 | (Bowne et al. 1999) |
| adRP | 4 | c.2035C>T in <i>cis</i> with c.5377C>T | p.Gln679X in <i>cis</i> with p.Pro1793Ser | (Sullivan et al. 1999; Berson et al. 2001) |
| adRP | 4 | c.2056C>T | p.Gln686X | (Gamundi et al. 2006) |
| adRP | 4 | c.2065C>T | p.Gln689X | This study: CIC01529, F1332 |

| | | | | |
|-------|---|--|---|---|
| adRP | 4 | c.2098G>T | p.Glu700X | (Bowne et al. 1999; Payne et al. 2000) |
| adRP4 | 4 | c.2115delA | p.Gly706ValsfsX7 previously p.Lys705fsX712 | (Gamundi et al. 2006) |
| adRP | 4 | c.2164_2165delinsG | p.Lys722GlufsX16 previously p.Lys722fsX737 | (Gamundi et al. 2006) |
| adRP | 4 | c.2167G>T | p.Gly723X | (Berson et al. 2001; Sohocki et al. 2001; Sullivan et al. 2006) |
| adRP | 4 | c.2168_2181del | p.Ile725ArgfsX6 | (Bowne et al. 1999; Payne et al. 2000) |
| adRP | 4 | c.2169delA | p.Ile725TyrfsX13 | This study: CIC0009, F8 and CIC02805, F1039 |
| adRP | 4 | c.2172dupG Previously c.2169_2170insG | p.Ile725AspfsX4 | (Bowne et al. 1999; Sohocki et al. 2001) |
| adRP | 4 | c.2171_2186del | p.Gly724GlufsX9 | (Payne et al. 2000) |
| adRP | 4 | c.2185delG | p. Glu729LysfsX9 | (Berson et al. 2001) |
| adRP | 4 | c.2206_2207insT | p.Thr736IlefsX4 | (Payne et al. 2000) |
| adRP | 4 | c.2232T>A | p.Cys744X | (Bowne et al. 1999; Payne et al. 2000) |

| | | | | |
|------|---|---|---|---|
| adRP | 4 | c.2239delA | p.Ser747ValfsX16 | (Jacobson et al. 2000) |
| adRP | 4 | c.2275A>T | p.Arg759X | This study: CIC01239, F749 |
| adRP | 4 | c.2284_2289del | p.Leu762_Asn763del previously mentioned frameshift with premature X? | (Payne et al. 2000) |
| adRP | 4 | c.2285_2289del previously c.2280_2284del | Leu762TyrfsX17 | (Bowne et al. 1999; Pierce et al. 1999; Jacobson et al. 2000; Payne et al. 2000; Berson et al. 2001; Sohocki et al. 2001; Sullivan et al. 2006) |
| adRP | 4 | c.2287_2290del | p.Asn763LeufsX11 | (Pierce et al. 1999; Berson et al. 2001) |
| adRP | 4 | c.2304delC previously c.2303delC | p.Lys769ArgfsX6 | (Bowne et al. 1999; Sohocki et al. 2001) |
| adRP | 4 | c.2332A>T | p.Lys778X incomplete penetrence | (Dietrich et al. 2002) |
| adRP | 4 | c.2336_2337delCT | p.Ser779X | (Kawamura et al. 2004) |
| adRP | 4 | c.2590_2599del | p.Ile864LysfsX11 incomplete penetrence | (Roberts et al. 2006) |

| | | | | |
|------------------|---|--|--|-------------------------|
| adRP | 4 | c.2594_2596del | p.Thr865_Leu866delinsIle | (Payne et al. 2000) |
| adRP | 4 | c.2613dupA previously c.2608_2609insA | p.Arg872ThrfsX2 | (Payne et al. 2000) |
| adRP | 4 | c.2732C>A | p.Ser911X incomplete penetrance | (Roberts et al. 2006) |
| arRP | 4 | c.2847delT | p.Asn949LysfsX32 | (Singh et al. 2009) |
| adRP | 4 | c.2951A>G | p.Asp984Gly phenotype variations in one family | (Chiang et al. 2006) |
| adRP | 4 | c.3157delT | p.Tyr1053ThrfsX4 | (Jacobson et al. 2000) |
| Simplex RP | 4 | c.4108A>G | p.Lys1370Glu | (Zhang et al. 2010) |
| arRP | 4 | c.4555delA previously c.4703delA | p.Arg1519GlufsX2 previously p.Arg1519fsX1521 | (Riazuddin et al. 2005) |
| non syndromic RP | 4 | c.4941dupT previously c.4941_4942insT | p.Pro1648SerfsX13 | (Chen et al. 2010) |
| Simplex RP | 4 | c.4955G>T | p.Arg1652Leu | (Zhang et al. 2005) |
| arRP | 4 | c.5252delA previously c.5400delA | p.Asn1751IlefsX4 previously p.Asn1751fsX1754 | (Riazuddin et al. 2005) |

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence NM_006269.1, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Table 2: Clinical data of affected index patients from families with adRP due to *R1* mutations

| Patient | Family | Mutation | Age at time of testing | Age at time of diagnosis | Sex | Relevant medical and ophthalmology history | Family history of incomplete penetrance or variable expressivity | Symptoms at time of diagnosis | BCVA OD/OS Refraction | Lens | Fundus examination | OCT | AF imaging |
|----------|--|---|------------------------|--------------------------|-----|---|---|---|--|--|--|---|--|
| CIC009 | 8 (rest of the family reported elsewhere) | c.2169delA p.Ile725TyrfsX13 | 70 | 15 | M | Trabeculectomy Bilateral cataract surgery age 60 | Incomplete penetrance | Night vision problems | 20/125 20/32 -5.75(-0.75)90° -6(-1)145° | Bilateral IOL | Narrowed blood vessels, pale optic discs, RPE changes in the periphery, perifoveal atrophy OD | Foveal thinning OD; relative preservation of foveal lamination OS | Patchy loss of AF outside the vascular arcades; perifoveal loss of AF OD |
| CIC00864 | 556 | c.2025delA p.Lys675AsnfsX7 | 48 | 25 | F | None | Possible incomplete penetrance with asymptomatic 19-year old daughter and variable expressivity with father diagnosed at age 55 | Initially decrease visual field and mild night vision problem | 20/25 20/40 -5.75(-1)60° -5.75(-1)150° | Bilateral posterior subcapsular cataract | Narrowed blood vessels, relatively well colored optic nerve discs and bone spicule pigment migration in the periphery and relatively spared macula | Preservation of foveal lamination | Low quality due to cataract Normal macular autofluorescence |
| CIC01234 | 745 | Uncertain pathogenic variant: c.4193C>G p.Ser1398 | 46 | 6 | F | Pneumothorax Bilateral cataract surgery age 44 | Not reported | No symptoms at diagnosis (family investigation) | 20/200 20/160 +2.50 +1.50(-0.50)170° | Bilateral IOL | Narrowed blood vessels, pale optic nerve disc, widespread RPE changes in the periphery and macular atrophy | Macular thinning with loss of ONL | Loss of AF outside the arcades and within the foveal region |
| CIC01239 | 749 | | 60 | 25 | M | Bilateral cataract surgery age 45 | Not reported | Night vision problems Mainly bothered | 20/25 20/40 -2.50(- | Bilateral IOL | Narrowed blood vessels, relatively well colored optic | OD Preservation of foveal lamination | Patchy loss of AF along the vascular arcades; OD |

| | | | | | | | | | | | | | |
|-----------------|------|--------------------------------|----|----|---|----------|---|--|--|---|---|--|--|
| | | c.2275A>T p.Arg759X | | | | | | at age 55 by constricted visual field | 0.50)95° -5(-0.50)90° | | nerve discs, little pigment migration in the periphery; OD relatively spared macula. OS CME | OS CME | Normal macular autofluorescence; OS perifoveal loss of AF on the temporal side and abnormal foveal af related to the CMO |
| CIC02805 | 1039 | c.2169delA p.Ile725TyrfsX13 | 58 | 30 | M | None | Variable age of onset | Night vision problem Decreased vision | 20/40 20/32 -2.25(-0.75)90° -2.75(-0.50)90) | Bilateral posterior subcapsular cataract | Narrowed blood vessels, pale optic discs, little RPE changes in the periphery | Relative conservation of foveal lamination | Patchy loss of AF outside the vascular arcades and some perifoveal loss |
| CIC01529 | 1332 | c.2065C>T p.Gln689X | 51 | 30 | M | None | Later age of onset reported in other family members | Night vision problem | 20/32 20/25 -2.50(-1.25)20° -3(-1)140° | Mild bilateral posterior subcapsular cataract | Narrowed blood vessels, pale optic nerve discs, RPE changes in the periphery and in perifoveal region | Relative conservation of foveal lamination | Patchy loss of AF outside the vascular arcades and in the perifoveal area |
| CIC03407 | 1452 | c.2025delA p.Lys675AsnfsX7 | 48 | 27 | F | Deafness | Not reported | Night vision problem | 20/200 20/800 Plano -0.25(-0.50)130° | Significant bilateral posterior subcapsular cataract OD>OS | Narrowed blood vessels, relatively well coloured optic nerve heads, little RPE changes in the periphery, macular atrophic changes | Macular thinning with loss of ONL | Patchy loss of AF in periphery and foveal region |

BCVA: best corrected visual acuity; OD: Oculis dextra (right eye); OS: Oculis Sinistra (left eye); OCT: Optical Coherence Tomography; AF: autofluorescence; IOL: intra ocular lens; CME: cystoid macular edema; RPE: retinal pigment epithelium; ONL: Outer Nuclear Layer

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence NM_006269.1, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

Table 3: Function data

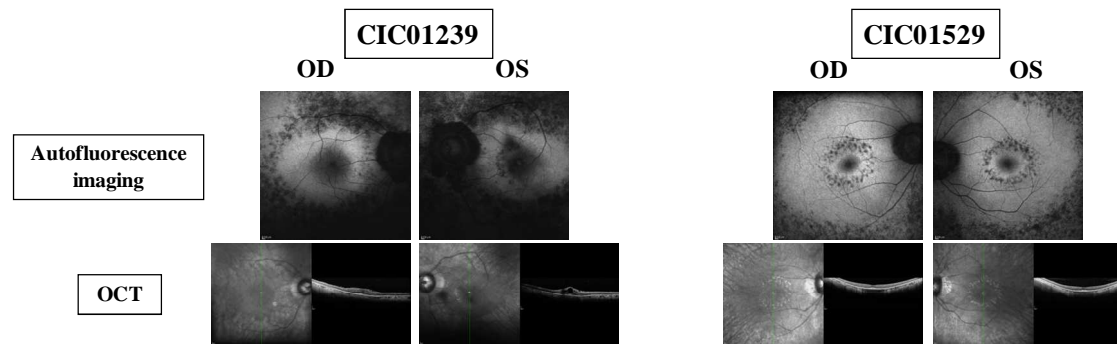
| Patient | Mutation | Colour vision (15 desaturated Hue) | Binocular Goldman visual field, III4 isopter | Full field ERG | Multifocal ERG |
|----------|---|---------------------------------------|---|-------------------|---|
| CIC0009 | c.2169delA p.Ile725TyrfsX13 | Dyschromatopsia with tritan axis | 10° horizontally and vertically | ND | ND |
| CIC00864 | c.2025delA p.Lys675AsnfsX7 | normal | 35° horizontally and 30° vertically | ND | Only residual responses to central hexagons |
| CIC01234 | Uncertain pathogenic variant: c.4193C>G p.Ser1398 | Dyschromatopsia with no specific axis | 40° horizontally and 20° vertically | ND | ND |
| CIC01239 | c.2275A>T p.Arg759X | Few mistakes without axis | 10° horizontally and vertically | ND | Only residual responses to central hexagons |
| CIC02805 | c.2169delA p.Ile725TyrfsX13 | Few mistakes of protan axis | 20° horizontally and vertically | ND | Only residual responses to central hexagons |
| CIC01529 | c.2065C>T p.Gln689X | Normal | 10° horizontally and vertically | ND | Only residual responses to central hexagons |
| CIC03407 | c.2025delA p.Lys675AsnfsX7 | Dyschromatopsia of tritan axis | Inferior to 10 central degree | ND | ND |

ND: not detectable

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence NM_006269.1, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.

FIGURE LEGENDS

Figure 1: Fundus autofluorescence and optical coherence appearance in 2 patients from this French cohort with *RPI* truncating mutations.



Supplementary data

Methods

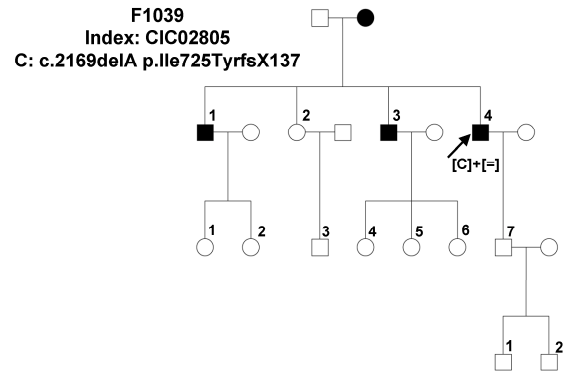
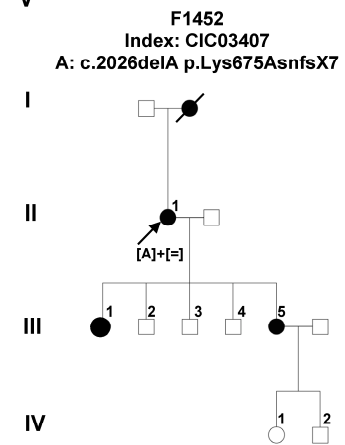
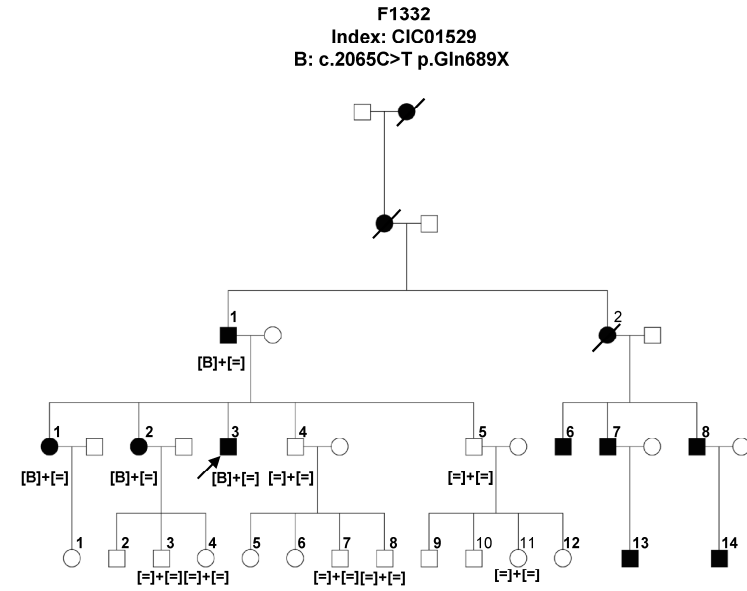
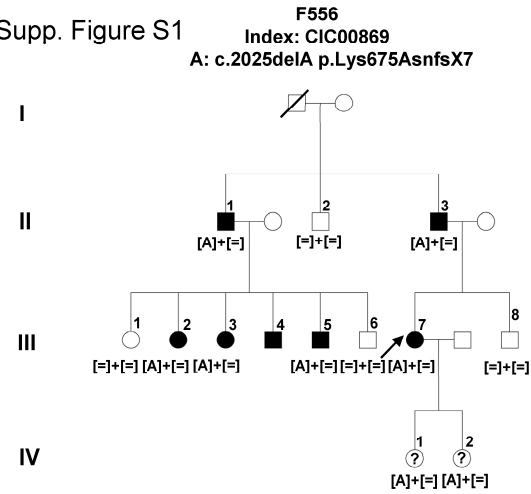
Clinical assessment

Patients with a provisional diagnosis of autosomal dominant rod-cone dystrophy, (adRP) were ascertained in the Clinical Investigating Centre of Quinze-Vingts Hospital. Informed consent was obtained from each patient and normal 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 clinical assessment as described earlier (**Audo et al., 2010, *RHO* mutations**). For additional family members who could not come to our centre for examination, ophthalmic records were obtained from local ophthalmologists.

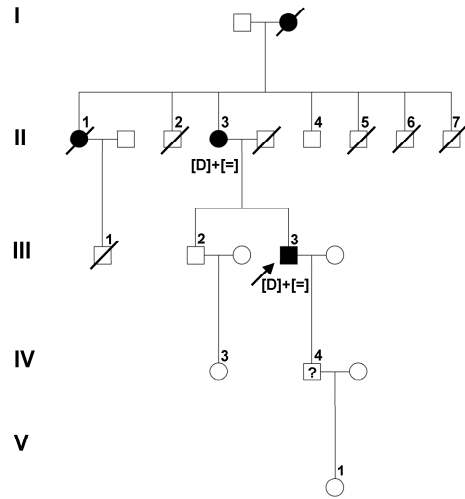
Mutation detection

Total genomic DNA was extracted from peripheral blood leucocytes according to manufacturer recommendation (Puregen Kit, Qiagen, Courtaboeuf, France). Subsequently, direct genomic sequencing of *RPI* (Accession Number: NM_006269.1) was performed. All 4 exons of which exons 2-4 are coding, and flanking intronic regions of *RPI* were PCR amplified in 20 fragments (*RPI* RefSeq NM_006269.1) using a polymerase (HotFire, Solis Biodyne, Estonia) in the presence of 1.5 mM MgCl₂ and at an annealing temperature of 58°C (Primers can be obtained by request). The 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). 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). For the missense mutation 190 commercially available control samples were used to validate the pathogenicity (Human random control panel 1-3, Health Protection Agency Culture Collections, Salisbury, United Kingdom).

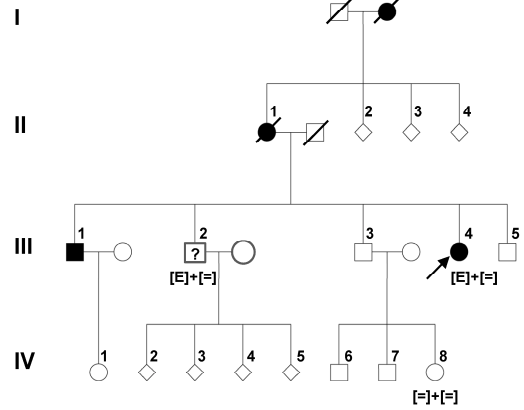
Supp. Figure S1



F749
Index: C1C01239
D: c.2275A>T p.Arg759X



F745
Index: C1C01234
E: c.4193C>G p.Ser1398Lys



Supp. Figure 1: Pedigrees of families with different novel *RPI* 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. Equation symbols represent unaffected alleles.

Supplementary Table 1: Novel *RPI* mutations in a French adRP cohort.

| Index (families) | Exon | Nucleotide Exchange | Protein Effect | controls | SIFT | POLYPHEN |
|---|------|---------------------|-----------------|----------|------|----------|
| CIC00864 (F556) (III.7) | 4 | c.2025delA | p.Lys675AsnfsX7 | nd | na | na |
| CIC00865 (IV.2) asymptomatic daughter | | c.2025delA | p.Lys675AsnfsX7 | | | |
| CIC04903 (IV.8) unaffected brother | | no | no | | | |
| CIC04950 (II.1) affected uncle | | c.2025delA | p.Lys675AsnfsX7 | | | |
| CIC04968 (II.2) unaffected uncle | | no | no | | | |
| CIC04969 (II.3) affected father | | c.2025delA | p.Lys675AsnfsX7 | | | |
| CIC05003, (III.3) affected cousin | | c.2025delA | p.Lys675AsnfsX7 | | | |
| CIC05043, (III.6) unaffected cousin | | no | no | | | |

| | | | | | | |
|---|---|------------|-----------------|----|----|----|
| CIC05051, (III.5) affected cousin | | c.2025delA | p.Lys675AsnfsX7 | | | |
| CIC05053, (III.1) unaffected cousin | | no | no | | | |
| CIC05093 (IV.2) asymptomatic daughter | | c.2025delA | p.Lys675AsnfsX7 | | | |
| CIC05168 (III.2) affected cousin | | c.2025delA | p.Lys675AsnfsX7 | | | |
| CIC03407 (F1452) (II.1) | 4 | c.2025delA | p.Lys675AsnfsX7 | nd | na | na |
| CIC01529 (F1332) (IV.3) | 4 | c.2065C>T | p.Gln689X | nd | na | na |
| CIC05213 (III.1) affected father | | c.2065C>T | p.Gln689X | | | |
| CIC05179 (IV.1) affected sister | | c.2065C>T | p.Gln689X | | | |
| CIC05185 (IV.2) affected sister | | c.2065C>T | p.Gln689X | | | |
| CIC05312 (IV.4) unaffected brother | | no | no | | | |

| | | | | | | |
|---------------------------------------|---|------------|------------------|----|----|----|
| CIC05193 (IV.5) unaffected brother | | no | no | | | |
| CIC05310 (V.3) unaffected nephew | | no | no | | | |
| CIC05186 (V.4) unaffected niece | | no | no | | | |
| CIC05253 (V.7) unaffected nephew | | no | no | | | |
| CIC05311 (V.8) unaffected nephew | | no | no | | | |
| CIC05192 (V.11) unaffected nephew | | no | no | | | |
| CIC00009 (F8)* | 4 | c.2169delA | p.Ile725TyrfsX13 | nd | na | na |
| CIC00010 affected son | | c.2169delA | p.Ile725TyrfsX13 | | | |
| CIC02805 (F1039) (II.4) | 4 | c.2169delA | p.Ile725TyrfsX13 | nd | na | na |
| CIC01239 (F749) (III.3) | 4 | c.2275A>T | p.Arg759X | nd | na | na |
| CIC04884 (II.3) affected mother | | c.2275A>T | p.Arg759X | | | |

*This family was also investigated by another French group (Christian Hamel, U583, INSERM, Institute for Neurosciences of Montpellier, France) and thus further details are given elsewhere.

Supplementary Table 2: Previously described *RPI* variants with unclear pathogenicity

| Disorder | Exon | Nucleotide Exchange | Protein Effect | Why unclear/comment | Literture |
|----------|------|------------------------|----------------------------|---|--|
| adRP | 3 | c.652G>A | p.Ala218Thr | No other family members available for co-segregation | (Berson et al. 2001) |
| adRP | 4 | c.1222A>C | p.Ile408Leu | PolyPhen and SIFT benign but did not appear in 190 controls | (Zhang et al. 2010) |
| adRP | 4 | c.1437G>T | p.Met479Ile | Insufficient data to conclude whether associated with RP | (Baum et al. 2001; Chiang et al. 2006) |
| adRP? | 4 | c.1989G>T | p.Lys663Asn | no other family members for testing, non-conserved residue in human and mouse | (Bowne et al. 1999; Payne et al. 2000) |
| adRP | 4 | c.2376A>C c.2393G>C | p.Lys792Gln p.Arg798Asn | no other family members, Lys conserved, Arg not | (Payne et al. 2000) |
| adRP | 4 | c.2700A>C | p.Lys900Thr | conserved residue, no other family members | (Payne et al. 2000) |
| adRP | 4 | c.2707T>A | p.Pro903Leu | 2 families, 1. 2 affected carried the mutation, while the unaffected did not show the mutation. 2. 1 affected and one at-risk individual incomplete | (Sheng et al. 2008) |

| penetrance | | | | | |
|------------|---|-----------|--------------|--|--|
| RP | 4 | c.4955G>T | p.Arg1652Leu | Conserved residue, no other family member | (Zhang et al. 2010) |
| adRP | 4 | c.5377C>T | p.Pro1793Ser | patient carried also a nonsense mutation in <i>cis</i> , which was considered to be pathogenic | (Payne et al. 2000; Berson et al. 2001) |
| adRP? | 4 | c.5423T>C | p.Leu1808Pro | no other family members for testing | (Bowne et al. 1999) |
| adRP | 4 | c.6338C>A | p.Thr2113Asn | no other family members for testing | (Payne et al. 2000; Sohocki et al. 2001) |

Supplementary Table 3: Previously described rare *RPI* variants which are unlikely to be pathogenic

| Disorder | Exon | Nucleotide Exchange | Protein Effect | Why | Literture |
|----------|----------|---------------------|----------------|---|--|
| adRP | 2 | c.502C>G | p.Arg168Gly | did not co-segregate | (Berson et al. 2001) |
| RP | 3 | c.746G>A | p.Arg249His | did not co-segregate | (Baum et al. 2001; Chiang et al. 2006) |
| RP | intron 3 | c.787+34T>C | - | no evidence to be pathogenic | (Zhang et al. 2010) |
| RP | 4 | c.2116G>C | p.Gly706Arg | appeared in controls | (Baum et al. 2001; Chiang et al. 2006; Zhang et al. 2010) |
| adRP | 4 | c.2255C>T | p.Thr752Met | did not co-segregate, appeared in controls | (Roberts et al. 2006) |
| adRP | 4 | c.2953A>T | p.Asn985Tyr | Segregated in one family but also reported as a | (Sullivan et al. 1999; Sohocki et al. 2001; Sheng et al. 2008) |

| | | | | | |
|----------|---|---|--------------|--|---|
| | | | | SNP rs2293869 and in specific cohorts appeared in controls | |
| adRP | 4 | c.3215A>G | p.Asp1072Gly | did not co-segregate | (Berson et al. 2001) |
| RP | 4 | c.3024G>A | p.Gln1008Gln | appeared in controls | (Baum et al. 2001; Zhang et al. 2010) |
| RP | 4 | Wrong annotation, not clear previously: c.3188G>A | p.Gln1063Arg | appeared in controls | (Baum et al. 2001; Chiang et al. 2006) |
| adRP | 4 | c.4067T>C | p.Leu1356Ser | did not co-segregate | (Berson et al. 2001) |
| adRP | 4 | c.4250T>C | p.Leu1417Pro | did not co-segregate, 1/95 controls | (Berson et al. 2001) |
| adRP | 4 | c.4784G>A | p.Arg1595Gln | no other family members now documented as SNP rs35084330 | (Bowne et al. 1999; Payne et al. 2000; Sohocki et al. 2001) |
| controls | 4 | c.5797C>T | p.Arg1933X | appeared in controls | (Baum et al. 2001; Chiang et al. 2006) |
| adRP | 4 | c.5805T>G | p.Phe1935Leu | did not co-segregate | (Berson et al. 2001) |
| RP | 4 | c.6045A>G | p.Leu2015Leu | no evidence to be pathogenic | (Zhang et al. 2010) |
| adRP | 4 | c.6196G>A | p.Asp2066Asn | did not co-segregate, 1/91 controls | (Berson et al. 2001) |
| controls | 4 | c.6423A>G | p.Ile2141Met | appeared in controls | (Baum et al. 2001; Chiang et al. 2006) |
| RP | 4 | c.6542C>T | - | appeared in controls | (Baum et al. 2001; Zhang et |

Supplementary Table 4: Rare *RPI* variants with uncertain pathogenicity in a French adRP cohort.

| Index (families) | Exon | Nucleotide Exchange | Protein Effect | controls | SIFT | POLYPHEN |
|--|------|---------------------|----------------|----------|-------------------------|-------------------|
| CIC01234 (F745) (III.4) | 4 | c.4193C>G | p.Ser1398Cys | 0/190 | affect protein function | possibly damaging |
| CIC04861 (IV.8) unaffected niece | | no | no | | | |
| CIC04983, (III.2) asymptomatic brother | | c.4193C>G | p.Ser1398Cys | | | |

Supplementary Table 5: Rare *RPI* variants in a French adRP cohort.

| Index (families) | Exon | Nucleotide Exchange | Protein Effect | controls | SIFT | POLYPHEN |
|--|------|---------------------|----------------|----------|-----------|----------|
| CIC00869 (F558) | 1 | c.1-51A>G | - | nd | na | na |
| Other CIC04860 affected daughter | 1 | no | | | | |
| CIC02802 (F933) | 4 | c.6139G>A | p.Val12047Met | nd | tolerated | benign |
| CIC03164 affected son | 4 | no | no | | | |
| CIC03224 unaffected daughter | 4 | no | no | | | |
| CIC03420 unaffected son | 4 | no | no | | | |
| CIC02630 | 4 | no | no | | | |

affected
cousin

| | | | | | | |
|--------------------|---|-----------|--------------|----|-----------|--------|
| CIC01239 (F749) | 4 | c.5962A>G | p.Ile1988Val | nd | tolerated | benign |
|--------------------|---|-----------|--------------|----|-----------|--------|

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence NM_006269.1, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1.