·Basic Research·

Identification of a novel p.R1443W mutation in *RP1* gene associated with retinitis pigmentosa *sine pigmento*

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Abstract

• AIM: To screen mutations in the *retinitis pigmentosa 1* (*RP1*) gene and the *rhodopsin* (*RHO*) gene in Chinese patients with retinitis pigmentosa *sine pigmento* (RPSP) and describe the genotype-phenotype relationship of the mutations.

• METHODS: Twenty affected, unrelated Chinese individuals with RPSP (4 autosomal dominant RPSP, 12 autosomal recessive RPSP and 4 unknown inheritance pattern) were recruited between 2009 and 2012. The clinical features were determined by complete ophthalmologic examinations. Polymerase chain reaction (PCR) and direct DNA sequencing were used to screen the entire coding region and splice junctions of the *RP1* gene and the *RHO* gene. The cosegregation analysis and population frequency studies were performed for patients with identified mutations.

• RESULTS: Five variants in the *RP1* gene and one in the *RHO* gene were detected in 20 probands. Four missense changes (rs444772, rs446227, rs414352, rs441800) and one non-coding variant (rs56340615) were common SNPs and none of them showed a significant relationship with RPSP. A missense mutation p.R1443W was identified in the *RP1* gene in three affected individuals from a family with autosomal dominant RPSP and was found to cosegregate with the phenotype in this family, suggestive of pathogenic. In addition, population frequency analysis showed the p.R1443W mutation was absent in 300 healthy controls.

• CONCLUSION: The identification of p.R1443W mutation cosegregating in a family with autosomal dominant RPSP highlights an atypical phenotype of the *RP1* gene mutation, while RHO gene is not associated with the pathogenesis of RPSP in this study. To our knowledge, this is the fist mutation identified to associate with RPSP.

• **KEYWORDS:** retinitis pigmentosa *sine pigmento*; *RP1* and *RHO* gene; gene mutation

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INTRODUCTION

Retinitis pigmentosa (RP; OMIM 268000, Mendelian Inheritance in Man; National Center for Biotechnology Information, Bethesda, MD) is a heterogeneous group of genetic retinal degeneration characterized by night blindness, progressive impairment of visual field and retinal pigmented changes [1]. Prevalence of RP varies among different ethnic populations, estimated to be between 1/6000 and 1/1000 [2,3]. RP is divided into typical RP and atypical RP according to the fundus manifestation and retinitis pigmentosa *sine pigmento* (RPSP) is designated to atypical RP. RPSP, a special phenotype and less common in clinic, is used to refer to no intraretinal pigmentation despite documented abnormalities of photoreceptor function [4]. Characteristics of this discord are as same as the typical RP including night blindness, reduced visual acuity, visual field constriction, optic disc pallor, attenuated retinal vessels and typical electroretinography (ERG) changes, but no pigment deposits in the retina.

Genetic factors play an important role in the pathogenesis of RP. To date, at least 63 genes and loci have been identified for the non-syndromic forms of RP, including 24 for autosomal dominant RP (adRP), 39 for autosomal recessive RP (arRP) and 6 for X-linked RP (xlRP) (RetNet, the Retinal Information Network, provided by the University of Texas Houston Health Science Center, Houston, TX, accessed on July 3, 2012). Nevertheless, none of genes was confirmed to be associated with RPSP.

Table 1 Clinical features of the probands with RPSP

Code	Gender	Onset[1]	Age of diagnosis	BCVA:RE/LE	VF:RE/LE	ERG:Cone/Rod
SP1	F	28	57	0.3/0.4	Central/10°	Reduction
SP2	F	30	50	0.3/0.8	Tunnel	Reduction
SP3	F	10	20	0.3/0.5	Central/10°	Reduction
SP4	F	30	60	0.1/0.2	Tunnel	Seriously reduction
SP5	M	3	12	1.0/1.0	Central/10°	Reduction
SP6	M	3	11	0.6/0.6	Central/10°	Reduction
SP7	F	17	22	0.02/0.02	NA	Seriously reduction
SP8	M	17	28	0.1/0.1	Tunnel	Seriously reduction
SP9	M	7	58	0.15/0.15	NA	Reduction
SP10	F	3	27	0.1/0.1	Tunnel	Seriously reduction
SP11	M	26	27	1.0/0.6	Central/10°	Reduction
SP12	F	16	36	0.2/0.3	Tunnel	Seriously reduction
SP13	F	21	53	0.3/0.4	Central/10°	Reduction
SP14	F	15	47	0.6/0.6	Tunnel	Reduction
SP15	F	10	30	0.5/0.4	Tunnel	Seriously reduction
SP16	M	40	58	0.2/HM	NA	Seriously reduction
SP17	M	10	31	0.5/0.4	Tunnel	Reduction
SP18	F	28	29	1.0/0.8	Central/10°	Reduction
SP19	F	9	62	0.6/0.3	Tunnel	Reduction
SP20	M	21	31	0.8/0.5	Tunnel	Reduction

BCVA: Best-corrected visual acuity; ERG: Electroretinogram; HM: Hand motion; LE: The left eye; RE: The right eye; VF: Visual field. [1]Onset (year): Age of onset, defined as the self-reported age in which night blindness was perceived. ERG seriously reduction means rod and cone electroretinogram amplitudes were below 10% of normal electroretinography (norms for scotopic ERG: b-wave 27.6 μ V±5.2; norms for photopic ERG: b-wave 70 μ V±8.9). NA: Data not available.

The *retinitis pigmentosa 1* gene (*RP1*, OMIM603937) and the *rhodopsin gene* (*RHO*, OMIM180380) are the most commonly investigated in Chinese populations. The *RP1* protein, which consists of 2 156 amino acids, is a microtubule-associated protein at the connecting cilia of photoreceptors and may play a significant role in cilia structure and photoreceptor function [5-7]. *RP1* mutations were estimated to account for over 5% of adRP and 1% of arRP in Caucasian and approximately 2% of overall RP patients in Chinese[8-11]. Another main gene, the *RHO* gene, first identified to be pathogenesis of RP, encodes rhodopsin, a visual pigment that regulates vision in scotopia through interaction with 11-cis-retinal in retinal rod photoreceptors [12]. Over 150 mutations have been identified so far, with a majority of missense or nonsense mutations (RetNet). In Chinese populations, *RHO* mutations are responsible for about 2% of overall RP, while prevalence ranges from 0% to 50% of adRP patients in different origins [13, 14].

In this study, we have conducted a mutational screening of the *RP1* gene and the *RHO* gene in a cohort of RPSP patients from Ningxia Hui Autonomous Region located in the northwest of China, with an objective of further depicting the mutation profile of the genes and evaluate the genotype-phenotype relationship of the mutations in the Ningxia populations.

SUBJECTS AND METHODS

Subjects All study subjects were recruited from the Department of Ophthalmology in the People's Hospital of Ningxia Hui Autonomous Region. All patients underwent complete ophthalmologic examinations including measurements of best-corrected visual acuity (BCVA), slit-lamp biomicroscopy, ophthalmoscopy, intraocular pressure by noncontact tonometer, visual field by automated perimetry, electroretinography (ERG), and color fundus photography. The individuals were diagnosed with RPSP if they had a typical clinical history and features of RPSP, such as night blindness, constricted visual field, optic disc pallor and attenuation of retinal vessels, with an exclusion of syndromic RP such as Usher's syndrome, Leber congenital amaurosis, and Bardet-Biedl syndrome. A total of 20 RPSP probands were recruited, including 8 males and 12 females, with the age at diagnosis ranging from 11 to 62 years and a mean (standard deviation, SD) of 37.45 (16.61) years (Table 1). Based on the family history, 4 (20%) patients were classified as autosomal dominant RPSP (adRPSP), 12 (60%) as autosomal recessive RPSP (arRPSP) and 4 unknown inheritance pattern because probands were from small families with no other affected members. The controls consisted of 300 individuals (including 142 males and 158 females) confirmed to be free of any major eye diseases except mild senile cataract in some individuals. They were older than 60 years, ranging from 60 to 89 years. When a mutation was detected, other family members were also recruited and all received complete ophthalmic examinations. The study protocol was approved by the Institutional Ethics Committees in the both collaborating centers. Informed consents were obtained from all participants. All procedures in this study were performed in accordance with the tenets of the Declaration of Helsinki.

Table 2 Sequence variants detected in the *RP1* gene and *RHO* gene

No.	Gene name	Location	Nucleotide change	Residual change	Description	Distribution in RPSP patients[1]	Distribution in controls
1	*RP1*	Exon 4	c.2615G>A	p.R872H	rs444772	0/10/10	48/98/154
2	*RP1*	Exon 4	c.4327C>T	p.R1443W	Novel	0/1/19	0/0/300
3	*RP1*	Exon 4	c.5008G>A	p.A1670T	rs446227	3/7/10	48/99/153
4	*RP1*	Exon 4	c.5071T>C	p.S1691P	rs414352	3/7/10	48/99/153
5	*RP1*	Exon 4	c.5175A>G	p.Q1725Q	rs441800	3/7/10	48/99/153
6	*RHO*	Intron 3	c.696+4C>T	/	rs56340615	0/8/12	0/29/271

[1]Homozygote/Heterozygote/Wild type.

Methods

Mutational screening of the *RP1* gene and *RHO* gene in patients and controls Peripheral venous blood samples were drawn from all patients and control subjects. Genomic DNA was extracted from whole blood by using a commercial kit (QIAamp DNA Blood kit; Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. All patients were screened for *RP1* and *RHO* mutations. Twenty-six amplicons covering the three coding exons and the splice junctions of the *RP1* and 4 amplicons covering five exons of *RHO* were amplified by polymerase chain reaction (PCR), and analyzed by direct DNA sequencing using the dye-termination chemistry (Big-Dye Terminator Cycle Sequencing Reaction Kit; ver. 3.1; Applied Biosystems, Inc., Foster City, CA, USA) on an ABI 3130XL DNA Genetic Analyzer (Applied Biosystem)[15,16]. The DNA sequence was compared with human *RP1* (ENSG00000104237) and *RHO* (ENSG00000163914) sequences from the Ensembl database (http://asia.ensembl.org/). All variants detected in patients were genotyped in control subjects and the putative disease-causing mutations were genotyped in the family members by sequencing the corresponding amplicons. Detected sequence variants, except common SNPs, were confirmed by bi-directional sequencing.

Analysis of Variants A variant was defined "novel" if the variant had not been reported in the literature or registered in the Single Nucleotide Polymorphism (SNP) database. A variant was regarded as potentially disease causing if it was 1) predicted to alter the amino acid sequence of the RP1 or RHO protein; 2) found exclusively in patients with RPSP and absent in controls; 3) predicted to alter the protein structure or function through *in silico* analysis; and 4) completely cosegregated with the disease in the family members, if available.

Statistical Analysis For common variants with a minor allele frequency >5% detected in patients or controls, the genotype frequencies were compared between the two groups using χ^2 test in SPSS (ver. 16.0; SPSS Inc., Chicago, IL, USA). For rare missense variants detected exclusively in patients, we used four web-based programs to predict the impact of each amino acid substitution on the structure and function of the RP1 or RHO protein: PolyPhen (http://genetics.bwh.harvard.edu/pph/), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/) and PANTHER (http://www.pantherdb.org/) [17-20].We also accessed the Prosite database (http://prosite.expasy.org/) to predict the protein domains and post-translational modification sites in the RP1 wide-type protein and proteins with the missense variants[21].

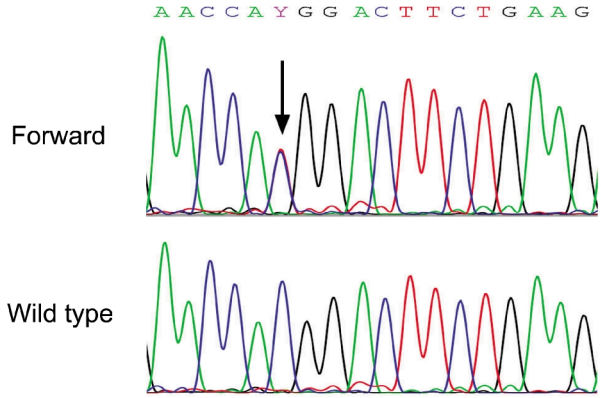

Figure 1 Chromatograms (forward sequence) of p.R1443W in the *RP1* gene. Sequence showed the heterozygous variant in patient SP4.

RESULTS

Variants Detected in the *RP1* Gene and *RHO* Gene and Functional Prediction of the Candidate Causative Mutation in the *RP1* Gene Among the 20 RPSP probands and 300 control subjects, a total of 5 heterozygous sequence changes in the *RP1* gene and 1 in the *RHO* gene were detected (Table 2). Five variants, p.R872H (rs444772), p.A1670T (rs446227), p.S1691P (rs414352), p.Q1725Q (rs441800) and c.696+4C>T (rs56340615), were common single nucleotide polymorphisms (SNPs) and found in both patients and controls. All these SNPs followed Hardy-Weinberg Equivalence (HWE), but none of them showed a significant difference in frequence between RPSP patients and controls (P_{corr}>0.05). A novel missense variant (Figure 1), p.R1443W, was identified exclusively as a heterozygote in a proband of an adRPSP family but none in controls, which was thus candidate causative mutation for RPSP. By using web-based programs, p.R1443W was

Table 3 Clinical features of the family members in pedigree with p.R1443W mutation in the *RP1* gene

Code	Gender	Birth date	Onset[1]	Age of diagnosis	BCVA:RE/LE	VF:RE/LE	ERG: Cone/Rod
SP4	F	1950	30	60	0.1/0.2	Tunnel	Seriously reduction
SP4-1	F	1974	23	24	0.6/0.5	Tunnel	Seriously reduction
SP4-2	F	1976	-	-	1.0/1.0	Normal	Normal
SP4-3	M	1979	-	-	1.0/0.8	Normal	Normal
SP4-4	F	1982	NA	26	0.3/0.4	Central/10°	Reduction
SP4-5	F	1995	-	-	0.8/0.8	Normal	Normal

BCVA: Best-corrected visual acuity; ERG: Electroretinogram; LE: The left eye; RE: The right eye; VF: Visual field. [1]Onset (year): age of onset, defined as the self-reported age in which night blindness was perceived. NA: Data not available. -: Normal family members.

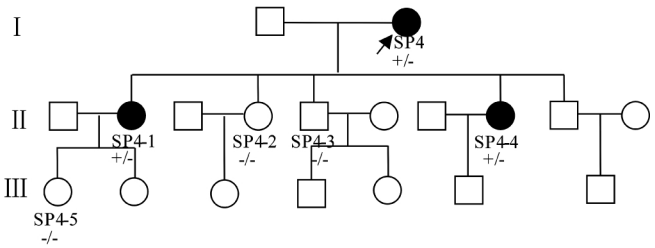

Figure 2 Pedigree with p.R1443W mutation in the *RP1* gene The circles represent females, and the squares represent males; slashed symbols indicate deceased family members. The filled symbols denote family members with retinitis pigmentosa *sine pigmento*; open symbols indicate unaffected individuals. The arrow marks the proband. +/- : Heterozygote; -/-: Wild-type.

predicted to be "Probably damaging" by PolyPhen, "Probably damaging" by PolyPhen 2, "Damaging" by SIFT, "Pathological" by PMUT, with a PANTHER score of -5.63 by means of deleterious.

Clinical Implication of the Family with p.R1443W in the *RP1* Gene The genotype-phenotype relationship with p.R1443W mutation was notable in the proband. After p.R1443W was identified in the proband, direct DNA sequence analysis was performed for all other family members. p.R1443W was also detected in other two affected family members and was found to segregate with the phenotype in this family (Figure 2). In this three-generation pedigree, six members consented to participate in this study (Table 3). The proband (SP4) had had poor visual acuity, experiencing night blindness for approximately 30 years and diagnosed at age of 60 years. She presented tunnel visual field. Fundus examination suggested a phenotype of RPSP, including waxy pallor of optic disc and attenuated blood vessels without pigment deposits (Figure 3). Both a and b waveform amplitude of full-field ERG were prominently reduced and nearly non-detectable, suggesting a severe impairment of photoreceptors. Her two daughters had similar fundus manifestation and ERG changes. The elder one (SP4-1) was a 38-year-old female with the best central visual acuity (BCVA) being 20/30 on the right eye and 20/40 on the left eye. She presented night blindness at age 23, and

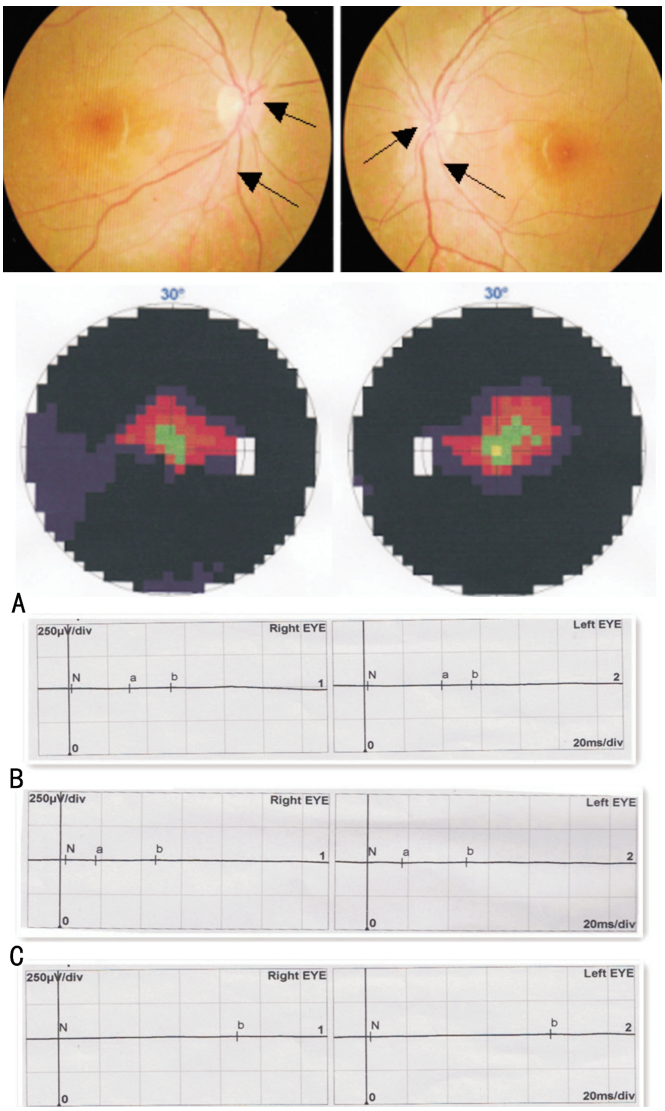

Figure 3 Fundus photographs, visual field and ERG results in proband (SP4) with p.R1443W A: ERG Cone/Rod response a-, b-wave; B: Photopic ERG; C: Scotopic ERG. Fundus photographs showed optic disc pallor, attenuated blood vessels, but no bone-spicular pigmentary changes. Visual field examination revealed tunnel visual field. Both rod and cone electroretinogram amplitudes were below 10% of normal electroretinography (norms for scotopic ERG: b-wave 27.6μV±5.2; norms for photopic ERG: b-wave 70μV±8.9).

diagnosed at age 24. Tunnel visual field and seriously

reduced waveforms uncovered at present. The younger one (SP4-4) was diagnosed at age 26 and the onset was not very clear. She had central 10° of radius remained by the examination of visual field and reduced a and b waveforms in ERG report. BCVA was 20/60 on the right eye and 20/40 on the left eye.

DISCUSSION

Ever since the discovery of *RP1* gene and *RHO* gene, it has been reported that at least 200 mutations induced the occurrence of RP. However, none of them was detected to be associated with RPSP. Previous studies showed most *RP1* mutations were predicted to lead to a premature termination which results in a truncated protein lacking 50% to 70% of original length, while no truncated mutation was found in Ningxia populations in this study[22]. Some missense mutations in *RP1* gene were reported in Chinese RP patients, including p.D984G, p.N985Y, p.K1370E, p.R1652L [9]. So missense *RP1* mutations might have played a major role in the molecular genetics of RP in ethnic Chinese. The *RHO* mutations occurred frequently in RP patients in Caucasian population, but no causative mutation was found in Ningxia RPSP patients[8].

In this mutational screening of the *RP1* gene in 20 affected unrelated RPSP patients, a total of 5 variants were identified with one novel. Four variants, p.R872H (rs444772), p.A1670T (rs446227), p.S1691P (rs414352) and p.Q1725Q (rs441800), were SNPs registered in the dbSNP database. All of them were identified in both patients and controls with a high frequency, and they were not statistically associated with RP. In contrast, one missense variant, p.R1443W, probably detrimental change by silico analysis, may play a vital role in changing the protein structure or function and play a vital role in function and localization, regulating the protein conformation. It was found exclusively in patients and to cosegregate with the phenotype in one Chinese family with autosomal dominant RPSP, is likely to be causative mutations for RPSP.

Our results enrich our growing understanding of the molecular genetics of RPSP. To our knowledge, this is the first time that a *RP1* mutation is found to correlate with this RP subtype. In a previous report by Niemeyer *et al* [23], a mutation, p.P347R in the *rhodopsin* gene was found to be associated with adRP in one six-generation family, in which the RP was characterized by early onset of night blindness before age 11, relatively preserved usable visual fields until about age 30, blindness at ages 40 to 60, and change from an initial apparently *sine pigmento* to a hyperpigmented and atrophic fundus picture between 30 and 50 years of age. This family probably should be diagnosed with classical RP, and pigment deposits started to occur from 30 years olds. In contrast, the patients with p.R1443W in the *RP1* gene showed a late onset of night blindness in the second decade or third decade and progress slowly without pigment changes detected on the retina. Some reports indicated that there were some RPSP patients emerged retinal pigmented changes after long-term observations [24]. They might be absent, especially early in the course of disease. Pigment deposits are created when the retinal pigment epithelium (a pigmented cell layer adjacent to photoreceptors) migrates into the neural retina in response to photoreceptor-cell death [8]. However, in this study, the oldest subject, SP4, was 61 years old by the time of recruitment, at which fundus examinations confirmed RPSP. Therefore, the RP phenotypes in this family could be specific. The further follow-up should be considered for the two young patients to confirm the diagnosis of RPSP and characterize the clinical manifestations of the p.R1443W mutation.

In contrast to *RP1*, the *RHO* gene made no contribution to RPSP in Ningxia populations according to the present study. One non-coding variant c.696+4C>T (rs56340615) was detected in 6 adRPSP families and 2 arRPSP families. To date at least 150 mutations have been implicated to cause autosomal dominant and recessive retinitis pigmentosa. However, in our study, the substitution of c.696+4C>T in the splicing site of intron 3 was not considered to be associated with RPSP there were no significant difference in allele frequencies between patients and controls.

In summary, the identification of the p.R1443W mutation in a pedigree with RPSP reveals a new genotype-phenotype relationship of the *RP1* gene. What's more, to our knowledge, this is the fist time to detect gene mutations to cause RPSP. On the contrary, the *RHO* gene was not associated with the pathogenesis of RPSP. As over 90% of patients do not find causative mutations, our study suggests that the presence of further novel genes or loci is involved in the etiology of RPSP.

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REFERENCES

1 Milam AH, Li ZY, Fariss RN. Histopathology of the human retina in retinitis pigmentosa. *Prog Retin Eye Res* 1998;17(2):175-205

2 Peterlin B, Canki-Klain N, Morela V, Stirn B, Rainer S, Cerar V. Prevalence of retinitis pigmentosa in Slovenia. *Clin Genet* 1992;42 (3): 122-123

3 Xu L, Hu L, Ma K, Li J, Jonas JB. Prevalence of retinitis pigmentosa in urban and rural adult Chinese: The Beijing Eye Study. *Eur J Ophthalmol* 2006;16(6):865-866

4 Pagon RA, Daiger SP. Retinitis Pigmentosa Overview. 2000 Aug 04 [updated 2005 Sep 16]. In: Pagon RA, Bird TD, Dolan CR, Stephens K, Adam MP, editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2013. Available from http://www.ncbi.nlm.nih.

gov/books/NBK1417/

5 Pierce EA, Quinn T, Meehan T, McGee TL, Berson EL, Dryja TP. Mutations in a gene encoding a new oxygen-regulated photoreceptor protein cause dominant retinitis pigmentosa. *Nat Genet* 1999;22 (3): 248-254

6 Liu Q, Zhou J, Daiger SP, Farber DB, Heckenlively JR, Smith JE, Sullivan LS, Zuo J, Milam AH, Pierce EA. Identification and subcellular localization of the RP1 protein in human and mouse photoreceptors. *Invest Ophthalmol Vis Sci* 2002;43(1):22-32

7 Liu Q, Zuo J, Pierce EA. The retinitis pigmentosa 1 protein is a photoreceptor microtubule-associated protein. *J Neurosci* 2004;24 (29): 6427-6436

8 Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet* 2006; 368(9549):1795-1809

9 Zhang X, Chen LJ, Law JP, Lai TY, Chiang SW, Tam PO, Chu KY, Wang N, Zhang M, Pang CP. Differential pattern of RP1 mutations in retinitis pigmentosa. *Mol Vis* 2010;16:1353-1360

10 Baum L, Chan WM, Yeung KY, Lam DS, Kwok AK, Pang CP. RP1 in Chinese: Eight novel variants and evidence that truncation of the extreme C-terminal does not cause retinitis pigmentosa. *Hum Mutat* 2001;17(5):436

11 Chiang SW, Wang DY, Chan WM, Tam PO, Chong KK, Lam DS, Pang CP. A novel missense RP1 mutation in retinitis pigmentosa. *Eye (Lond)* 2006;20(5):602-605

12 Dryja TP, McGee TL, Reichel E, Hahn LB, Cowley GS, Yandell DW, Sandberg MA, Berson EL. A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. *Nature* 1990;343(6256):364-366

13 Chan WM, Yeung KY, Pang CP, Baum L, Lau TC, Kwok AK, Lam DS. Rhodopsin mutations in Chinese patients with retinitis pigmentosa. *Br J Ophthalmol* 2001;85(9):1046-1048

14 Audo I, Manes G, Mohand-Saïd S, Friedrich A, Lancelot ME, Antonio A, Moskova-Doumanova V, Poch O, Zanlonghi X, Hamel CP, Sahel JA, Bhattacharya SS, Zeitz C. Spectrum of rhodopsin mutations in French autosomal dominant rod-cone dystrophy patients. *Invest Ophthalmol Vis Sci* 2010;51(7):3687-3700

15 Sheng X, Zhang X, Wu W, Zhuang W, Meng R, Rong W. Variants of RP1 gene in Chinese patients with autosomal dominant retinitis pigmentosa. *Can J Ophthalmol* 2008;43(2):208-212

16 Kim KJ, Kim C, Bok J, Kim KS, Lee EJ, Park SP, Chung H, Han BG, Kim HL, Kimm K, Yu HG, Lee JY. Spectrum of rhodopsin mutations in Korean patients with retinitis pigmentosa. *Mol Vis* 2011;17:844-853

17 Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. *Nucleic Acids Res* 2002;30(17):3894-3900

18 Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7(4):248-249

19 Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009;4(7):1073-1081

20 Mi H, Lazareva-Ulitsky B, Loo R, Kejariwal A, Vandergriff J, Rabkin S, Guo N, Muruganujan A, Doremieux O, Campbell MJ, Kitano H, Thomas PD. The PANTHER database of protein families, subfamilies, functions and pathways. *Nucleic Acids Res* 2005;33(Database issue):D284-288

21 Sigrist CJ, Cerutti L, de Castro E, Langendijk-Genevaux PS, Bulliard V, Bairoch A, Hulo N. PROSITE, a protein domain database for functional characterization and annotation. *Nucleic Acids Res* 2010;38 (Database issue):D161-166

22 Chen LJ, Lai TY, Tam PO, Chiang SW, Zhang X, Lam S, Lai RY, Lam DS, Pang CP. Compound heterozygosity of two novel truncation mutations in RP1 causing autosomal recessive retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2010;51(4):2236-2242

23 Niemeyer G, Trüb P, Schinzel A, Gal A. Clinical and ERG data in a family with autosomal dominant RP and Pro-347-Arg mutation in the rhodopsin gene. *Doc Ophthalmol* 1992;79(4):303-311

24 Chang S, Vaccarella L, Olatunji S, Cebulla C, Christoforidis J. Diagnostic challenges in retinitis pigmentosa: genotypic multiplicity and phenotypic variability. *Curr Genomics* 2011;12(4):267-275