Journal of Clinical & Experimental Ophthalmology

Lafont et al. J Clinic Experiment Ophthalmol 2011, 2:12
DOI: 10.4172/2155-9570.1000194

Research Article Open Access

Patients with Retinitis Pigmentosa due to *RP1* Mutations Show Greater Severity in Recessive than in Dominant Cases

Estèle Lafont[1,2,3#], Gaël Manes[1,2,3#], Audrey Sénéchal[1,2,3], Béatrice Bocquet[1,2,3], Delphine Coustès-Chazalette[4], Corinne Baudoin[4], Mohamed Ksantini[1,2,3], Jérôme Bourien[1,2,3], Aurore Devos[5], Hélène Dollfus[6], Xavier Zanlonghi[7], Isabelle Meunier[1,2,3,4], Claire-Marie Dhaenens[5], Christian P. Hamel[1,2,3,4*] and the French research group for autosomal dominant retinitis pigmentosa (Isabelle Audo[8], Francine Behar-Cohen[9], Sabine Defoort-Dhellemmes[10], Jean-Louis Dufier[11], Hubert Journel[12], Josseline Kaplan[11], Guylène Le Meur[13], Saddek Mohand-Saïd[8], Sylvie Odent[12], Valérie Pelletier[6], Bernard Puech[10], Jean-Michel Rozet[11], José Alain Sahel[8], Michel Weber[13] and Christina Zeitz[8])

[1]*INSERM U1051, Institute for Neurosciences of Montpellier, Montpellier, France*
[2]*Université Montpellier 1, Montpellier, France*
[3]*Université Montpellier 2, Montpellier, France*
[4]*Genetics of Sensory Diseases, CHRU, Montpellier, France*
[5]*Biochemistry and Molecular Biology Department (Genopathy unit), CHRU, Lille, France*
[6]*Rare Genetic Ocular Diseases, CHRU, Strasbourg, France*
[7]*ElectroDiagnostic Imaging Unit, Clinique Sourdille, Nantes, France*
[8]*Inherited Retinal Dystrophies, Hôpital des 15/20, Paris, France*
[9]*Department of Ophthalmology, Hôtel-Dieu, Paris, France*
[10]*Department of Visual Investigations, CHRU, Lille, France*
[11]*Ophthalmic Genetics, INSERM U781, Paris, France*
[12]*Department of Medical Genetics, CHRU, Rennes, France*
[13]*Department of Ophthalmology, CHRU, Nantes, France*
[#]*Contributed equally to this work*

Abstract

Study background: *RP1* is a major gene for autosomal dominant retinitis pigmentosa and was reported in a few recessive families. Taken together, patients with *RP1* mutations of both types of inheritance show a large spectrum in the severity of the disease. To get better insight in these clinical variations, patients with dominant and recessive retinitis pigmentosa due to *RP1* mutations were investigated and their clinical features were compared.

Methods: *RP1* exons 2 and 3 were sequenced in 324 unrelated patients with presumed recessive retinitis pigmentosa (213 simplex, 68 multiplex) or cone rod dystrophy (27 simplex, 16 multiplex) and *RP1* exon 4 hot spot (nt 1500-3216) was sequenced in 174 probands with dominant retinitis pigmentosa. Visual acuity and visual field were correlated with age using Pearson's linear coefficient and compared with a non parametric Wilcoxon test.

Results: Two novel recessive null mutations (p.His31GlnfsX47, p.Val157TrpfsX16) were found in exon 2. Five novel dominant mutations (p.Lys673ArgfsX9, p.Tyr685X, p.Ile725TyrfsX13, p.Asn748IlefsX15, p.Ser862X) and the recurrent p.Gln679X and p.Ser911X mutations were found in exon 4. In recessive cases, decrease in visual acuity was at 21.8±5.8 years with visual acuity of 0.32±0.28. In dominant cases, decrease in visual acuity occurred later at 45.2±10.4 years in one group (0.54±0.28) and at 61.0±5.2 years in a second group (0.71±0.14). Visual field decrease was noticed earlier in recessive than in dominant cases (20.9±7.2 *vs* 49.0±16.3) but decrease level was similar (41.8±33.3% *vs* 34.5±31.7%). The rate of decrease was similar for visual acuity while for visual field it was higher in recessive than in dominant cases (3.93% per year *vs* 1.65% per year).

Conclusions: The recessive patients had much more severe disease than dominant patients, with higher decrease rate in visual field and earlier onset in visual acuity decrease.

Keywords: Retinitis pigmentosa; Autosomal dominant inheritance; Autosomal recessive inheritance; RP1; Visual field; Visual acuity

Introduction

Retinitis Pigmentosa (RP) is a group of inherited degenerative disorders of the retina characterized by night blindness, progressive loss of peripheral vision and pigment deposits predominant in the peripheral retina. To date, 54 disease causing genes have been identified in nonsyndromic RP and 7 loci have been mapped, including 22 in autosomal dominant (ad) RP, 36 in autosomal recessive (ar) RP, 2 in X-linked RP, 1 in digenic RP and 1 in mitochondrially inherited RP (http://www.sph.uth.tmc.edu/retnet/sum-dis.htm). In recent years, several of these genes have been found both in ad and arRPs, indicating that genetics of RP is more complex than previously anticipated. The transcription factor *NRL* first described in adRP was later found also involved in arRPs [1-3]. Similarly, the transcription factor *NR2E3* initially described in the recessive enhanced S-cone syndrome and in arRP was also reported in dominant forms of RP [4-8]. *PROM1*, which could play a role in nascent photoreceptor outer segment discs, was first reported in severe arRP and later in dominant macular dystrophy or cone rod dystrophy [9,10]. *SEMA4A* was also described in dominant and recessive RP and cone rod dystrophies [11]. More recently, *RDH12* and *RPE65*, whose mutations lead to Leber congenital amaurosis and

***Corresponding author:** Christian P. Hamel, INSERM U1051, Institut des Neurosciences de Montpellier, Hôpital Saint-Eloi, BP 7410380, rue Augustin Fliche 34091 Montpellier Cedex 5, France, Tél: (33) 499 636 010 ; Fax: (33) 499 636 020; E-mail: christian.hamel@inserm.fr

Received October 29, 2011; **Accepted** December 01, 2011; **Published** December 02, 2011

Citation: Lafont E, Manes G, Sénéchal A, Bocquet B, Coustès-Chazalette D, et al. (2011) Patients with Retinitis Pigmentosa due to *RP1* Mutations Show Greater Severity in Recessive than in Dominant Cases. J Clinic Experiment Ophthalmol 2:194. doi:10.4172/2155-9570.1000194

rarely to arRP cases, were each found to be the causal gene in one adRP family [12,13]. Finally, mutations of the *RP1* gene, responsible for 3-6 % of the adRPs [14-21] were repeatedly observed in several arRP cases [20] [22-25].

RP1 is a photoreceptor-specific protein expressed in both rod and cone photoreceptors [26]. This 2156 amino acid-long protein belongs to the axoneme of the photoreceptor outer segment, and hence plays an important role in the organization of the outer segments [27,28]. It interacts, through two doublecortin domains located in tandem in its N-terminal part (amino acids 33-228), with the axonemal microtubules [28]. Downstream doublecortin domains, another domain homologous to drosophila bifocal (amino acids 486-635) is known to be important for the development of rhabdomeres in drosophila eyes [18]. Recently, RP1 was also shown to interact with the ciliary male germ cell-associated kinase MAK, a regulator of the ciliary length [29] and to share, in the photoreceptor connecting cilium, overlapping expression with the photoreceptor-specific RP1L1, the paralog of *RP1* [30].

Patients with *RP1* mutations of both types of inheritance show a large spectrum in the severity of the disease, spanning intrafamilial variations of penetrance to severe, early onset RP cases [14-25]. To get better insight in these clinical variations, *RP1* was screened in both recessive and dominant families. We found arRP families with novel, presumably null *RP1* mutations and compared them to adRP families with novel, presumably dominant negative mutations. We show that the disease course is much more severe in autosomal recessive than in autosomal dominant cases and that decrease in visual acuity shows a progression that is different from that in visual field.

Materials and Methods

Patients

Autosomal dominant RP families were recruited at outpatient clinics from Western (Clinique Sourdille, Nantes), Eastern (CARGO, Strasbourg), and Southern (MAOLYA, Montpellier) parts of France. Autosomal recessive RP families were all recruited in the Montpellier centre. The study (# 2008-A01238-47) had received the authorization from the Sud méditerranée IV ethical board committee (# 08 10 05 from 04/11/2008), was approved by the French regulation agency for medication (AFSSAPS # B81319-70) and is registered at http://clinicaltrials.gov (# NCT01235624). The investigators followed the tenets of the Declaration of Helsinki.

Clinical investigations

Patients had standard ophthalmologic examination (refractometry, visual acuity, slit-lamp examination, applanation tonometry, funduscopy). Kinetic visual fields were determined with a Goldman perimeter with targets V4e, III4e and I4e. OCT measurement of the macula was performed using an OCT-3 system (STRATUS model 3000, Carl Zeiss Meditec, CA) with the software version 3.0. Autofluorescence measurements were obtained with the HRA2 Heidelberg retinal confocal angiograph (HEIDELBERG Engineering, Dossenheim, Germany) and fundus pictures were taken. Full-fields ERG was recorded using a ganzfeld apparatus (METROVISION, Pérenchies, France) with a bipolar contact lens electrode on maximally dilated pupils according to the ISCEV protocol [31].

For numerical values, visual acuity was measured with snellen charts in decimal numbers. Goldman visual field was quantified by counting the number of subdivisions of the Goldman grid within the areas of the V4e isopter and expressed as a percentage of the normal visual field. Correlations between visual ability (acuity or field) and age were investigated with the Pearson's linear coefficient. In case of significant correlation, the slope of the linear fit was calculated. Two given samples were compared with a non parametric Wilcoxon test. Means were expressed ± s.d.

Molecular investigations

DNA extraction and genotyping: Informed consent and blood samples were obtained from the patients. Genomic DNA was extracted from 10-ml peripheral blood samples by a standard salting out procedure [32] and stored at -20°C before use. Members of one family were genotyped for 262,270 SNPs (GeneChip Mapping 250K Nsp Array, AFFYMETRIX, Santa Clara, CA) at the Centre National de Génotypage (www.cng.fr, Evry, France) and homozygous regions were searched using the TASE software [33].

Screening and sequencing: *RP1* exons 2 and 3 (Genbank # NT-008183) were amplified in a single 1534-nt fragment using primers (available upon request) flancking the 5' splice site junction of exon 2 and 3' splice site junction of exon 3. Each PCR was performed in a 10-µl reaction mix containing 50 ng genomic DNA, 10 % 360 GC Enhancer (APPLIED BIOSYSTEMS, Foster City, CA) 0.16 µM of each primer and 1U of AmpliTaq Gold DNA Polymerase (APPLIED BIOSYSTEMS, Foster City, CA) in its appropriate buffer. Following the first denaturation at 95°C for 10 min, amplification was carried out in 35 cycles at 95°C for 30 s, 58°C for 30 s and 72 °C for 1 min, ending with a final extension step for 7 min. PCR products were purified with ExoSap-it cleen up (AMERSHAM BIOSCIENCES, Piscataway, NJ).

Part of *RP1* exon 4 was amplified in 4 overlapping fragments (primer sets available upon request) covering 1863 nt, encompassing codons 500 to 1072. Each PCR was performed in a 25-µl reaction mix containing 50 ng genomic DNA, 20 µM of each primers, 1.5 mM (fragments 1 and 3) or 2.5 mM (fragment 2 and 4) $MgCl_2$, 20 µM dNTPs and 0.25 U of Taq Polymerase (INVITROGEN, Carlsbad, CA) in its appropriate buffer. Following the first denaturation at 94°C for 3 min, amplification was carried out in 35 cycles at 94°C for 30 s, 58°C for 40 s and 72 °C for 45 s, ending with a final extension step for 7 min. Excess primers and dNTPs were removed using 0.5 unit of Shrimp Alkaline Phosphatase (AMERSHAM PHARMACIA) and 2 units of Exonuclease I (AMERSHAM PHARMACIA).

Sequencing of all amplified fragments was performed using the BigDye Terminator Cycle Sequencing Ready Reaction kit V3.1 (APPLIED BIOSYSTEMS) on an ABI PRISM 3130 capillary sequencer (APPLIED BIOSYSTEMS). Sequencing results were analyzed using SeqScape Software Version 2.5 (APPLIED BIOSYSTEMS).

Results

Null *RP1* mutations in autosomal recessive retinitis pigmentosa

Parents and 5/6 children of the Moroccan consanguineous family RP94 (Figure 1) were genotyped for 262,270 SNPs. Two homozygous chromosomal segments shared only by the 3 affected siblings were identified; one in chromosome 3 (region 3p12) between SNPs rs1007414 and rs7631290 defining a 1.3-Mb region and one in chromosome 8 (region 8q11-q13) between SNPs rs11984645 and rs2726551 defining a 4.6-Mb region. This latter region contained *RP1* which was consequently sequenced. Four amino acid changes were found including c.2833G>T

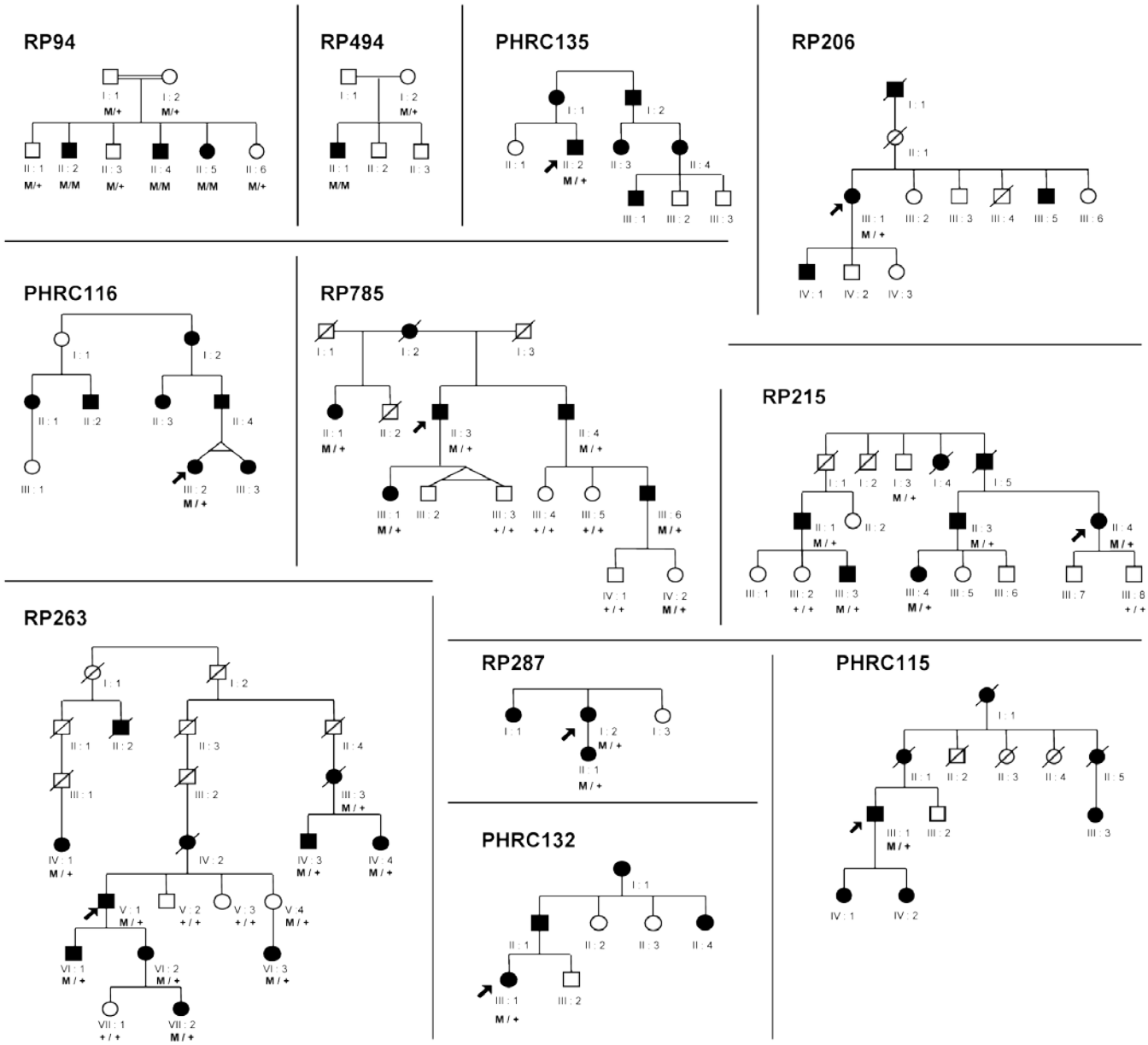

Figure 1: Pedigrees of 11 families with retinitis pigmentosa and *RP1* mutations. Squares indicate male, circles indicate female. Blackened symbols are individuals affected with RP. A double horizontal line between the mating pair indicates consanguinity. *M* means mutated allele (refers to Table 1 for mutation description), + means wild type allele. Patient numbers indicate generation in roman typeset and order in generation in arabic typeset. RP94 and RP494 are consanguineous families with autosomal recessive retinitis pigmentosa; the 9 other retinitis pigmentosa families show autosomal dominant inheritance.

(p.Val945Leu), c.2953A>T (p.Asn985Tyr), c.6098G>A (p.Cys2033Tyr) which are referenced as rs16920621, rs2293869 and rs61739567 polymorphisms, respectively and c.1769C>G (p.Thr590Ser) which was unlikely to be pathogenic as it was conservatory. In exon 2, a 13-nt deletion (TCCTGTTGTGGCC) with a 3-nt insertion (AAA) at position 92 was also found at the homozygous state in the 3 affected individuals, II:2, II:4 and II:5 (Figure 1). This c.93_105del13insAAA leads to a premature stop codon at position 77, resulting in a severely truncated protein of 76 instead of 2156 amino acids (p.His31GlnfsX47). This mutation was present at the heterozygous state in the obligate carrier parents and in the 3 unaffected siblings. None of 54 normal chromosomes carried this change.

Since *RP1* could potentially carry null mutations in RP, we then screened 324 unrelated patients from presumed autosomal recessive RP (213 simplex, 68 multiplex) or CRD (27 simplex, 16 multiplex) families in exons 2 and 3. In exon 2, we found a c.469delG variant at the homozygous state in the simplex case from the Moroccan family RP494 (Figure 1). This deletion causes a premature stop codon at position 172, resulting in a severely truncated protein of 171 instead of 2156 amino acids (p.Val157TrpfsX16). This mutation was present at the heterozygous state in the unaffected mother and was absent from 54 normal chromosomes. We also found among the 324 probands several non pathogenic rare variants at the heterozygous state, including c.228C>T (p.Leu76Leu) in 1 patient, c.466C>T (p.Leu156Leu) in 2

patients, c.615+16G>T in 1 patient, c.616-6T>C in 8 patients and c.764A>G (p.Asn255Ser) in 1 patient.

Truncating *RP1* mutations in autosomal dominant retinitis pigmentosa

As virtually all dominant *RP1* mutations described so far are clustered in the first half of exon 4, we screened the probands from 174 adRP families in this region of the gene. Seven mutations (4 nonsenses, 3 frameshifts) were found in 9 families, among which 5 were novel (Table 1). All of them were truncating mutations, and therefore likely to be pathogenic. When additional family members were available, they were found to segregate with the RP phenotype (Figure 1), although a few asymptomatic carriers were encountered. Thus, the prevalence of families with *RP1* mutations in this adRP cohort was 5.2 %. Three additional non pathogenic variants were also found. Two were frequent, c.2615G>A leading to p.Arg872His (rs444772) in 29.9 % of the chromosomes and c.2953A>T leading to p.Asn985Tyr (rs2293869) in 42.3 % of the chromosomes. A third one, c.3101A>T (p.His1034Leu), was found in only one case and was probably non pathogenic as it was poorly conserved among species (human, chimpanzee, dog, cow, mouse, rat).

Clinical finding in autosomal recessive retinitis pigmentosa

Apparent onset of symptoms occurred variably, from early infancy to 17 (Table 2). All 4 patients were myopic, with a mean spherical equivalent of -6.26 dioptries ± 5.41 (range -1.00 to -13.75). At 20 years of age they had visual acuity < 0.5 in average and had < 50 % of remaining

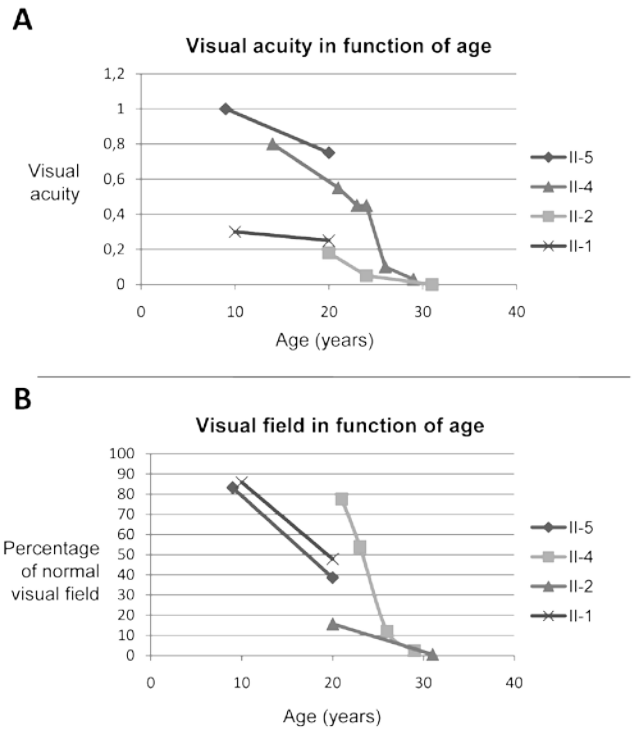

Figure 2: Evolution of visual acuity (A) and visual field (B) in function of age for each patient with autosomal recessive retinitis pigmentosa. II:1 refers to the simplex patient in family RP494; II:2, II:4 and II:5 refer to the patients of the multiplex family RP94 (see Figure 1). **A**: visual acuity measured in decimal values with Snellen chart was plotted with age; **B**: percentage of the remaining visual field compared to normal was determined with the Goldman perimeter V4e stimulus and plotted with age.

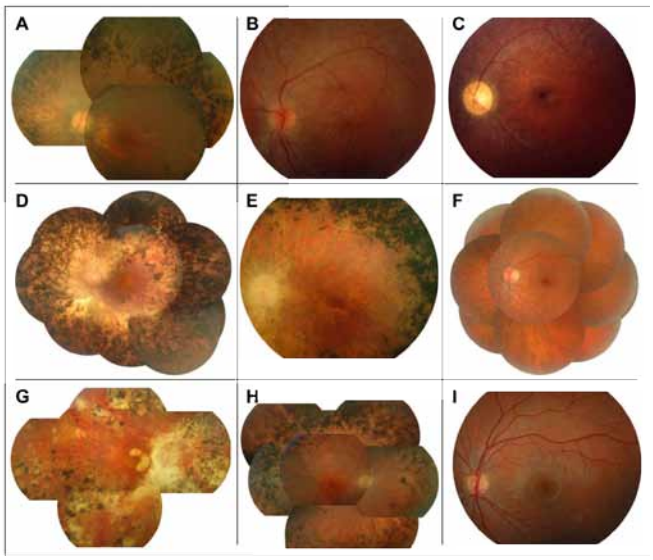

Figure 3: Fundus images of *RP1* patients with recessive (A-C) or dominant (D-I) retinitis pigmentosa (see Figure 1 for pedigrees). **A**: family RP94, left eye of 31 year-old patient II:2, VA (visual acuity) = light perception; **B**: family RP94, left eye of 29 year-old patient II:4, VA = 0.05; **C**: family RP494, left eye of 20 year-old patient II:1, VA = 0.30; **D**: family PHRC115, left eye of 63 year-old patient III:1, VA = no light perception; **E**: family RP785, left eye of 58 year-old patient II:3, VA = 0.50; **F**: family PHRC116, left eye of 36 year-old patient III:2, VA = 0.80; **G**: family RP263, right eye of 58 year-old patient V:1, VA = 0.62; **H**: family RP785, right eye of 44 year-old patient III:6, VA = 0.62; **I**: family RP785, left eye of 15 year-old patient IV:2, VA = 1.00.

visual field in average (Table 2). Both the scotopic and photopic ERGs were unrecordable for 3 of them and showed only traces for the fourth patient. Follow up was available from age 10 to 20 for II:1, 20 to 31 for II:2, 14 to 29 for II:4 and 9 to 20 for II:5. They had moderate decrease in visual acuity during the second decade (from 1 to 0.18) but underwent dramatic decrease in the third decade (from 0.75 to light perception) (Figure 2A). Similarly, the peripheral visual field decreased to a tubular vision in the third decade (Figure 2B). At 20 years of age, the fundus of all 4 patients showed typical bone spicule-shaped pigment deposits covering more or less densely the entire periphery (Figure 3). There was a variable degree of narrowing of retinal vessels. Moderate macular involvement was present at ages 20 and 29 in II:1 and II:4, respectively, while the atrophy spread out to the entire macula in patient II:5. A bilateral epiretinal membrane was present in patients II:4 and II:5. The photoreceptor IS/OS layer was observable only in the fovea in the second decade and disappeared with a severe thinning of the macula in the third decade.

Since *RP1* mutations are usually encountered heterozygously in dominant RP, we paid a particular attention to the available parents and siblings who were heterozygote for the causal mutation. All six available heterozygote family members (parents and the 3 unaffected children from RP94 and mother from RP494) had normal visual acuity, funduscopy, fundus autofluorescence and macular thickness. ERG responses were within normal limits.

Clinical finding in autosomal dominant retinitis pigmentosa

Among 23 patients carrying a dominant *RP1* mutation for whom clinical data were obtained, 4 were asymptomatic. The apparent age of onset, based on night blindness, peripheral visual field restriction or

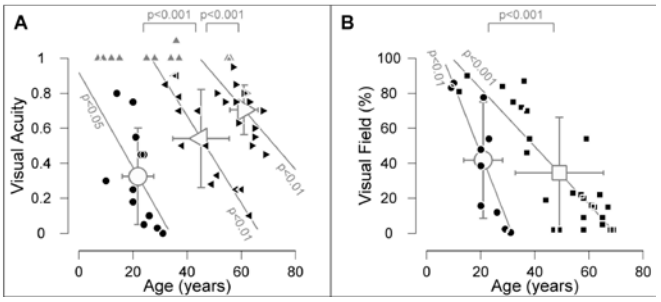

Figure 4: Visual acuity and visual field in function of age. Visual acuity Snellen chart decimal values (**A**) and percentage of normal visual field (**B**) were plotted in function of age for autosomal recessive (circles) and autosomal dominant (triangles or squares) retinitis pigmentosa cases. Linear fit lines are shown. For autosomal recessive cases, the mean visual acuity and mean percentage of the remaining visual field, and mean age for each of both values are indicated as large circles on the fit line. Standard deviations are shown. For autosomal dominant cases, the same data are indicated as a large square for visual field (B), or as a left-oriented (moderate disease) or a right-oriented (mild disease) triangle for visual acuity (A). Ligth grey upper-oriented triangles in A correspond to normal visual acuity values (>1) which have not been considered in the calculations.

photophobia occurred in average at 35 and age at first examination was at 43 in average (Table 2), later than in arRP cases. At this age, almost half the visual field remained and ERG responses were still recordable (Table 2). Some patients had no pigment deposits in fundus (Figure 3). Some others had densely distributed deposits, but they generally retained enough visual acuity for reading.

RP1 autosomal recessive cases are more severe than autosomal dominant cases

Values of visual acuity and visual field (defined as a percentage of normal visual field) were plotted in function of age for arRP and adRP cases. For both forms of inheritance, visual acuity decreased significantly with age (Pearson's linear coefficient test, $p<0.05$) (Figure 4A). Patients with decreasing visual acuity (defined as less than 1) could be separated in 3 different groups depending on the age of visual acuity decrease (non parametric Wilcoxon test, $p<0.001$). The arRP patients (# 1) showed the earliest decrease occurring at mean age 20.9 ± 7.2, while the first group of adRP patients (# 2) underwent decrease at mean age 45.2 ± 10.4, followed by the second group of adRP patients (# 3) showing decrease at mean age 61.0 ± 5.2. Moreover, the mean visual acuity in group # 1 was low (0.32 ± 0.28), while it was better preserved in group # 2 (0.54 ± 0.28) and even better in group # 3 (0.71 ± 0.14). By contrast with the differences observed in mean visual acuity and age of decrease, the rate (linear fit slope) at which visual acuity decreased was similar in the 3 groups, i.e. 0.027 per year for group # 1, 0.025 per year for group # 2 and 0.018 per year for group # 3. The visual field also decreased significantly with age in both arRP and adRP cases (Pearson's linear coefficient test, $p<0.01$) (Figure 4B). However, the distribution of percentage of remaining visual field with age and the rate of visual field decrease were different from what was found for visual acuity. Indeed, for visual field, all adRP cases fitted in only one group and, in average, underwent a decrease later than arRP cases (non parametric Wilcoxon test, $p<0.001$), the mean age of decreasing visual field being 20.9 ± 7.2 for recessive cases while it was 49.0 ± 16.3 for dominant cases. However, the mean percentage of decreasing visual field at mean age was not different between recessive and dominant cases, being 41.8 ± 33.3 *vs* 34.5 ± 31.7, respectively. Yet, the rate (linear fit slope) of visual field decrease was much higher for arRP cases (3.93 % per year) than for adRP cases (1.65 % per year), indicating that arRP cases had a faster decrease in visual field than adRP cases, even though the onset in visual field decrease was at the same age.

Discussion

Genotyping and systematic sequencing of autosomal recessive RP families revealed new *RP1* mutations. Novel mutations were also found by screening exon 4 *RP1* in autosomal dominant RP families. Comparison of clinical data from autosomal recessive and autosomal dominant RP cases showed that the presence of two mutated *RP1* alleles leads to a more severe disease than if only one *RP1* allele is mutated.

Today, 37 *RP1* mutations (including the 6 reported herein) have been described in ad RP [14-16], [18], [34-42], accounting for 3.3 to 6 % of adRPs [17-21]. As such, *RP1* belongs to the second most frequent group of genes causing adRP (with *PRPF31*), following *RHO* which has the highest mutation rate [43,44]. Among the 37 dominant mutations, only 6 are missense. Except for D984G found in one patient and her presumably affected son [40], validation of these missense variants as pathogenic mutations is still awaited since there were no family history and familial segregation available [23,34,45]. The remaining 31 dominant mutations, i.e. the majority, are frameshift or nonsense and are all localized in the first part of exon 4, most of them clustering just downstream the sequence homologous to bifocal, from codons 635 to 900 (Figure 5). Since they are localized in the last exon, mRNA decay is not activated, and therefore mutants should encode truncated proteins containing the doublecortin and bifocal domains (Figure 5). They are thus hypothesized to be dominant negative mutations. Only 9 recessive mutations (including those reported herein) have been identified so far [22-25]. Like for dominant mutations, they are mostly frameshift or nonsense, only one amino acid change being reported whose pathogenicity remains uncertain since it was found in the control population [23]. By contrast with dominant mutations however, they are distributed throughout the coding *RP1* sequence and none of them is found in the dominant cluster (Figure 5). Five are located in exon 4 and possibly lead, as dominant mutations, to expression of C-terminal truncated proteins. The reason why these 5 mutations are recessive remains unclear. One possibility would be that they have a milder dominant effect than the currently reported dominant mutations. This hypothesis could be supported by the finding of a mild atrophy in the peripheral retina in some heterozygote parents [22]. The 3 remaining recessive mutations (among which two are described in this study) are located in exon 2, leading probably to the absence of the mutated protein by mRNA decay, and as such can be considered as loss-of-function (null) mutation. None of the heterozygote members of these three arRP families had signs of retinal degeneration, indicating that a 50 % content of normal RP1 protein is indeed not deleterious to photoreceptors as was reported from studies on heterozygous $Rp1^{+/-}$ mice [27].

In adRP caused by *RP1* mutations, wide variations in severity of the disease were previously reported [34]. In fact, the presence of asymptomatic mutation carriers in a family is a clinical feature that should lead to *RP1* screening. In the study presented here, we also found asymptomatic carriers and significant variations in disease severity. Yet, in general, adRP patients from our series had a relatively moderate form of RP, with average age at first presentation of 43, visual acuity decrease between 40 and 70 years of age, and visual field loss slowly progressing from 20 to 70 years of age. These results are comparable to those reported in a large scale study of adRP patients with *RP1* mutations [35] in which most patients retained a visual acuity

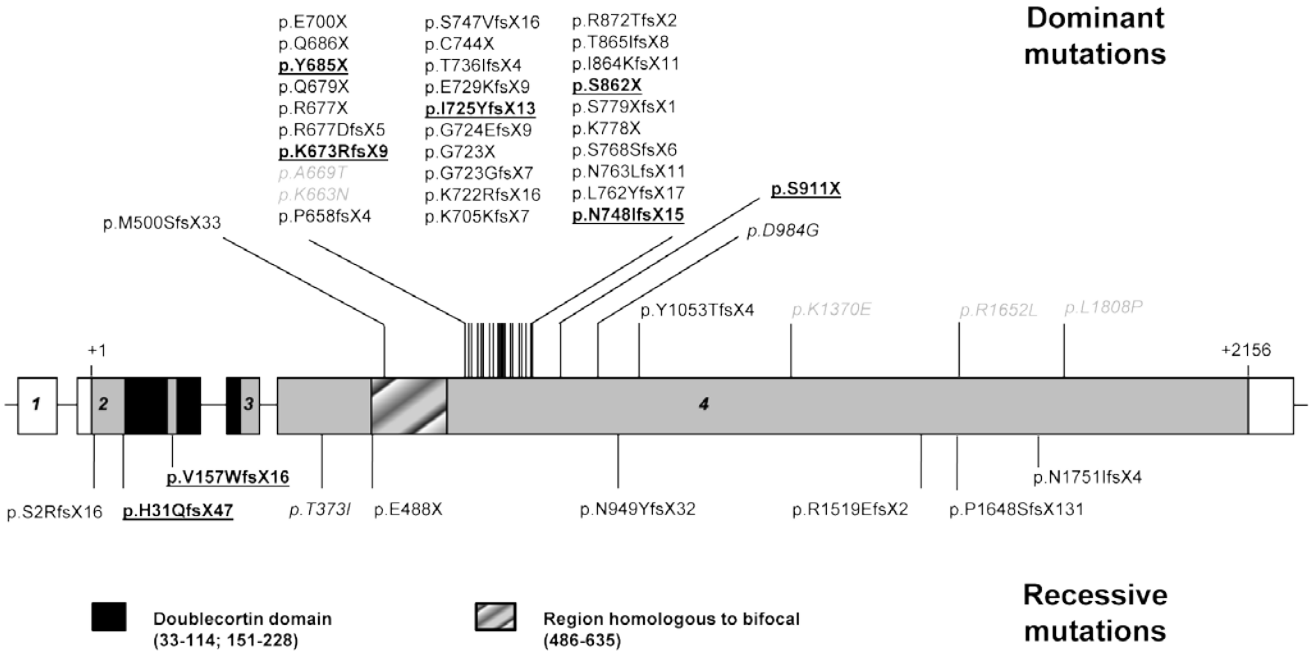

Figure 5: Schematic diagram of the *RP1* gene showing the location of published dominant (*above*) and recessive (*below*) mutations. Exon numbers are in italics and grayscale denotes coding sequence. Mutations found in this study are underlined and in bold type. Amino acid changes are in italic, and in light grey if pathogenicity is uncertain. Homologous regions to doublecortin and bifocal are indicated.

	Night Blindness	Visual field defect		Photophobia	Cataract	Pigment deposits in fundus	
	autosomal recessive retinitis pigmentosa						
Positive patients/total	4/4	4/4		4/4	2/4	4/4	
	autosomal dominant retinitis pigmentosa						
Positive patients/total	14/22	15/22	14/22		12/22	17/23	
	Age at first exam	**Age at onset**	**Visual acuity**	**Percentage of remaining visual field**	**Dim blue scotopic ERG**	**White photopic ERG**	**30-Hz flickers photopic ERG**
	autosomal recessive retinitis pigmentosa						
Mean Standard deviation Range	10 ±1 8-10 *n=4*	5 ±8 1-17 *n=4*	0.43* ±0.27 0.18-0.75 *n=4*	45* ±26 16-78 *n=4*	0 *n=4*	0 *n=4*	1 ±3 0-5 *n=4*
	autosomal dominant retinitis pigmentosa						
Mean Standard deviation Range	43 ±16 7-64 *n=23*	35 ±12 13-51 *n=13*	0.79** ±0.26 0.2-1.25 *n=23*	44** ±28 9-87 *n=15*	91 ±88 0-313 *n=16*	47 ±39 0-131 *n=16*	38 ±31 0-96 *n=16*

*determined for each arRP patient at 20 years of age; ** determined for each adRP at first examination age; ERG values are b wave amplitude in μV; "n" means the number of analyzed patients

Table 2: Summary of clinical data for RP1 patients.

higher that 20/30 between 27 to 64 years and more than 40 % of their visual field. However, closer examination of the distribution of visual acuity loss in function of age in our patient series suggested two groups of patients; one undergoing visual acuity decrease between 40 to 60 and another, milder disease group, experiencing visual acuity decrease between 55 to 70. Although not detailed, the presence of some *RP1* adRP patients with late onset and slow progression whereas others had onset at teen age followed by rapid progression, was previously suggested [34]. Interestingly, examination of the visual field loss in function of age did not show such a dichotomy. One explanation could be that, while peripheral visual field restriction is observable as soon as cones start to die, that is relatively early in the disease course, the decrease in visual acuity is observable only when cone degeneration reach the macula, that is relatively late in the course of the disease. Therefore, it is conceivable that patients with a severe condition have an earlier onset in visual acuity decrease that those with a milder condition.

By contrast, arRP patients with *RP1* mutations had a much more severe disease than adRP patients. arRP patients underwent a dramatic decrease in the visual acuity during their third decade and had tubular vision with less than 15 % of the remaining visual field by the end the third decade. Also, the ERG responses were virtually absent by the age

Family	Mutation	Exon	DNA	Protein	Remark
autosomal recessive retinitis pigmentosa					
RP94	Ho	2	c.93_105del13ins3	p.His31GlnfsX47	Novel
RP494	Ho	2	c.469delG	p.Val157TrpfsX16	Novel
autosomal dominant retinitis pigmentosa					
PHRC135	He	4	c.2018delA	p.Lys673ArgfsX9	Novel
RP206	He	4	c.2035C>T	p.Gln679X	Recurrent
PHRC116	He	4	c.2035C>T	p.Gln679X	Recurrent
RP785	He	4	c.2055T>G	p.Tyr685X	Novel
RP215	He	4	c.2169delA	p.Ile725TyrfsX13	Novel
RP263	He	4	c.2169delA	p.Ile725TyrfsX13	Novel
RP287	He	4	c.2243delA	p.Asn748IlefsX15	Novel
PHRC115	He	4	c.2585C>A	p.Ser862X	Novel
PHRC132	He	4	c.2732C>G	p.Ser911X	Recurrent

He: heterozygote, Ho: homozygote

Table 1: *RP1* mutations found in the present study.

of 20 in patients with arRP, while many dominant patients retained recordable ERGs. Previous reported cases showed onset in childhood [15,24], flat ERG by 18 [15], macular involvement before 20 [22, 23], or even total blindness before 20 [23]. Although arRP patients from our series underwent loss in visual acuity much earlier than adRP patients, the speed at which visual acuity decreased was not different than that of adRP, indicating that the progression of macular cone degeneration was similar in both series, albeit earlier in arRP. As observed in the *Rp1*$^{-/-}$ mice or homozygote mice with a truncation after the bifocal domain, the absence of a normal RP1 protein leads to rapid photoreceptor degeneration [28,46]. Interestingly, the 4 arRP patients described herein and the only arRP patient previously reported in whom refraction was mentioned [25] were myopic. Myopia is known to be associated with *RP2* and *RPGR*, the two X-linked retinitis pigmentosa causing genes [47]. Both RPGR and RP2 are ciliairy proteins, as is RP1. Thus, it is tempting to speculate that mechanisms common to RP1 and X-linked RP genes may cause myopia. Further reports of arRP patients due to *RP1* mutations are necessary to confirm the association of myopia to this gene-specific form of RP.

Acknowledgments

We thank the family members. The work was supported by private foundations (Fondation des Aveugles et Handicapés Visuels de France, Information Recherche sur la Rétinite Pigmentaire, Retina France, SOS Rétinite and UNADEV), Centre National de Génotypage, INSERM and the European EVI-GENORET contract # LSHG-CT-2005-512036 which supports fellowship for EL.

References

1. Bessant DA, Payne AM, Mitton KP, Wang QL, Swain PK, et al. (1999) A mutation in NRL is associated with autosomal dominant retinitis pigmentosa. Nature Genet 21: 355-356
2. Kanda A, Friedman JS, Nishiguchi KM, Swaroop A (2007) Retinopathy mutations in the bZIP protein NRL alter phosphorylation and transcriptional activity. Hum Mutat. 28: 589-598.
3. Nishiguchi KM, Friedman JS, Sandberg MA, Swaroop A, Berson EL, et al. (2004) Recessive NRL mutations in patients with clumped pigmentary retinal degeneration and relative preservation of blue cone function. Proc Natl Acad Sci USA 101: 17819-17824.
4. Haider NB, Jacobson SG, Cideciyan AV, Swiderski R, Streb LM, et al. (2000) Mutation of a nuclear receptor gene, NR2E3, causes enhanced S cone syndrome, a disorder of retinal cell fate. Nature Genet 24: 127-131.
5. Gerber S, Rozet JM, Takezawa SI, Dos Santos LC, Lopes L, et al. (2000) The photoreceptor cell-specific nuclear receptor gene (PNR) accounts for retinitis pigmentosa in the Crypto-Jews from Portugal (Marranos), survivors from the Spanish Inquisition. Hum Genet 107: 276-284.
6. Coppieters F, Leroy BP, Beysen D, Hellemans J, De Bosscher K, et al. (2007) Recurrent mutation in the first zinc finger of the orphan nuclear receptor NR2E3 causes autosomal dominant retinitis pigmentosa. Am J Hum Genet 81: 147-157.
7. Gire AI, Sullivan LS, Bowne SJ, Birch DG, Hughbanks-Wheaton D, et al. (2007) The Gly56Arg mutation in NR2E3 accounts for 1-2% of autosomal dominant retinitis pigmentosa. Mol Vis 13: 1970-1975.
8. Escher P, Gouras P, Roduit R, Tiab L, Bolay S, et al. (2009) Mutations in NR2E3 can cause dominant or recessive retinal degenerations in the same family. Hum Mutat 30: 342-351.
9. Maw MA, Corbeil D, Koch J, Hellwig A, Wilson-Wheeler JC, et al. (2000) A frameshift mutation in prominin (mouse)-like 1 causes human retinal degeneration. Hum Mol Genet. 9: 27-34.
10. Yang Z, Chen Y, Lillo C, Chien J, Yu Z, et al. (2008) Mutant prominin 1 found in patients with macular degeneration disrupts photoreceptor disk morphogenesis in mice. J Clin Invest 118: 2908-2916.
11. Abid A, Ismail M, Mehdi SQ, Khaliq S (2006) Identification of novel mutations in the SEMA4A gene associated with retinal degenerative diseases. J Med Genet 43: 378-381.
12. Fingert JH, Oh K, Chung M, Scheetz TE, Andorf JL, et al. (2008) Association of a novel mutation in the retinol dehydrogenase 12 (RDH12) gene with autosomal dominant retinitis pigmentosa. Arch Ophthalmol 126: 1301-1307.
13. Bowne SJ, Humphries MM, Sullivan LS, Kenna PF, Tam LC, et al. (2011) A dominant mutation in RPE65 identified by whole-exome sequencing causes retinitis pigmentosa with choroidal involvement. Eur J Hum Genet 19: 1074-1081.
14. Pierce EA, Quinn T, Meehan T, McGee TL, Berson EL, et al. (1999) Mutations in a gene encoding a new oxygen-regulated photoreceptor protein cause dominant retinitis pigmentosa. Nature Genet 22: 248-254.
15. Sullivan LS, Heckenlively JR, Bowne SJ, Zuo J, Hide WA, et al. (1999) Mutations in a novel retina-specific gene cause autosomal dominant retinitis pigmentosa. Nature Genet 22 : 255-259.
16. Guillonneau X, Piriev NI, Danciger M, Kozak CA, Cideciyan AV, et al. (1999) A nonsense mutation in a novel gene is associated with retinitis pigmentosa in a family linked to the RP1 locus. Hum Mol Gene. 8: 1541-1546.
17. Berson EL, Grimsby JL, Adams SM, McGee TL, Sweklo E, et al. (2001) Clinical features and mutations in patients with dominant retinitis pigmentosa-1 (RP1). Invest Ophthalmol Vis Sci 42: 2217-2224.
18. Payne A, Vithana E, Khaliq S, Hameed A, Deller J, et al. (2000) RP1 protein truncating mutations predominate at the RP1 adRP locus. Invest Ophthalmol Vis Sci 41: 4069-4073.
19. Milla E, Maseras M, Martinez-Gimeno M, Gamundi MJ, Assaf H, et al. (2002) Genetic and molecular characterization of 148 patients with autosomal dominant retinitis pigmentosa (ADRP). Arch Soc Esp Oftalmol 7: 481-484.
20. Sullivan LS, Bowne SJ, Birch DG, Hughbanks-Wheaton D, Heckenlively JR, et al. (2006) Prevalence of disease-causing mutations in families with autosomal dominant retinitis pigmentosa: a screen of known genes in 200 families. Invest Ophthalmol Vis Sci 47: 3052-3064.
21. Ziviello C, Simonelli F, Testa F, Anastasi M, Marzoli SB, et al. (2005) Molecular genetics of autosomal dominant retinitis pigmentosa (ADRP): a comprehensive study of 43 Italian families. J Med Genet 42: e47.
22. Riazuddin SA, Zulfiqar F, Zhang Q, Sergeev YV, Qazi ZA, et al. (2005) Autosomal recessive retinitis pigmentosa is associated with mutations in RP1 in three consanguineous Pakistani families. Invest Ophthalmol Vis Sci 46: 2264-2270.
23. Khaliq S, Abid A, Ismail M, Hameed A, Mohyuddin A, et al. (2005) Novel association of RP1 gene mutations with autosomal recessive retinitis pigmentosa. J Med Genet 42: 436-438.
24. Singh HP, Jalali S, Narayanan R, Kannabiran C (2009) Genetic analysis of Indian families with autosomal recessive retinitis pigmentosa by homozygosity screening. Invest Ophthalmol Vis Sci 50: 4065-4071.
25. Chen LJ, Lai TY, Tam PO, Chiang SW, Zhang X, et al. (2010) Compound heterozygosity of two novel truncation mutations in RP1 causing autosomal recessive retinitis pigmentosa. Invest Ophthalmol Vis Sci 51: 2236-2242.
26. Liu Q, Zhou J, Daiger SP, Farber DB, Heckenlively JR, et al. (2002)

Identification and subcellular localization of the RP1 protein in human and mouse photoreceptors. Invest Ophthalmol Vis Sci 43: 22-32.

27. Gao J, Cheon K, Nusinowitz S, Liu Q, Bei D, et al. (2002) Progressive photoreceptor degeneration, outer segment dysplasia, and rhodopsin mislocalization in mice with targeted disruption of the retinitis pigmentosa-1 (Rp1) gene. Proc Natl Acad Sci USA 99: 5698-5703.

28. Liu Q, Zuo J, Pierce EA (2004) The retinitis pigmentosa 1 protein is a photoreceptor microtubule-associated protein. J Neurosci 24: 6427-6436.

29. Omori Y, Chaya T, Katoh K, kajimura N, Sato S, et al. (2010) Negative regulation of ciliary length by ciliary male germ cell-associated kinase (Mak) is required for retinal photoreceptor survival. Proc Natl Acad Sci U S A 107: 22671-22676.

30. Yamashita T, Liu J, Gao J, LeNoue S, Wang C, et al. (2009) Essential and synergistic roles of RP1 and RP1L1 in rod photoreceptor axoneme and retinitis pigmentosa. J Neurosci 29: 9748-9760.

31. Marmor MF, Holder GE, Seeliger MW, Yamamoto S (2004) Standard for clinical electroretinography (2004 update). Doc Ophthalmol 108: 107-114.

32. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleid Acids Res 16: 1215.

33. Hebrard M, Manes G, Bocquet B, Meunier I, Coustes-Chazalette D, et al (2011) Combining gene mapping and phenotype assessment for fast mutation finding in non-consanguineous autosomal recessive retinitis pigmentosa families. Eur J Hum Genet 19: 1256-1263.

34. Bowne SJ, Daiger SP, Hims MM, Sohocki MM, Malone KA, et al. (1999) Mutations in the RP1 gene causing autosomal dominant retinitis pigmentosa. Hum Mol Genet 8: 2121-2128.

35. Jacobson SG, Cideciyan AV, Iannaccone A, Weber RG, Fishman GA, et al. (2000) Disease expression of RP1 mutations causing autosomal dominant retinitis pigmentosa. Invest Ophthalmol Vis Sci 41: 1898-1908.

36. Baum L, Chan WM, Yeung KY, Lam DS, Kwok AK, et al. (2001) RP1 in Chinese: Eight novel variants and evidence that truncation of the extreme C-terminal does not cause retinitis pigmentosa. Hum Mutat 17: 436.

37. Dietrich K, Jacobi FK, Tippmann S, Schmid R, Zrenner E, et al. (2002) A novel mutation of the RP1 gene (Lys778ter) associated with autosomal dominant retinitis pigmentosa. Br J Ophthalmol 86: 328-332.

38. Kawamura M, Wada Y, Noda Y, Itabashi T, Ogawa S, et al. (2004) Novel 2336-2337delCT mutation in RP1 gene in a Japanese family with autosomal dominant retinitis pigmentosa. Am J Ophthalmol 137: 1137-1139.

39. Gamundi MJ, Hernan I, Martinez-Gimeno M, Maseras M, Garcia-Sandoval B, et al. (2006) Three novel and the common Arg677Ter RP1 protein truncating mutations causing autosomal dominant retinitis pigmentosa in a Spanish population. BMC Med Genet 7: 35.

40. Chiang SW, Wang DY, Chan WM, Tam PO, Chong KK, et al. (2006) A novel missense RP1 mutation in retinitis pigmentosa. Eye 20: 602-605.

41. Roberts L, Bartmann L, Ramesar R, Greenberg J (2006) Novel variants in the hotspot region of RP1 in South African patients with retinitis pigmentosa. Mol Vis 12: 177-183.

42. Sheng X, Zhang X, Wu W, Zhuang W, Meng R, et al. (2008) Variants of RP1 gene in Chinese patients with autosomal dominant retinitis pigmentosa. Can J Ophthalmol 43: 208-212.

43. Daiger SP, Shankar SP, Schindler AB, Sullivan LS, Bowne SJ, et al. (2006) Genetic factors modifying clinical expression of autosomal dominant RP. Adv Exp Med Biol 572: 3-8.

44. Hartong DT, Berson EL, Dryja TP (2006) Retinitis pigmentosa. Lancet 368: 1795-1809.

45. Zhang X, Chen LJ, Law JP, Lai TY, Chiang SW, et al. (2010) Differential pattern of RP1 mutations in retinitis pigmentosa. Mol Vis. 16: 1353-1360.

46. Liu Q, Lyubarsky A, Skalet JH, Pugh EN, Pierce EA (2003) RP1 is required for the correct stacking of outer segment discs. Invest Ophthalmol Vis Sci 44: 4171-4183.

47. Kaplan J, Pelet A, Martin C, Delrieu O, Aymé S, et al. (1992) Phenotype-genotype correlations in X-linked retinitis pigmentosa. J Med Genet 29: 615-623.