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ORIGINAL INVESTIGATION

Mutations of 60 known causative genes in 157 families with retinitis pigmentosa based on exome sequencing

Yan Xu · Liping Guan · Tao Shen · Jianguo Zhang · Xueshan Xiao · Hui Jiang · Shiqiang Li · Jianhua Yang · Xiaoyun Jia · Ye Yin · Xiangming Guo · Jun Wang · Qingjiong Zhang

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Abstract Retinitis pigmentosa (RP) is the most common and highly heterogeneous form of hereditary retinal degeneration. This study was to identify mutations in the 60 genes that were known to be associated with RP in 157 unrelated Chinese families with RP. Genomic DNA from probands was initially analyzed by whole exome sequencing. Sanger sequencing was used to confirm potential candidate variants affecting the encoded residues in the 60 genes, including heterozygous variants from genes that are related to autosomal dominant RP, homozygous or compound heterozygous variants from genes that are related to autosomal recessive RP, and hemizygous variants from genes that are related to X-linked RP. Synonymous and intronic variants were also examined to confirm whether they could affect splicing. A total of 244 candidate variants were detected by exome sequencing. Sanger sequencing confirmed 240 variants out of the 244 candidates. Informatics and segregation analyses suggested 110 potential pathogenic mutations in 28 out of the 60 genes involving 79 of the 157 (50 %) families, including 31 (39 %, 31/79) families with heterozygous mutations in autosomal dominant genes, 37 (47 %, 37/79) families with homozygous (9) or compound heterozygous (28) mutations in autosomal recessive genes, and 11 (14 %, 11/79) families with hemizygous mutations in X-linked genes. Of the 110 identified variants, 74 (67 %) were novel. The genetic defects in approximately half of the 157 studies families were detected by exome sequencing. A comprehensive analysis of the 60 known genes not only expanded the mutation spectrum and frequency of the 60 genes in Chinese patients with RP, but also provided an overview of the molecular etiology of RP in Chinese patients. The analysis of the known genes also supplied the foundation and clues for discovering novel causative RP genes.

Introduction

Retinitis pigmentosa (RP, MIM: 268000), with a worldwide incidence of approximately one in 3,500–5,000 individuals (Boughman et al. 1980; Chizzolini et al. 2011; Hu 1982), is a group of hereditary retinal degeneration diseases that are characterized by night blindness, a constriction of the visual field (Jacobson et al. 1986), a gradual reduction of the visual acuity, waxy pale optic discs, attenuated retinal artery, and pigment abnormality initially from the mid-peripheral retina. Electroretinogram (ERG) recordings are usually extinguished or severely reduced, with the rods being affected before the cones (Berson 1987). Retinitis pigmentosa can be inherited in different patterns. Approximately 10–19 % of the cases are autosomal dominant (adRP), 6–8 % are X-linked (xlRP), and the remaining cases are most likely autosomal recessive (arRP) (Boughman et al. 1980; Bunker et al. 1984; Daiger 2004; Daiger

Y. Xu and L. Guan contributed equally. J. Wang and Q. Zhang contributed equally.

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Y. Xu · T. Shen · X. Xiao · S. Li · X. Jia · X. Guo · Q. Zhang (✉)
State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 54 Xianlie Road, Guangzhou 510060, China
e-mail: zhangqji@mail.sysu.edu.cn

L. Guan · J. Zhang · H. Jiang · J. Yang · Y. Yin · J. Wang
BGI-Shenzhen, Shenzhen 518083, China

et al. 2007; Grondahl 1987). Rarely, RP may be transmitted as a mitochondrial or digenic trait (Beales et al. 2003; Goldberg and Molday 1996; Kajiwara et al. 1994; Katsanis et al. 2001).

Molecular genetic studies of RP have produced great progress in recent years. To date, at least 62 genes have been reported to be associated with nonsyndromic RP as listed in RetNet (http://www.sph.uth.tmc.edu/retnet/). Of these genes, mutations in *RHO* (MIM: 180380), *USH2A* (MIM: 608400), and *RPGR* (MIM: 312610) were the most frequently reported in previous studies to be responsible for approximately 30 % of all of the RP cases (Hartong et al. 2006). Because of the highly heterogeneous nature of RP and the potential ethnic variety of individual gene variations, genes that are frequently mutated in one population, such as *RHO* (MIM: 180380) and *CYP4V2* (MIM: 608614), may not be common causes other populations (Li et al. 2010; Xiao et al. 2011). Due to the large number of identified genes, the systematic analysis of all these genes in a cohort of patients has been difficult. Subsequently, the genetic basis for most patients with RP in Chinese patients remains unknown. A frequency analysis of the mutations and genes that are responsible for retinitis pigmentosa in this population can serve as a reference for researchers who are investigating the mutation spectrum and frequency of RP genes in other populations and will provide a guide for rapid and efficient molecular diagnostic approaches. In addition, the identification of the genetic defect has not been applicable for most patients in clinical practice due to the highly heterogeneous genetically and clinically complicated features of RP (Berger et al. 2010; den Hollander et al. 2010).

In this study, whole exome sequencing was used to detect variations in 60 of the 62 known causative genes in probands from 157 unrelated Chinese families with RP. *FSCN2* (MIM: 607643) was excluded from this study because it was previously excluded as a causative gene for RP (Jin et al. 2008; Zhang et al. 2007). *EMC1*, however, was not captured by whole exome sequencing. Although mutations in *USH2A* (MIM: 608400) can cause autosomal recessive Usher syndrome (mainly RP and deafness), some mutations in *USH2A* (MIM: 608400) do cause autosomal recessive nonsyndromic RP (Avila-Fernandez et al. 2010; Bernal et al. 2003; Rivolta et al. 2000; Seyedahmadi et al. 2004). *USH2A* (MIM: 608400) mutations are the most common cause of autosomal recessive RP (including syndromic and nonsyndromic RP) (Daiger et al. 2007; Hartong et al. 2006). Another gene that is associated with Usher syndrome, *CLRN1* (MIM: 606397) and a causative gene of Bardet-Biedl syndrome, *TTC8* (MIM: 608132) are also associated with nonsyndromic RP (Khan et al. 2011; Riazuddin et al. 2010). Therefore, it is reasonable to include *USH2A* (MIM: 608400), *CLRN1* (MIM: 606397), and *TTC8* (MIM: 608132) in our study. The potential candidate variants were initially confirmed by Sanger sequencing and then further evaluated in the available family members and normal controls.

Materials and methods

Probands with RP

All of the 157 probands with RP, as well as their available family members and controls, were recruited from our Pediatric and Genetic Clinic at the Zhongshan Ophthalmic Center. Written informed consent was obtained from the participants or their guardians before the collection of their venous blood and clinical data. This study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center. The genomic DNA from each participating individuals was prepared from the leukocytes of peripheral venous blood as previously described (Wang et al. 2010). Ninety-six unrelated healthy individuals were recruited as controls.

Whole exome sequencing

Exome sequencing was completed using a commercial service from BGI Shenzhen (http://www.genomics.cn/index.php) as previously described (Chen et al. 2013; Huang et al. 2013). In brief, the exome capture was carried out using a NimbleGen SeqCap EZ Exome (44M) array. The sequences of the exon-enriched DNA fragments were determined using the Illumina Genome Analyzer II platform. The average depth of each base in the targeted regions was set to 60-fold. SOAP aligner (Li et al. 2008, 2009b) was used to arrange the sequencing reads on the NCBI hg19. The software SOAPsnp was used to assemble the consensus sequences and call the genotypes in target regions. (Li et al. 2009a). The variants in the 60 RP-associated genes (Supplementary Table S1) were detected by exome sequencing were selected for validation.

Sanger sequencing

Sanger sequencing was used to confirm the candidate variants that were identified by whole exome sequencing, including heterozygous variants in the genes that are associated with adRP, homozygous or compound heterozygous variants in the genes that are associated with arRP, and hemizygous variants in the genes that are associated with xlRP. The primers (Supplementary Table S2) that were used to amplify the fragments harboring the variants were designed using Primer3 (http://frodo.wi.mit.edu/) (Rozen and Skaletsky 2000). The procedures that were

Table 1 The 110 causative variants in the 79 of 157 Chinese RP probands

Gene	Inheritance	Patient ID	Nucleotide change	Amino acid change	State	Computational prediction				Allele frequency in		Reported
						P/SS	SIFT	phastCons	GERP	Patients	Controls	
RHO	AD	RP314	c.403C>T	p.R135W	Het	PrD	D	1.000	2.050	1/314	ND	Sung et al. (1991), Mokarzel-Falcon et al. (2008), Rakoczy et al. (2011)
		RP209	c.481T>C	p.W161R	Het	PrD	D	1.000	5.260	1/314	ND	Gardner et al. (2010)
		RP221	c.541G>A	p.E181K	Het	PoD	D	0.914	5.190	2/314	ND	Dryja et al. (1991), Rakoczy et al. (2011)
		RP295	c.541G>A	p.E181K	Het	PoD	D	0.914	5.190	2/314	ND	Dryja et al. (1991), Rakoczy et al. (2011)
		RP253	c.632A>G	p.H211R	Het	PrD	D	1.000	5.040	1/314	ND	Macke et al. (1993), Rakoczy et al. (2011)
		RP373	c.1033G>T	p.V345L	Het	PoD	D	1.000	5.420	1/314	0/192	Novel
		RP042	c.1040C>T	p.P347L	Het	PrD	D	0.993	5.420	3/314	0/192	Dryja et al. (1990), Mokarzel-Falcon et al. (2008), Rakoczy et al. (2011)
		RP100	c.1040C>T	p.P347L	Het	PrD	D	0.993	5.420	3/314	0/192	Dryja et al. (1990), Mokarzel-Falcon et al. (2008), Rakoczy et al. (2011)
		RP343	c.1040C>T	p.P347L	Het	PrD	D	0.993	5.420	3/314	0/192	Dryja et al. (1990), Mokarzel-Falcon et al. (2008), Rakoczy et al. (2011)
SNRNP200	AD	RP311	c.2036+1G>T	Splicing defect	Het	SSA	–	1.000	6.170	1/314	0/192	Novel
		RP144	c.2041C>T	p.R681C	Het	PrD	D	1.000	6.060	2/314	ND	Benaglio et al. (2011)
		RP230	c.2041C>T	p.R681C	Het	PrD	D	1.000	6.060	2/314	ND	Benaglio et al. (2011)
		RP399	c.2359G>A	p.A787T	Het	PrD	D	1.000	5.680	1/314	0/192	Novel
PRPF8	AD	RP335	c.5804G>A	p.R1935H	Het	PrD	D	1.000	5.910	1/314	0/192	Novel
		RP353	c.6910T>C	p.F2304L	Het	PoD	D	1.000	5.860	1/314	0/192	Novel
		RP305	c.6985G>A	p.D2329N	Het	PrD	D	0.193	4.900	1/314	0/192	Novel
PRPH2	AD	RP325	c.535T>C	p.W179R	Het	PrD	D	1.000	5.630	1/314	ND	Bareil et al. (2000)
		RP387	c.914delG	p.G305Afs*19	Het	–	–	–	–	2/314	0/192	Novel
		RP405	c.914delG	p.G305Afs*19	Het	–	–	–	–	2/314	0/192	Novel
TOPORS	AD	RP316	c.2550_2553del	p.D850Efs*15	Het	–	–	–	–	1/314	0/192	Novel
		RP282	c.2554_2557del	p.E852Qfs*13	Het	–	–	–	–	1/314	0/192	Novel
		RP021	c.2556_2557del	p.E852Dfs*20	Het	–	–	–	–	1/314	0/192	Chakarova et al. (2007)
NR2E3	AD	RP215	c.166G>C	p.G56R	Het	PrD	D	0.986	3.980	2/314	0/192	Novel
		RP291	c.166G>C	p.G56R	Het	PrD	D	0.986	3.980	2/314	0/192	Novel
CRX	AD	RP327	c.541delG	p.A181Pfs*6	Het	–	–	–	–	1/314	ND	Zhang et al. (2001)
GUCA1B	AD	RP289	c.361A>G	p.I121V	Het	B	D	0.863	4.960	1/314	0/192	Novel
IMPDH1	AD	RP339	c.931G>A	p.D311N	Het	B	D	0.991	5.290	1/314	ND	Bowne et al. (2002), Bischof et al. (2006), Wang et al. (2011)
PRPF3	AD	RP259	c.1481C>T	p.T494M	Het	PrD	D	1.000	5.820	1/314	ND	Chakarova et al. (2002), Vaclavik et al. (2010); Tanackovic et al. (2011)
PRPF31	AD	RP379	c.736G>A	p.A246T	Het	PrD	D	0.999	5.210	1/314	0/192	Novel

Table 1 continued

Gene	Inheritance	Patient ID	Nucleotide change	Amino acid change	State	Computational prediction				Allele frequency in		Reported
						P/SS	SIFT	phastCons	GERP	Patients	Controls	
ROM1	AD	RP053	c.667C>T	p.R223W	Het	PrD	D	0.998	2.280	1/314	0/192	Novel
RP1	AD/AR	RP245	c.2117delG	p.G706Vfs*7	Het	–	–	–	–	1/314	0/192	Novel
		RP382	c.426dupA	p.A143Sfs*86	Het	–	–	–	–	1/314	0/192	Novel
			c.607G>C	p.G203R	Het	PrD	D	0.727	4.380	1/314	0/192	Novel
			c.830C>T	p.S277F	Het	PoD	D	0.999	5.050	1/314	0/192	Novel
USH2A	AR	RP331	c.997T>C	p.S333P	Het	PoD	T	0.005	−1.020	1/314	0/192	Novel
			c.9371+1G>C	Splicing defect	Het	SSA	–	0.999	5.010	2/314	ND	Le Quesne Stabej et al. (2012)
		RP385	c.1142A>G	p.Q381R	Het	PoD	D	1.000	5.360	1/314	0/192	Novel
			c.2802T>G	p.C934W	Het	PrD	D	0.432	3.740	7/314	ND	Xu et al. (2011)
		RP337	c.1340A>G	p.Y447C	Het	PrD	D	0.992	4.260	1/314	0/192	Novel
			c.5572+1G>A	Splicing defect	Het	SSA	–	0.895	5.010	1/314	0/192	Novel
			c.14914C>T	p.R4972C	Het	PrD	D	0.022	4.670	1/314	0/192	Novel
		RP296	c.1397G>T	p.G466V	Het	PrD	D	1.000	5.500	1/314	0/192	Novel
			c.2439delG	p.Q814Sfs*42	Het	–	–	–	–	1/314	0/192	Novel
		RP309	c.2653C>T	p.H885Y	Het	PrD	T	1.000	6.030	2/314	0/192	Novel
			c.9371+1G>C	Splicing defect	Het	SSA	–	0.999	5.010	2/314	ND	Le Quesne Stabej et al. (2012)
		RP202	c.2653C>T	p.H885Y	Het	PrD	T	1.000	6.030	2/314	0/192	Novel
			c.13811+1G>A	Splicing defect	Het	SSA	–	0.993	4.990	1/314	0/192	Novel
		RP238	c.2802T>G	p.C934W	Het	PoD	D	0.432	3.740	7/314	ND	Xu et al. (2011)
			c.10903A>C	p.T3635P	Het	PoD	T	0.935	3.560	1/314	0/192	Novel
		RP377	c.2802T>G	p.C934W	Het	PrD	D	0.432	3.740	7/314	ND	Xu et al. (2011)
			c.8368delT	p.S2790Lfs*40	Het	–	–	–	–	1/314	0/192	Novel
		RP275	c.2802T>G	p.C934W	Het	PrD	D	0.432	3.740	7/314	ND	Xu et al. (2011)
			c.8730dupT	p.T2911Yfs*27	Het	–	–	–	–	1/314	0/192	Novel
		RP219	c.2802T>G	p.C934W	Het	PrD	D	0.432	3.740	7/314	ND	Xu et al. (2011)
			c.9570+1G>A	Splicing defect	Het	SSA	–	0.998	5.550	1/314	0/192	Novel
		RP315	c.2802T>G	p.C934W	Het	PrD	D	0.432	3.740	7/314	ND	Xu et al. (2011)
			c.14285A>G	p.N4762S	Het	PrD	T	0.948	5.740	1/314	0/192	Le Quesne Stabej et al. (2012)
		RP338	c.5375G>A	p.G1792E	Het	PrD	T	0.663	4.290	2/314	0/192	Novel
			c.9565G>T	p.A3189S	Het	B	T	0.781	−1.980	1/314	0/192	Novel
		RP307	c.7451+3G>A	Splicing defect	Het	SSA	–	0.997	3.210	1/314	0/192	Novel
			c.9244A>G	p.I3082V	Het	B	T	0.445	−4.930	1/314	0/192	Novel
		RP313	c.9958G>T	p.G3320C	Het	PrD	D	1.000	5.800	1/314	0/192	Novel
			c.11140C>T	p.Q3714*	Het	–	–	–	–	1/314	0/192	Novel

Table 1 continued

Gene	Inheritance	Patient ID	Nucleotide change	Amino acid change	State	Computational prediction				Allele frequency in		Reported
						P/SS	SIFT	phastCons	GERP	Patients	Controls	
ABCA4	AR	RP342	c.858+2T>A	Splicing defect	Het	SSA	–	1.000	5.590	1/314	0/192	Novel
			c.5318C>T	p.A1773V	Het	PoD	T	0.983	4.720	1/314	ND	Stenirri et al. (2008)
		RP365	c.983A>T	p.E328V	Het	B	D	1.000	5.260	1/314	ND	Rivera et al. (2000)
			c.2063dupA	p.N689Kfs*78	Het	–	–	–	–	1/314	0/192	Novel
			c.3106G>A	p.E1036K	Het	B	T	1.000	4.830	1/314	ND	Nasonkin et al. (1998)
		RP005	c.1760+1G>T	Splicing defect	Hom	SSA	–	1.000	5.040	2/314	0/192	Novel
		RP250	c.4537dupC	p.Q1513Pfs*42	Hom	–	–	–	–	2/314	ND	Briggs et al. (2001), Stenirri et al. (2008)
PDE6B	AR	RP016	c.1133G>A	p.W378*	Hom	–	–	0.795	4.980	2/314	0/192	Novel
		RP022	c.1133G>A	p.W378*	Het	–	–	0.795	4.980	1/314	0/192	Novel
			c.1615−1G>C	Splicing defect	Het	SSA	–	0.989	4.290	1/314	0/192	Novel
		RP397	c.313G>A	p.E105K	Het	PrD	T	0.988	4.980	1/314	0/192	Novel
			c.2047G>A	p.V683M	Het	PrD	D	0.997	3.940	1/314	0/192	novel
		RP281	c.610G>T	p.E204*	Het	–	–	0.267	3.590	1/314	0/192	Novel
			c.1615−1G>C	Splicing defect	Het	SSA	–	0.989	4.290	1/314	0/192	Novel
EYS	AR	RP195	c.904C>T	p.L302F	Het	B	D	0.000	−0.279	1/314	0/192	Novel
			c.6416G>A	p.C2139Y	Het	PoD	D	0.950	3.060	1/314	ND	Audo et al. (2010)
		RP303	c.1211dupA	p.N404Kfs*3	Het	–	–	–	–	1/314	ND	Bandah-Rozenfeld et al. (2010)
			c.6416G>A	p.C2139Y	Het	PoD	D	0.950	3.060	1/314	ND	Audo et al. (2010)
		RP151	c.7376dupA	p.N2459Kfs*2	Hom	–	–	–	–	2/314	0/192	Novel
MERTK	AR	RP300	c.1186G>T	p.E396*	Hom	–	–	0.033	0.711	2/314	0/192	Novel
		RP191	c.1691−1G>A	Splicing defect	Hom	SSA	–	0.366	5.370	2/314	0/192	Novel
RDH12	AR	RP201	c.598T>C	p.Y200H	Hom	PrD	D	1.000	6.170	2/314	0/192	Novel
		RP193	c.599A>G	p.Y200C	Hom	PrD	D	1.000	6.170	2/314	0/192	Stone et al. (2007)
PDE6A	AR	RP398	c.2198delinsGG	p.Q733Rfs*9	Hom	–	–	–	–	2/314	0/192	Novel
CERKL	AR	QT770	c.398T>C	p.L133P	Het	PrD	T	0.989	5.220	1/314	0/192	Novel
			c.1561_1564dup	p.Y504Sfs*19	Het	–	–	–	–	2/314	0/192	Novel
CNGB1	AR	RP101	c.2361C>A	p.Y787*	Het	–	–	0.479	1.010	1/314	0/192	Novel
			c.2888_2889del	p.F963Sfs*4	Het	–	–	–	–	1/314	0/192	novel
CRB1	AR	RP336	c.2711C>G	p.S904C	Het	PoD	D	0.668	5.550	1/314	0/192	Novel
			c.2809G>A	p.A937T	Het	B	T	0.610	3.490	2/314	0/192	Novel
MAK	AR	RP298	c.553G>A	p.A185T	Het	PrD	D	1.000	5.430	1/314	0/192	Novel
			c.1105C>T	p.Q369*	Het	–	–	0.001	3.010	1/314	0/192	Novel
PROM1	AR	RP233	c.139delC	p.H47Ifs*12	Het	–	–	–	–	2/314	1/192	Novel

Table 1 continued

Gene	Inheritance	Patient ID	Nucleotide change	Amino acid change	State	Computational prediction				Allele frequency in		Reported
						P/SS	SIFT	phastCons	GERP	Patients	Controls	
			c.1238T>A	p.V413D	Het	PoD	D	0.013	5.520	1/314	0/192	Novel
SPATA7	AR	RP236	c.322C>T	p.R108*	Het	–	–	0.005	4.210	1/314	ND	Wang et al. (2009)
			c.1183C>T	p.R395*	Het	–	–	0.996	1.610	1/314	ND	Wang et al. (2009), Li et al. (2011)
RPGR	XL	RP367	c.310+1G>A	Splicing defect	Hemi	SSA	–	1.000	6.070	1/205*	0/137#	Novel
		RP122	c.530dupT	p.S178Kfs*2	Hemi	–	–	–	–	1/205*	0/137#	Novel
		RP044	c.1234C>T	p.R412*	Hemi	–	–	0.365	3.240	1/205*	0/137#	Breuer et al. (2002)
		RP292	c.2075dupG	p.E693Rfs*77	Hemi	–	–	–	–	1/205*	0/137#	Novel
		RP207	c.2405_2406delAG	p.E802Gfs*32	Hemi	–	–	–	–	1/205*	0/137#	Vervoort et al. (2000), Bader et al. (2003); Ji et al. (2010)
		RP231	c.2420_2435del	p.E807Gfs*3	Hemi	–	–	–	–	1/205*	0/137#	Novel
		RP396	c.2476_2477delAG	p.R826Gfs*8	Hemi	–	–	–	–	1/205*	0/137#	Bader et al. (2003)
RP2	XL	RP263	c.49C>T	p.P17S	Hemi	B	T	0.922	0.514	1/205*	0/137#	Novel
		RP056	c.115G>A	p.D39N	Hemi	B	T	1.000	5.620	1/205*	0/137#	Novel
		RP223	c.428T>C	p.I143T	Hemi	PoD	D	1.000	5.620	1/205*	0/137#	Novel
		RP288	c.591_597del	p.Y198Lfs*38	Hemi	–	–	–	–	1/205*	0/137#	Novel

AR autosomal recessive, *AD* autosomal dominate, *XL* X-linked, *P/SS* Polyphen-2/Splice Site Prediction, *Het* heterozygous, *Hom* homozygous, *Hemi* hemizygous, *PrD* probably damaging, *PoD* possibly damaging, *B* benign, *SSA* splicing site abolished, *D* damaging, *T* tolerated, *ND* not done

* In the 157 patients, 109 are male and 48 are female, and there are 205 alleles for genes on the X chromosome

In the 96 normal controls, 55 are male and 41 are female, and there are 137 alleles for genes on the X chromosome

used for the amplification, sequencing, and analysis of the target fragments were previously described (Chen et al. 2013; Huang et al. 2013).

Putative pathogenic heterozygous or hemizygous variants in the genes that are associated with adRP or xlRP, respectively, were selected based on the bioinformatics analysis and the pedigree structure. Unlikely pathogenic variants in *RP1* (MIM: 603937), *RHO* (MIM: 180380) and *RPGR* (MIM: 312610) were excluded according to the previous description (Buraczynska et al. 1997; Davies et al. 2012; Siemiatkowska et al. 2012; Vervoort et al. 2000). Considering the presence of the normal carriers in the genes that are associated with arRP, we assumed that the affected individuals were likely homozygous or compound heterozygous. Subsequently, the variants that were absent in the dbSNP138, 1000 Genome, Exome Variant Server database or with allelic frequencies of $\leq$0.5 % were considered putative pathogenic [the frequency of heterozygote carriers was calculated based on a disease prevalence of 1:4,000 multiplied by 10 %, the maximum frequency in the RP gene of all of the RP cases (Avila-Fernandez et al. 2010; Hartong et al. 2006; Maubaret and Hamel 2005; Seyedahmadi et al. 2004) and the square root of this value]. Each novel potential causative variant was further evaluated in 96 normal individuals.

Results

Whole exome sequencing detected 244 candidate variants in 60 of the 62 genes that are associated with RP. The variants in the remaining two genes, *EMC1* and *FSCN2* (MIM: 607643), were not considered because *EMC1* was not captured by the NimbleGen SeqCap EZ Exome (44M) array while the mutation in *FSCN2* (MIM: 607643) was excluded as a cause of adRP in previous reports. Of the 244 variants, 240 (98 %) were confirmed by Sanger sequencing. The other four (2 %) were absent (false positive). Based on the informatics analysis and pedigree structure, 110 of the 240 variants, including 74 novel variants, in 79 families (50 %, 79/157) were considered potential pathogenic mutations [Table 1 (Audo et al. 2010; Bader et al. 2003; Bandah-Rozenfeld et al. 2010; Bareil et al. 2000; Benaglio et al. 2011; Bischof et al. 2006; Bowne et al. 2002; Breuer et al. 2002; Briggs et al. 2001; Chakarova et al. 2002; 2007; Dryja et al. 1990, 1991; Gardner et al. 2010; Ji et al. 2010; Le Quesne Stabej et al. 2012; Macke et al. 1993; Mokarzel-Falcon et al. 2008; Nasonkin et al. 1998; Rakoczy et al. 2011; Rivera et al. 2000; Stenirri et al. 2008; Stone 2007; Sung et al. 1991; Tanackovic et al. 2011; Vaclavik et al. 2010; Vervoort et al. 2000; Wang et al. 2009, 2011; Xu et al. 2011; Zhang et al. 2001)].

The 110 mutations in the 79 families involved 28 of the 60 genes (Fig. 1 and Supplementary Table S3). The segregation analysis was available for 39 of the 79 families, and the mutations were segregated with the disease in their families (Fig. 2). Mutations in the 79 families consisted of 31 (39 %, 31/79) families with heterozygous mutations in the genes that are associated with adRP, 37 (47 %, 37/79) families with homozygous (9) or compound heterozygous (28) mutations in the genes that are associated with arRP, and 11 (14 %, 11/79) families with hemizygous mutations in the genes that are associated with xlRP.

The clinical data of the 79 affected individuals with potential pathogenic mutations are listed in Table 2. These patients showed a similar variable retinal appearance. The fundus changes mainly showed a waxy pale optic disc, the attenuation of retinal arteries, and bone spicule pigment deposits in the mid-periphery of the retina. Some fundus photos of patients with mutations are listed in Fig. 3, including patients with novel mutations as well as reported mutations. There were a few clinically interesting cases. For instance, patient RP250 carried the variant c.4537dupC (p.Q1513Pfs*42) in *ABCA4* (MIM: 601691) which was previously described as a cause of Stargardt disease (Briggs et al. 2001), but he was diagnosed with RP in the current study based on his fundus examination (Fig. 3). Different mutations in the same gene might demonstrate significantly different fundus appearance, such as those patients with mutations in *RDH12* and *SNRNP200*, respectively (Fig. 3).

Other variants that were detected in the 157 patients are listed in Table S4 and were divided into three subgroups: (1) additional less likely pathogenic compound heterozygous variants in the arRP genes; (2) additional potential pathogenic variants with only one hit in the arRP genes; and (3) other less likely pathogenic variants. The reasons that these variations as other variants were classified and not mutations are explained in the footnote of Table S4.

Discussion

In terms of the initial screening of the exome sequencing and of the confirmation by Sanger sequencing, 110 putative pathogenic mutations in 28 of the 60 genes were identified in 50 % of the 157 families with RP, including 68 % (23/34) adRP, 44 % (12/27) arRP, 70 % (7/10) xlRP, and 43 % (37/86) sporadic RP.

The genes that most frequently harbored mutations compared to the other genes that are associated with autosomal dominant, autosomal recessive and X-linked RP were *RHO* (MIM: 180380, 6 %), *USH2A* (MIM: 608400, 9 %), and *RPGR* (MIM: 312610, 4 %) genes, respectively. The overall mutation frequency of these three genes (19 %) was less than that which was previously reported

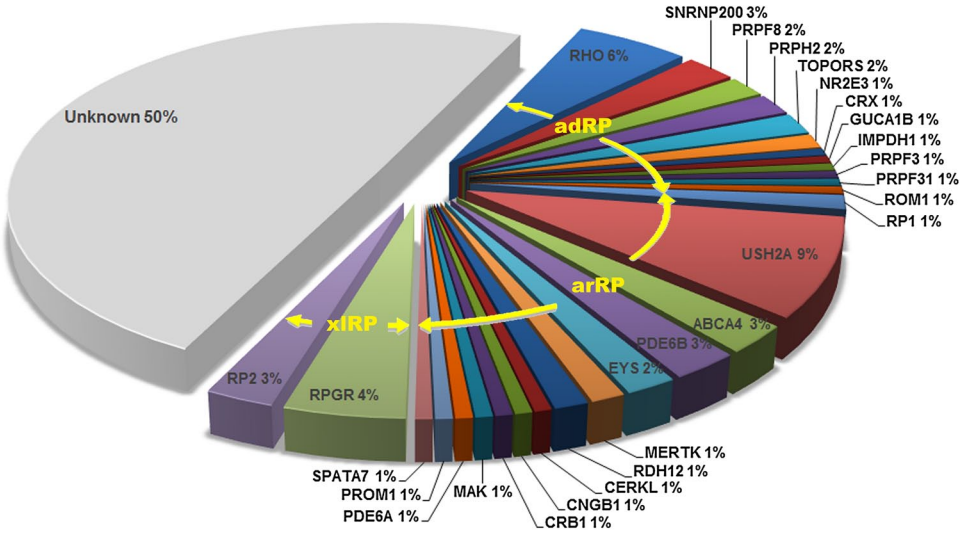

Fig. 1 Mutation proportions of individual genes in the 157 unrelated patients with RP

(Hartong et al. 2006). The frequency of the mutations that were detected in *RHO* (MIM: 180380) was higher than that in our previous study, wherein potential pathogenic mutations were detected only in six out of the 248 (2 %) Chinese families with RP by sequencing the coding exons (Li et al. 2010). In this study, seven of the nine probands with *RHO* (MIM: 180380) mutations were from the 34 adRP families, so that the frequency of the *RHO* (MIM: 180380) mutations in the adRP (21 %, 7/34) cases was significantly higher than that in another report of Asian adRP cohorts (Fujiki et al. 1995; Gandra et al. 2008), close to the frequency in Caucasians (16–29 %) (Dryja et al. 1991; Sullivan et al. 2006; Sung et al. 1991; Ziviello et al. 2005) and Mexicans (Matias-Florentino et al. 2009) considering the results of our previous study (Li et al. 2010) because families with previously identified mutations were excluded from the exome analysis. The mutation frequencies of *PRPH2* (MIM: 179605, 9 %, 3/34), *PRPF8* (MIM: 607300, 6 %, 2/34), *NR2E3* (MIM: 604485, 6 %, 2/34), and *TOPORS* (MIM: 609507, 6 %, 2/34) in adRP were also higher than those in other reports (Fahim et al. 1993; Ferrari et al. 2011; Hartong et al. 2006). The other adRP gene mutations were lower than those in previous studies (Sullivan et al. 2006), especially in *RP1* (MIM: 603937) and *IMPDH1* (MIM: 146690) where only one potential pathogenic mutation was identified. Mutations in *USH2A* (MIM: 608400) were involved in 12 % (14/113) of the arRP and sporadic cases, a greater frequency than those in North Americans (7 %) (Seyedahmadi et al. 2004) and Spaniards (7 %) (Avila-Fernandez et al. 2010). The mutation frequency of *RPGR* (MIM: 312610) and *RP2* (MIM: 300757) in X-linked RP patients (*RPGR*, 60 %; *RP2*, 10 %) was similar to that of other reports (Fahim et al. 1993; Ferrari et al. 2011; Hartong et al. 2006; Jin et al. 2006). However, the frequency of the mutations in *RPGR* (MIM: 312610) (4 %, 7/157) was significantly less than that previously reported (Hamel 2006), possibly because the families without identified mutations in our 2010 study (Ji et al. 2010) were recruited in the current study for further analysis by exome sequencing. Therefore, the frequency of mutations in *RPGR* (MIM: 312610) and *RP2* (MIM: 300757) may be underrepresented in the current study. Moreover, the inheritance pattern in the two families was wrongly assigned before the study as autosomal dominant and was later confirmed to be X-linked by the identification of the mutation. In some families that only had an affected mother and an affected son, such as RP044 and RP231 in our study, it is difficult to determine whether the inheritance pattern is adRP or xlRP. In these cases, molecular genetic testing can help to determine the actual mode of inheritance.

Apart from the causative mutations, some patients had additional heterozygous variants in other arRP genes. Patient QT770 had compound heterozygous variants in *CERKL* (MIM: 608381). He also had two variants in *CRB1* (MIM: 604210) but these two variants were in the same allele that was transmitted from his healthy father. His affected sister, with a phenotype that was similar to the proband, also had the same variants as those of the proband. So far, it is unclear whether the two variants in *CRB1* (MIM: 604210) would affect the phenotype of the *CERKL* (MIM: 608381) mutants, as we do not have any other families with *CERKL* (MIM: 608381) mutations alone for comparison. However, our previous study (Li et al. 2011) did not find an additive phenotypic effect of an additional third mutant allele. In the current study, these types of less likely pathogenic compound heterozygous variants were determined following the criteria that are described in the "Methods" section and are listed in Supplementary Table S4. These variants indicate that some sequence variants

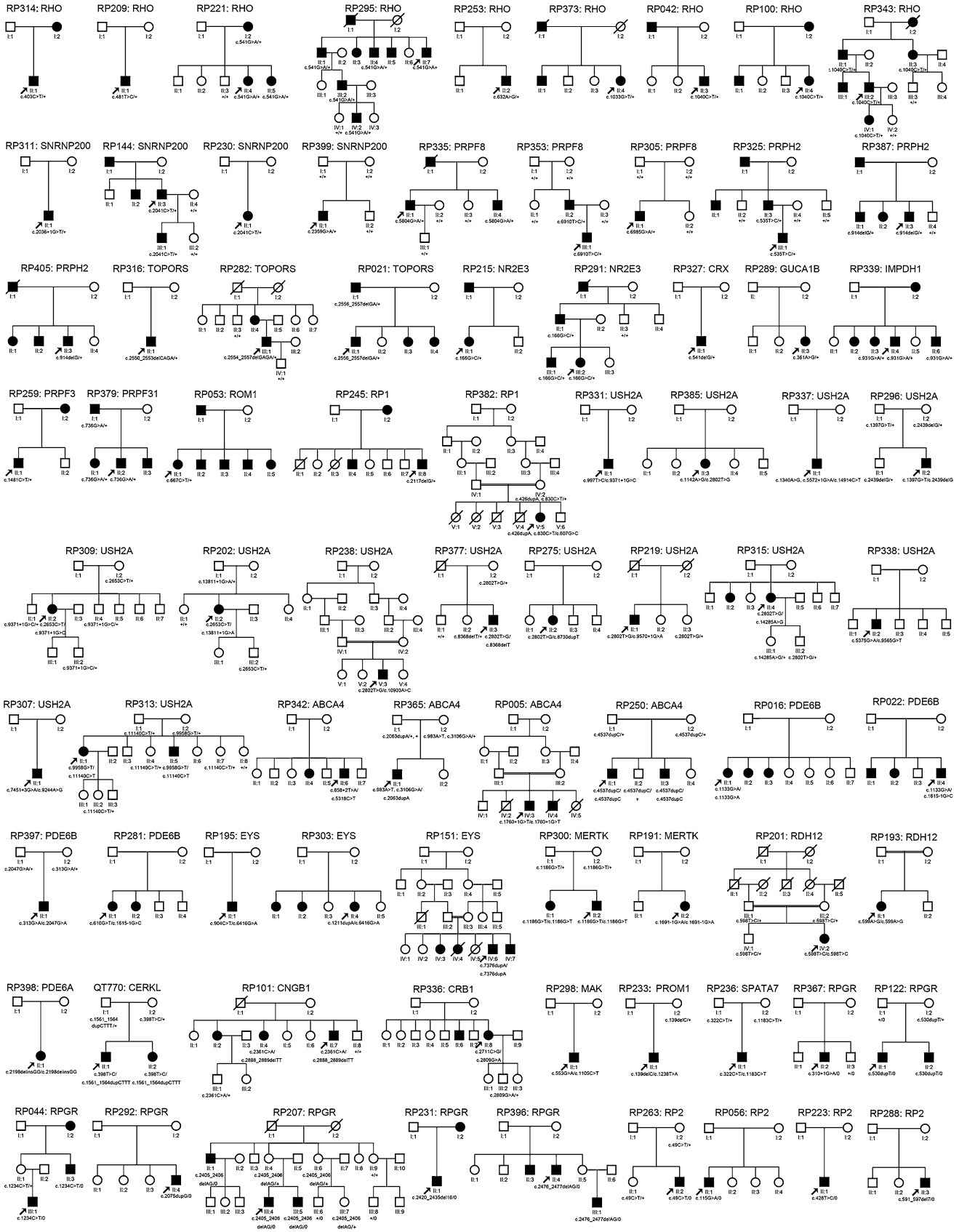

Fig. 2 Pedigrees of the 79 families with mutations. The family members and their corresponding mutations are shown just above the pedigrees (+: wild type allele)

Table 2 Clinical features of the 79 RP probands with mutations that were identified in this study

Patient ID	Gene	Variations	Inheritance	Gender	Age at		First	Visual acuity	Fundus	Electroretinography responses	
					Exam	Onset	Symptom	OD/OS	Examination	Rod	Cone
RP314	*RHO*	c.[403C>T];[=]	AD	M	28	EC	PV	0.2/0.05	ARA; PD	NA	NA
RP209	*RHO*	c.[481T>C];[=]	Sporadic	M	41	31	PV; NB	0.2/0.4	ARA; PBSL	NA	NA
RP221	*RHO*	c.[541G>A];[=]	AD	F	33	31	PV; NB	0.4/0.3	PBSL	Extinguished	Extinguished
RP295	*RHO*	c.[541G>A];[=]	AD	M	34	EC	PV	0.15/0.15	ARA; PBSL	NA	NA
RP253	*RHO*	c.[632A>G];[=]	Sporadic	F	9	NA	PV; NB	0.7/0.7	ARA; SP	Extinguished	Moderately reduced
RP373	*RHO*	c.[1033G>T];[=]	AD	F	45	12	NB	0.3/0.2	ARA; PBSL; NFR; TLR	NA	NA
RP042	*RHO*	c.[1040C>T];[=]	AD	M	26	9	PV; NB	0.2/0.1	ARA; TLR	Extinguished	Extinguished
RP100	*RHO*	c.[1040C>T];[=]	AD	F	24	NA	NB	0.2/0.3	ARA; PBSL	NA	NA
RP343	*RHO*	c.[1040C>T];[=]	AD	M	34	EC	PV	0.05/0.02	ARA; PBSL; NFR	Extinguished	Extinguished
RP311	*SNRNP200*	c.[2036+1G>T];[=]	Sporadic	M	31	26	PV; NB	1.0/FC	ARA; PD; MD	Severe reduced	Severe reduced
RP144	*SNRNP200*	c.[2041C>T];[=]	AD	M	5	EC	NA	NA	ARA; TLR	Extinguished	Moderately reduced
RP230	*SNRNP200*	c.[2041C>T];[=]	Sporadic	F	46	EC	PV	HM/0.05	ARA; PBSL; NFR	NA	NA
RP399	*SNRNP200*	c.[2359G>A];[=]	Sporadic	M	6	EC	PV	0.5/0.3	ARA; PD	NA	NA
RP335	*PRPF8*	c.[5804G>A];[=]	AD	M	33	EC	PV	HM/0.05	PD	Extinguished	Extinguished
RP353	*PRPF8*	c.[6910T>C];[=]	AD	M	5	EC	PV; NB	NA	ARA; PD	NA	NA
RP305	*PRPF8*	c.[6985G>A];[=]	Sporadic	M	24	EC	NB	0.1/0.07	ARA; PBSL	NA	NA
RP325	*PRPH2*	c.[535T>C];[=]	AD	M	22	15	PV; NB	0.6/0.7	ARA; PBSL; SP	Extinguished	Extinguished
RP387	*PRPH2*	c.[914delG];[=]	AD	M	34	EC	PV	0.7/0.8	ARA; PD; NFR	NA	NA
RP405	*PRPH2*	c.[914delG];[=]	AD	M	58	55	PV	0.2/0.2	ARA; PD	NA	NA
RP316	*TOPORS*	c.[2550_2553delCAGA];[=]	Sporadic	M	19	11	NB	0.6/0.7	PBSL	NA	NA
RP282	*TOPORS*	c.[2554_2557delGAGA];[=]	AD	M	30	18	PV; NB	0.5/0.4	ARA; PBSL	NA	NA
RP021	*TOPORS*	c.[2556_2557delGA];[=]	AD	M	39	20	NB	0.5/0.6	ARA; PD; NFR	NA	NA
RP215	*NR2E3*	c.[166G>C];[=]	AD	F	41	NA	NB	0.6/0.4	PBSL	Extinguished	Extinguished
RP291	*NR2E3*	c.[166G>C];[=]	AD	F	18	EC	PV	0.6/0.01	ARA; PBSL; NFR	Extinguished	Severe reduced
RP327	*CRX*	c.[541delG];[=]	AD	M	21	EC	PV	NLP/NLP	ARA; PD	Extinguished	Extinguished
RP289	*GUCA1B*	c.[361A>G];[=]	Sporadic	F	28	13	PV	0.1/0.2	ARA	Extinguished	Extinguished
RP339	*IMPDH1*	c.[931G>A];[=]	AD	M	22	NA	PV	HM/HM	ARA; PBSL	NA	NA
RP259	*PRPF3*	c.[1481C>T];[=]	AD	M	18	5	NB	0.4/0.6	ARA; PBSL; SP	NA	NA
RP379	*PRPF31*	c.[736G>A];[=]	AD	M	65	64	PV; NB	HM/0.2	ARA; PD; NFR	NA	NA
RP053	*ROM1*	c.[667C>T];[=]	AD	F	33	12	PV; NB	0.5/0.6	ARA; PBSL; NFR; TLR	NA	NA
RP245	*RP1*	c.[2117delG];[=]	AD	M	60	NA	NA	NA	NA	NA	NA

Table 2 continued

Patient ID	Gene	Variations	Inheritance	Gender	Age at		First	Visual acuity	Fundus	Electroretinography responses	
					Exam	Onset	Symptom	OD/OS	Examination	Rod	Cone
RP382	*RP1*	c.[426dupA;830C>T];[607G>C]	Sporadic/ Cons	F	15	5	PV; NB	NLP/0.3	ARA; PBSL	NA	NA
RP331	*USH2A*	c.[997T>C(;)9371+1G>C]	Sporadic	M	43	33	PV; NB	0.9/0.8	ARA; PBSL	Extinguished	Extinguished
RP385	*USH2A*	c.[1142A>G(;)2802T>G]	Sporadic	F	34	16	PV; NB	0.4/0.4	ARA; PBSL; TLR	NA	NA
RP337	*USH2A*	c.[1340A>(;)5572+1G>A(;)14914C>T]	Sporadic	M	24	12	NB	0.5/0.7	ARA; PD	Extinguished	Extinguished
RP296	*USH2A*	c.[1397G>T];[2439delG]	AR	M	26	11	NB	0.9/0.9	ARA; PD	Extinguished	Extinguished
RP309	*USH2A*	c.[2653C>T];[9371+1G>C]	Sporadic	F	53	25	PV; NB	0.6/0.7	ARA; PBSL	NA	NA
RP202	*USH2A*	c.[2653C>T];[13811+1G>A]	Sporadic	F	35	22	PV; NB; PA	0.3/0.2	ARA, PBSL	Extinguished	Extinguished
RP238	*USH2A*	c.[2802T>G(;)10903A>C]	Sporadic/ Cons	M	37	31	NB; PA	0.6/0.3	ARA; PBSL	NA	NA
RP377	*USH2A*	c.[2802T>G];[8368delT]	Sporadic	M	33	17	PV; NB	HM/0.1	ARA; PBSL; TLR	Extinguished	Extinguished
RP275	*USH2A*	c.[2802T>G(;)8730dupT]	Sporadic	F	23	16	PV; NB; CB	0.9/0.9	ARA	Extinguished	Extinguished
RP219	*USH2A*	c.[2802T>G[;]9570+1G>A]	Sporadic	M	46	40	PV; NB	0.6/0.6	PBSL; NFR	NA	NA
RP315	*USH2A*	c.[2802T>G];[14285A>G]	Sporadic	F	54	40	PV; NB	FC/FC	ARA; PBSL	Extinguished	Extinguished
RP338	*USH2A*	c.[5375G>A(;)9565G>T]	Sporadic	M	26	EC	PV	HM/1.5	ARA; PBSL	NA	NA
RP307	*USH2A*	c.[7451+3G>A(;)9244A>G]	Sporadic	M	12	EC	NB	1.0/1.0	ARA; PBSL	Extinguished	Extinguished
RP313	*USH2A*	c.[9958G>T];[11140C>T]	AR	F	45	30	PV; NB	0.4/0.7	ARA; PBSL; SP	Extinguished	Extinguished
RP342	*ABCA4*	c.[858+2T>A(;)5318C>T]	AR	M	19	13	PV	0.1/0.1	ARA; NFR;	NA	NA
RP365	*ABCA4*	c.[983A>T;3106G>A];[2063dupA]	Sporadic	M	7	7	PV	0.07/0.05	ARA; TLR	Extinguished	Extinguished
RP005	*ABCA4*	c.[1760+1G>T];[1760+1G>T]	AR/Cons	M	46	43	PV	0.2/0.01	ARA; PBSL; NFR	NA	NA
RP250	*ABCA4*	c.[4537dupC];[4537dupC]	AR	M	19	12	PV	0.04/0.03	ARA; MD	Extinguished	Severe reduced
RP016	*PDE6B*	c.[1133G>A];[1133G>A]	AR	F	28	EC	PV	0.1/0.1	ARA; PBSL	NA	NA
RP022	*PDE6B*	c.[1133G>A(;)1615−1G>C]	AR	M	38	7	PV; NB	0.5/0.3	ARA; PBSL; NFR	NA	NA
RP397	*PDE6B*	c.[313G>A];[2047G>A]	Sporadic	M	6	EC	PV; NB	0.6/0.3	ARA; PBSL	Extinguished	Moderately reduced
RP281	*PDE6B*	c.[610G>T(;)1615−1G>C]	Sporadic	F	16	6	PV; NB	0.8/0.9	PD	NA	NA
RP195	*EYS*	c.[904C>T(;)6416G>A]	Sporadic	M	NA	EC	PV	0.2/0.2	ARA; PBSL	NA	NA
RP303	*EYS*	c.[1211dupA(;)6416G>A]	Sporadic	F	43	43	PV	0.8/0.7	ARA; PD	NA	NA
RP151	*EYS*	c.[7376dupA];[7376dupA]	AR/Cons	M	30	13	PV; NB	0.3/0.4	ARA; PD; NFR	NA	NA
RP300	*MERTK*	c.[1186G>T];[1186G>T]	AR	M	27	24	PV; NB	HM/HM	ARA; PD; NFR	Extinguished	Extinguished
RP191	*MERTK*	c.[1691−1G>A];[1691−1G>A]	Sporadic	F	25	15	NB	0.1/0.2	TLR	Extinguished	Extinguished
RP201	*RDH12*	c.[598T>C];[598T>C]	Sporadic/ Cons	F	29	15	PV; NB	0.08/0.08	ARA; PBSL	Extinguished	Extinguished

Table 2 continued

Patient ID	Gene	Variations	Inheritance	Gender	Age at		First	Visual acuity	Fundus	Electroretinography responses	
					Exam	Onset	Symptom	OD/OS	Examination	Rod	Cone
RP193	*RDH12*	c.[599A>G];[599A>G]	Sporadic/ Cons	F	25	22	PV; NB	0.04/0.3	ARA; PBSL; NFR	Severe reduced	Severe reduced
RP398	*PDE6A*	c.[2198delinsGG];[2198delinsGG]	Sporadic	F	23	EC	PV	1.0/1.2	PBSL	NA	NA
QT770	*CERKL*	c.[398T>C];[1507_1510dupCTTT]	AR	M	15	11	PV; NB	0.01/0.05	ARA; PD	Extinguished	Extinguished
RP101	*CNGB1*	c.[2361C>A];[2888_2889delTT]	AR	M	34	NA	PV; NB	0.5/0.4	PBSL	Extinguished	Extinguished
RP336	*CRB1*	c.[2711C>G(;)2809G>A]	Sporadic	F	44	41	PV; NB	0.7/FC	ARA; PD; NFR	NA	NA
RP298	*MAK*	c.[553G>A(;)1105C>T]	Sporadic	M	33	28	PV; NB	1.0/0.03	ARA; PBSL; NFR; MD	Extinguished	Severe reduced
RP233	*PROM1*	c.[139delC];[1238T>A]	AR	M	20	15	PV; NB; CB	0.06/0.04	NFR	Extinguished	Severe reduced
RP236	*SPATA7*	c.[322C>T];[1183C>T]	Sporadic	M	3	EC	PV; NB; PA	0.4/0.6	ARA; PD	Extinguished	Extinguished
RP367	*RPGR*	c.[310+1G>A];[0]	Sporadic	M	20	EC	PV	0.1/0.08	ARA; PBSL	Extinguished	Extinguished
RP122	*RPGR*	c.[530dupT];[0]	XL	M	5	5	PV; NB	0.1/0.2	ARA; PD	Extinguished	Extinguished
RP044	*RPGR*	c.[1234C>T];[0]	XL	M	10	EC	NB	0.4/0.3	ARA; PD	NA	NA
RP292	*RPGR*	c.[2072_2073insG];[0]	XL	M	26	EC	PV	0.6/0.6	ARA; PBSL	Extinguished	Extinguished
RP207	*RPGR*	c.[2405_2406delAG];[0]	XL	M	27	EC	PV	0.3/0.2	ARA; PBSL	Extinguished	Extinguished
RP231	*RPGR*	c.[2420_2435del];[0]	XL	M	22	EC	NB	0.3/0.3	ARA; PBSL	NA	NA
RP396	*RPGR*	c.[2476_2477delAG];[0]	XL	M	40	NA	PV; NB	0.2/0.1	ARA; PD	NA	NA
RP263	*RP2*	c.[49C>T];[0]	Sporadic	M	22	EC	PV; NB	0.7/0.7	ARA; PD	Extinguished	Extinguished
RP056	*RP2*	c.[115G>A];[0]	Sporadic	M	34	6	PV; NB	0.1/0.1	ARA; PBSL; NFR	NA	NA
RP223	*RP2*	c.[428T>C];[0]	Sporadic	M	34	24	PV	0.9/1.0	PBSL	Extinguished	Severe reduced
RP288	*RP2*	c.[591_597delCTATGTT];[0]	XL	M	23	NA	NA	NA	ARA; PD	NA	NA

Cons consanguineous marriage of parents, *AR* autosomal recessive, *AD* autosomal dominant, *XL* X-linked, *M* male, *F* female, *EC* early childhood, *NA* not available, *OD* right eye, *OS* left eye, *NLP* no light perception, *FC* finger counting, *HM* hand movement, *PV* poor vision, *NB* night blindness, *PA* photoaversion, *CB* color blindness, *ARA* attenuated retinal arteries, *PD* pigment deposit, *PBSL* pigment bone spicule-like, *SP* salt-and-pepper like retinal degeneration, *NFR* no foveal reflex, *MD* macular degeneration, *TLR* tapetal-like retinal degeneration

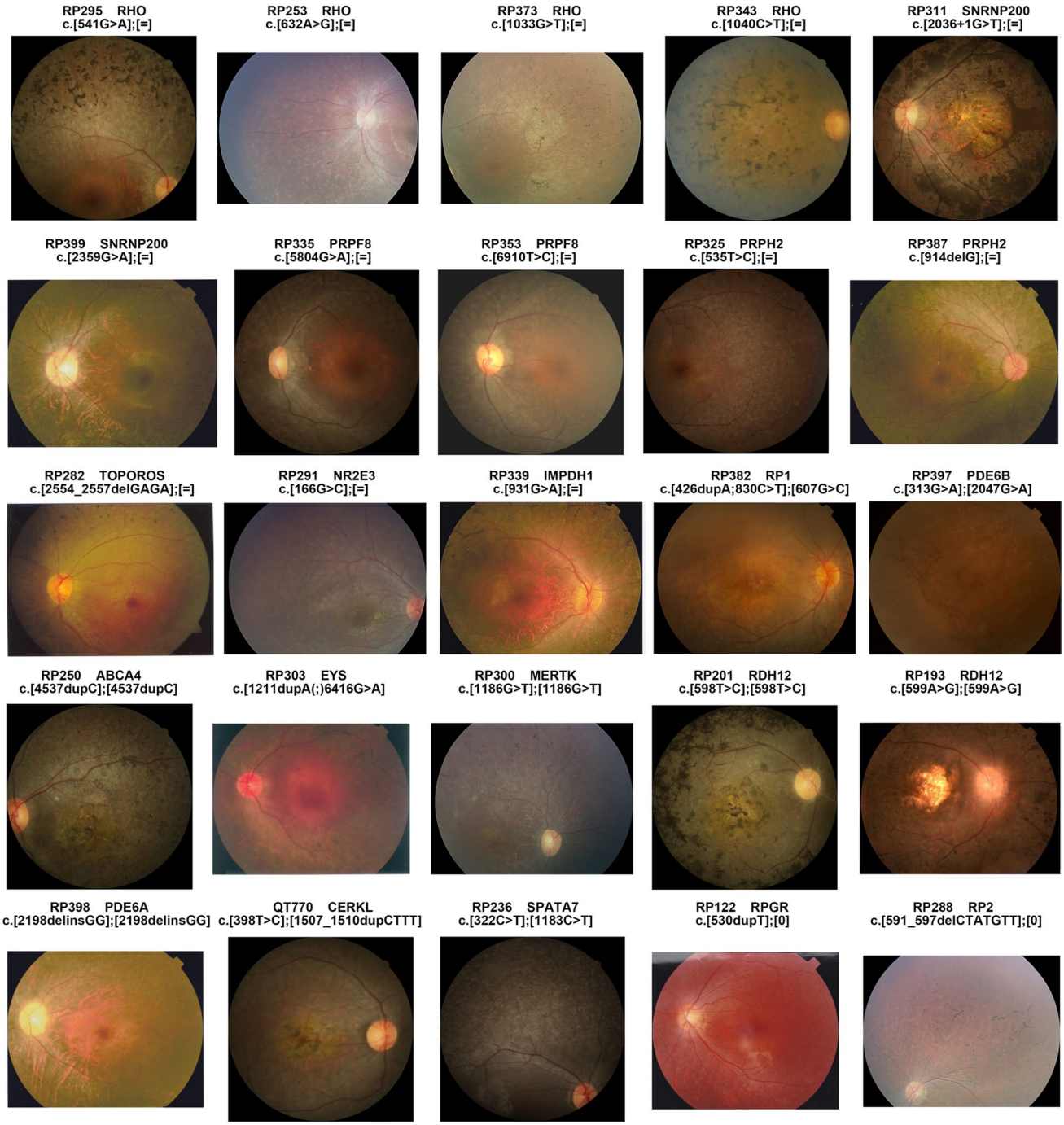

Fig. 3 Fundus photos of patients from 25 families with the mutations that were identified in this study. The corresponding patients' identification numbers and gene mutations are listed above each photo. Further clinical information of these patients is listed in Table 2

might mistakenly be considered as causative mutations for RP if only a single individual gene was analyzed.

The variants with only one hit in the arRP genes were also detected in this study. These variants might be causative in either a homozygous or compound heterozygous status. Furthermore, considering the limitations of whole exome sequencing, it is still possible that these cases might carry other pathogenic variants in the noncoding region of the corresponding gene and deletion involving whole exons. In addition, it is possible that mutations in some genes that are associated with other retinal diseases, such as Leber congenital amaurosis (LCA), might contribute to RP. Mutations in the syndromic RP genes may be associated with RP without noticeable systemic manifestation. These

views may be the reasons why mutations were not detected in the exons of the reported RP genes in the remaining 78 probands.

This study demonstrates that next-generation sequencing can be an effective tool for determining pathogenic mutation in RP patients. The novel mutations that were identified in the 157 RP probands were in the majority (67 %, 74/110) and could not be detected by an RP mutation chip (Kim et al. 2012). The mutation detection rate by exome sequencing was significantly higher than that obtained by microarray genotyping technology (Avila-Fernandez et al. 2010; Blanco-Kelly et al. 2012). Compared to the recently published Next generation sequencing (NGS)-based methods, the NimbleGen SeqCap EZ Exome (44M; Roche, Basil, Switzerland) array has a higher target exons coverage rate and mutation detection rate in nonsyndromic RP genes (Audo et al. 2012; Neveling et al. 2012; O'Sullivan et al. 2012).

In conclusion, pathogenic mutations in the 60 genes that are known to be associated with RP were detected by exome sequencing in approximately half of a cohort of Chinese families with RP. A comprehensive analysis of the mutation spectrum and frequency in the 60 genes not only expanded the mutation spectrum and frequency of the 60 genes in the Chinese patients with RP, but also provided an overview of the molecular etiology of RP in Chinese patients. The frequency analysis of the mutations and genes that are responsible for retinitis pigmentosa in this population can serve as a reference for researchers who are investigating the mutation spectrum and frequency of RP genes in the other populations and provide a guide for rapid and efficient molecular diagnostic approaches. The method of filtering and evaluating exome variants in our study, frequency analysis based on incidence, computational predictions, and cosegregation analysis can also provide researchers a simple way to target candidate pathogenic variants. Rare variants that are unlikely associated with RP may provide a useful reference for future studies in identifying genetic defects in this disease. The remaining information of those 78 patients without identified mutations is a valuable source for searching new genes that are responsible for RP. The clinical data in our manuscript provide valuable information not only for this study but also for future studies in understanding the relationships between genotypes and phenotypes. These data may not currently determine a firm genotype–phenotype correlation but they will be valuable toward the final goal.

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References

Audo I, Sahel JA, Mohand-Said S, Lancelot ME, Antonio A, Moskova-Doumanova V, Nandrot EF, Doumanov J, Barragan I, Antinolo G, Bhattacharya SS, Zeitz C (2010) EYS is a major gene for rod-cone dystrophies in France. Hum Mutat 31:E1406–E1435. doi:10.1002/humu.21249

Audo I, Bujakowska KM, Leveillard T, Mohand-Said S, Lancelot ME, Germain A, Antonio A, Michiels C, Saraiva JP, Letexier M, Sahel JA, Bhattacharya SS, Zeitz C (2012) Development and application of a next-generation-sequencing (NGS) approach to detect known and novel gene defects underlying retinal diseases. Orphanet J Rare Dis 7:8. doi:10.1186/1750-1172-7-8

Avila-Fernandez A, Cantalapiedra D, Aller E, Vallespin E, Aguirre-Lamban J, Blanco-Kelly F, Corton M, Riveiro-Alvarez R, Allikmets R, Trujillo-Tiebas MJ, Millan JM, Cremers FP, Ayuso C (2010) Mutation analysis of 272 Spanish families affected by autosomal recessive retinitis pigmentosa using a genotyping microarray. Mol Vis 16:2550–2558

Bader I, Brandau O, Achatz H, Apfelstedt-Sylla E, Hergersberg M, Lorenz B, Wissinger B, Wittwer B, Rudolph G, Meindl A, Meitinger T (2003) X-linked retinitis pigmentosa: RPGR mutations in most families with definite X linkage and clustering of mutations in a short sequence stretch of exon ORF15. Invest Ophthalmol Vis Sci 44:1458–1463

Bandah-Rozenfeld D, Littink KW, Ben-Yosef T, Strom TM, Chowers I, Collin RW, den Hollander AI, van den Born LI, Zonneveld MN, Merin S, Banin E, Cremers FP, Sharon D (2010) Novel null mutations in the EYS gene are a frequent cause of autosomal recessive retinitis pigmentosa in the Israeli population. Invest Ophthalmol Vis Sci 51:4387–4394. doi:10.1167/iovs.09-4732

Bareil C, Delague V, Arnaud B, Demaille J, Hamel C, Claustres M (2000) W179R: a novel missense mutation in the peripherin/RDS gene in a family with autosomal dominant retinitis pigmentosa. Hum Mutat 15:583–584. doi:10.1002/1098-1004(200006)15:6<583:AID-HUMU24>3.0.CO;2-X

Beales PL, Badano JL, Ross AJ, Ansley SJ, Hoskins BE, Kirsten B, Mein CA, Froguel P, Scambler PJ, Lewis RA, Lupski JR, Katsanis N (2003) Genetic interaction of BBS1 mutations with alleles at other BBS loci can result in non-Mendelian Bardet-Biedl syndrome. Am J Hum Genet 72:1187–1199. doi:10.1086/375178

Benaglio P, McGee TL, Capelli LP, Harper S, Berson EL, Rivolta C (2011) Next generation sequencing of pooled samples reveals new SNRNP200 mutations associated with retinitis pigmentosa. Hum Mutat 32:E2246–E2258. doi:10.1002/humu.21485

Berger W, Kloeckener-Gruissem B, Neidhardt J (2010) The molecular basis of human retinal and vitreoretinal diseases. Prog Retin Eye Res 29:335–375. doi:10.1016/j.preteyeres.2010.03.004

Bernal S, Ayuso C, Antinolo G, Gimenez A, Borrego S, Trujillo MJ, Marcos I, Calaf M, Del Rio E, Baiget M (2003) Mutations in USH2A in Spanish patients with autosomal recessive retinitis pigmentosa: high prevalence and phenotypic variation. J Med Genet 40:e8

Berson EL (1987) Electroretinographic findings in retinitis pigmentosa. Jpn J Ophthalmol 31:327–348

Bischof JM, Chiang AP, Scheetz TE, Stone EM, Casavant TL, Sheffield VC, Braun TA (2006) Genome-wide identification of pseudogenes capable of disease-causing gene conversion. Hum Mutat 27:545–552. doi:10.1002/humu.20335

Blanco-Kelly F, Garcia-Hoyos M, Corton M, Avila-Fernandez A, Riveiro-Alvarez R, Gimenez A, Hernan I, Carballo M, Ayuso C (2012) Genotyping microarray: mutation screening in Spanish

families with autosomal dominant retinitis pigmentosa. Mol Vis 18:1478–1483

Boughman JA, Conneally PM, Nance WE (1980) Population genetic studies of retinitis pigmentosa. Am J Hum Genet 32:223–235

Bowne SJ, Sullivan LS, Blanton SH, Cepko CL, Blackshaw S, Birch DG, Hughbanks-Wheaton D, Heckenlively JR, Daiger SP (2002) Mutations in the inosine monophosphate dehydrogenase 1 gene (IMPDH1) cause the RP10 form of autosomal dominant retinitis pigmentosa. Hum Mol Genet 11:559–568

Breuer DK, Yashar BM, Filippova E, Hiriyanna S, Lyons RH, Mears AJ, Asaye B, Acar C, Vervoort R, Wright AF, Musarella MA, Wheeler P, MacDonald I, Iannaccone A, Birch D, Hoffman DR, Fishman GA, Heckenlively JR, Jacobson SG, Sieving PA, Swaroop A (2002) A comprehensive mutation analysis of RP2 and RPGR in a North American cohort of families with X-linked retinitis pigmentosa. Am J Hum Genet 70:1545–1554. doi:10.1086/340848

Briggs CE, Rucinski D, Rosenfeld PJ, Hirose T, Berson EL, Dryja TP (2001) Mutations in ABCR (ABCA4) in patients with Stargardt macular degeneration or cone-rod degeneration. Invest Ophthalmol Vis Sci 42:2229–2236

Bunker CH, Berson EL, Bromley WC, Hayes RP, Roderick TH (1984) Prevalence of retinitis pigmentosa in Maine. Am J Ophthalmol 97:357–365

Buraczynska M, Wu W, Fujita R, Buraczynska K, Phelps E, Andreasson S, Bennett J, Birch DG, Fishman GA, Hoffman DR, Inana G, Jacobson SG, Musarella MA, Sieving PA, Swaroop A (1997) Spectrum of mutations in the RPGR gene that are identified in 20% of families with X-linked retinitis pigmentosa. Am J Hum Genet 61:1287–1292. doi:10.1086/301646

Chakarova CF, Hims MM, Bolz H, Abu-Safieh L, Patel RJ, Papaioannou MG, Inglehearn CF, Keen TJ, Willis C, Moore AT, Rosenberg T, Webster AR, Bird AC, Gal A, Hunt D, Vithana EN, Bhattacharya SS (2002) Mutations in HPRP3, a third member of pre-mRNA splicing factor genes, implicated in autosomal dominant retinitis pigmentosa. Hum Mol Genet 11:87–92

Chakarova CF, Papaioannou MG, Khanna H, Lopez I, Waseem N, Shah A, Theis T, Friedman J, Maubaret C, Bujakowska K, Veraitch B, Abd El-Aziz MM, de Prescott Q, Parapuram SK, Bickmore WA, Munro PM, Gal A, Hamel CP, Marigo V, Ponting CP, Wissinger B, Zrenner E, Matter K, Swaroop A, Koenekoop RK, Bhattacharya SS (2007) Mutations in TOPORS cause autosomal dominant retinitis pigmentosa with perivascular retinal pigment epithelium atrophy. Am J Hum Genet 81:1098–1103. doi:10.1086/521953

Chen Y, Zhang Q, Shen T, Xiao X, Li S, Guan L, Zhang J, Zhu Z, Yin Y, Wang P, Guo X, Wang J, Zhang Q (2013) Comprehensive mutation analysis by whole-exome sequencing in 41 Chinese families with Leber congenital amaurosis. Invest Ophthalmol Vis Sci 54(6):4351–4357. doi:10.1167/iovs.13-11606

Chizzolini M, Galan A, Milan E, Sebastiani A, Costagliola C, Parmeggiani F (2011) Good epidemiologic practice in retinitis pigmentosa: from phenotyping to biobanking. Curr Genomics 12:260–266. doi:10.2174/138920211795860071

Daiger SP (2004) Identifying retinal disease genes: how far have we come, how far do we have to go? Novartis Found Symp 255:17–27; discussion 27–36, 177–178

Daiger SP, Bowne SJ, Sullivan LS (2007) Perspective on genes and mutations causing retinitis pigmentosa. Arch Ophthalmol 125:151–158. doi:10.1001/archopht.125.2.151

Davies WI, Downes SM, Fu JK, Shanks ME, Copley RR, Lise S, Ramsden SC, Black GC, Gibson K, Foster RG, Hankins MW, Nemeth AH (2012) Next-generation sequencing in health-care delivery: lessons from the functional analysis of rhodopsin. Genet Med 14:891–899. doi:10.1038/gim.2012.73

den Hollander AI, Black A, Bennett J, Cremers FP (2010) Lighting a candle in the dark: advances in genetics and gene therapy of recessive retinal dystrophies. J Clin Invest 120:3042–3053. doi:10.1172/JCI42258

Dryja TP, McGee TL, Hahn LB, Cowley GS, Olsson JE, Reichel E, Sandberg MA, Berson EL (1990) Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. N Engl J Med 323:1302–1307. doi:10.1056/NEJM199011083231903

Dryja TP, Hahn LB, Cowley GS, McGee TL, Berson EL (1991) Mutation spectrum of the rhodopsin gene among patients with autosomal dominant retinitis pigmentosa. Proc Natl Acad Sci U S A 88:9370–9374

Fahim AT, Daiger SP, Weleber RG (1993) Retinitis Pigmentosa Overview. In: Pagon RA, Adam MP, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K (eds) GeneReviews, Seattle (WA)

Ferrari S, Di Iorio E, Barbaro V, Ponzin D, Sorrentino FS, Parmeggiani F (2011) Retinitis pigmentosa: genes and disease mechanisms. Curr Genomics 12:238–249. doi:10.2174/138920211795860107

Fujiki K, Hotta Y, Murakami A, Yoshii M, Hayakawa M, Ichikawa T, Takeda M, Akeo K, Okisaka S, Kanai A (1995) Missense mutation of rhodopsin gene codon 15 found in Japanese autosomal dominant retinitis pigmentosa. Jpn J Hum Genet 40:271–277. doi:10.1007/BF01876186

Gandra M, Anandula V, Authiappan V, Sundaramurthy S, Raman R, Bhattacharya S, Govindasamy K (2008) Retinitis pigmentosa: mutation analysis of RHO, PRPF31, RP1, and IMPDH1 genes in patients from India. Mol Vis 14:1105–1113

Gardner JC, Webb TR, Kanuga N, Robson AG, Holder GE, Stockman A, Ripamonti C, Ebenezer ND, Ogun O, Devery S, Wright GA, Maher ER, Cheetham ME, Moore AT, Michaelides M, Hardcastle AJ (2010) X-linked cone dystrophy caused by mutation of the red and green cone opsins. Am J Hum Genet 87:26–39. doi:10.1016/j.ajhg.2010.05.019

Goldberg AF, Molday RS (1996) Defective subunit assembly underlies a digenic form of retinitis pigmentosa linked to mutations in peripherin/rds and rom-1. Proc Natl Acad Sci USA 93:13726–13730

Grondahl J (1987) Estimation of prognosis and prevalence of retinitis pigmentosa and Usher syndrome in Norway. Clin Genet 31:255–264

Hamel C (2006) Retinitis pigmentosa. Orphanet J Rare Dis 1:40. doi:10.1186/1750-1172-1-40

Hartong DT, Berson EL, Dryja TP (2006) Retinitis pigmentosa. Lancet 368:1795–1809. doi:10.1016/S0140-6736(06)69740-7

Hu DN (1982) Genetic aspects of retinitis pigmentosa in China. Am J Med Genet 12:51–56. doi:10.1002/ajmg.1320120107

Huang L, Zhang Q, Li S, Guan L, Xiao X, Zhang J, Jia X, Sun W, Zhu Z, Gao Y, Yin Y, Wang P, Guo X, Wang J, Zhang Q (2013) Exome sequencing of 47 chinese families with cone-rod dystrophy: mutations in 25 known causative genes. PLoS ONE 8(6):e65546. doi:10.1371/journal.pone.0065546

Jacobson SG, Voigt WJ, Parel JM, Apathy PP, Nghiem-Phu L, Myers SW, Patella VM (1986) Automated light- and dark-adapted perimetry for evaluating retinitis pigmentosa. Ophthalmology 93:1604–1611

Ji Y, Wang J, Xiao X, Li S, Guo X, Zhang Q (2010) Mutations in RPGR and RP2 of Chinese patients with X-linked retinitis pigmentosa. Curr Eye Res 35:73–79. doi:10.3109/02713680903395299

Jin ZB, Liu XQ, Hayakawa M, Murakami A, Nao-i N (2006) Mutational analysis of RPGR and RP2 genes in Japanese patients with retinitis pigmentosa: identification of four mutations. Mol Vis 12:1167–1174

Jin ZB, Mandai M, Homma K, Ishigami C, Hirami Y, Nao-I N, Takahashi M (2008) Alleliccopy number variation in FSCN2 detected using allele-specific genotyping and multiplex real-time PCRs. Invest Ophthalmol Vis Sci 49(9):3799–3805. doi:10.1167/iovs.07-1656

Kajiwara K, Berson EL, Dryja TP (1994) Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. Science 264:1604–1608

Katsanis N, Ansley SJ, Badano JL, Eichers ER, Lewis RA, Hoskins BE, Scambler PJ, Davidson WS, Beales PL, Lupski JR (2001) Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. Science 293:2256–2259. doi:10.1126/science.1063525

Khan MI, Kersten FF, Azam M, Collin RW, Hussain A, Shah ST, Keunen JE, Kremer H, Cremers FP, Qamar R, den Hollander AI (2011) CLRN1 mutations cause nonsyndromic retinitis pigmentosa. Ophthalmology 118:1444–1448. doi:10.1016/j.ophtha.2010.10.047

Kim C, Kim KJ, Bok J, Lee EJ, Kim DJ, Oh JH, Park SP, Shin JY, Lee JY, Yu HG (2012) Microarray-based mutation detection and phenotypic characterization in Korean patients with retinitis pigmentosa. Mol Vis 18:2398–2410

Le Quesne Stabej P, Saihan Z, Rangesh N, Steele-Stallard HB, Ambrose J, Coffey A, Emmerson J, Haralambous E, Hughes Y, Steel KP, Luxon LM, Webster AR, Bitner-Glindzicz M (2012) Comprehensive sequence analysis of nine Usher syndrome genes in the UK National Collaborative Usher Study. J Med Genet 49:27–36. doi:10.1136/jmedgenet-2011-100468

Li R, Li Y, Kristiansen K, Wang J (2008) SOAP: short oligonucleotide alignment program. Bioinformatics 24:713–714. doi:10.1093/bioinformatics/btn025

Li R, Li Y, Fang X, Yang H, Wang J, Kristiansen K, Wang J (2009a) SNP detection for massively parallel whole-genome resequencing. Genome Res 19:1124–1132. doi:10.1101/gr.088013.108

Li R, Yu C, Li Y, Lam TW, Yiu SM, Kristiansen K, Wang J (2009b) SOAP2: an improved ultrafast tool for short read alignment. Bioinformatics 25:1966–1967. doi:10.1093/bioinformatics/btp336

Li S, Xiao X, Wang P, Guo X, Zhang Q (2010) Mutation spectrum and frequency of the RHO gene in 248 Chinese families with retinitis pigmentosa. Biochem Biophys Res Commun 401:42–47. doi:10.1016/j.bbrc.2010.09.004

Li L, Xiao X, Li S, Jiao X, Hejtmancik JF, Zhang Q (2011) Lack of phenotypic effect of triallelic variation in SPATA7 in a family with Leber congenital amaurosis resulting from CRB1 mutations. Mol Vis 17:3326–3332

Macke JP, Davenport CM, Jacobson SG, Hennessey JC, Gonzalez-Fernandez F, Conway BP, Heckenlively J, Palmer R, Maumenee IH, Sieving P et al (1993) Identification of novel rhodopsin mutations responsible for retinitis pigmentosa: implications for the structure and function of rhodopsin. Am J Hum Genet 53:80–89

Matias-Florentino M, Ayala-Ramirez R, Graue-Wiechers F, Zenteno JC (2009) Molecular screening of rhodopsin and peripherin/RDS genes in Mexican families with autosomal dominant retinitis pigmentosa. Curr Eye Res 34:1050–1056. doi:10.3109/02713680903283169

Maubaret C, Hamel C (2005) Genetics of retinitis pigmentosa: metabolic classification and phenotype/genotype correlations. J Fr Ophtalmol 28:71–92

Mokarzel-Falcon L, Padron-Garcia JA, Carrasco-Velar R, Berry C, Montero-Cabrera LA (2008) In silico study of the human rhodopsin and meta rhodopsin II/S-arrestin complexes: impact of single point mutations related to retina degenerative diseases. Proteins 70:1133–1141. doi:10.1002/prot.21873

Nasonkin I, Illing M, Koehler MR, Schmid M, Molday RS, Weber BH (1998) Mapping of the rod photoreceptor ABC transporter (ABCR) to 1p21-p22.1 and identification of novel mutations in Stargardt's disease. Hum Genet 102:21–26

Neveling K, Collin RW, Gilissen C, van Huet RA, Visser L, Kwint MP, Gijsen SJ, Zonneveld MN, Wieskamp N, de Ligt J, Siemiatkowska AM, Hoefsloot LH, Buckley MF, Kellner U, Branham KE, den Hollander AI, Hoischen A, Hoyng C, Klevering BJ, van den Born LI, Veltman JA, Cremers FP, Scheffer H (2012) Next-generation genetic testing for retinitis pigmentosa. Hum Mutat 33:963–972. doi:10.1002/humu.22045

O'Sullivan J, Mullaney BG, Bhaskar SS, Dickerson JE, Hall G, O'Grady A, Webster A, Ramsden SC, Black GC (2012) A paradigm shift in the delivery of services for diagnosis of inherited retinal disease. J Med Genet 49:322–326. doi:10.1136/jmedgenet-2012-100847

Rakoczy EP, Kiel C, McKeone R, Stricher F, Serrano L (2011) Analysis of disease-linked rhodopsin mutations based on structure, function, and protein stability calculations. J Mol Biol 405:584–606. doi:10.1016/j.jmb.2010.11.003

Riazuddin SA, Iqbal M, Wang Y, Masuda T, Chen Y, Bowne S, Sullivan LS, Waseem NH, Bhattacharya S, Daiger SP, Zhang K, Khan SN, Riazuddin S, Hejtmancik JF, Sieving PA, Zack DJ, Katsanis N (2010) A splice-site mutation in a retina-specific exon of BBS8 causes nonsyndromic retinitis pigmentosa. Am J Hum Genet 86:805–812. doi:10.1016/j.ajhg.2010.04.001

Rivera A, White K, Stohr H, Steiner K, Hemmrich N, Grimm T, Jurklies B, Lorenz B, Scholl HP, Apfelstedt-Sylla E, Weber BH (2000) A comprehensive survey of sequence variation in the ABCA4 (ABCR) gene in Stargardt disease and age-related macular degeneration. Am J Hum Genet 67:800–813. doi:10.1086/303090

Rivolta C, Sweklo EA, Berson EL, Dryja TP (2000) Missense mutation in the USH2A gene: association with recessive retinitis pigmentosa without hearing loss. Am J Hum Genet 66:1975–1978. doi:10.1086/302926

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365–386

Seyedahmadi BJ, Rivolta C, Keene JA, Berson EL, Dryja TP (2004) Comprehensive screening of the USH2A gene in Usher syndrome type II and non-syndromic recessive retinitis pigmentosa. Exp Eye Res 79:167–173. doi:10.1016/j.exer.2004.03.005

Siemiatkowska AM, Astuti GD, Arimadyo K, den Hollander AI, Faradz SM, Cremers FP, Collin RW (2012) Identification of a novel nonsense mutation in RP1 that causes autosomal recessive retinitis pigmentosa in an Indonesian family. Mol Vis 18:2411–2419

Stenirri S, Alaimo G, Manitto MP, Brancato R, Ferrari M, Cremonesi L (2008) Are microarrays useful in the screening of ABCA4 mutations in Italian patients affected by macular degenerations? Clin Chem Lab Med 46:1250–1255. doi:10.1515/CCLM.2008.248

Stone EM (2007) Leber congenital amaurosis—a model for efficient genetic testing of heterogeneous disorders: LXIV Edward Jackson Memorial Lecture. Am J Ophthalmol 144:791–811. doi:10.1016/j.ajo.2007.08.022

Sullivan LS, Bowne SJ, Birch DG, Hughbanks-Wheaton D, Heckenlively JR, Lewis RA, Garcia CA, Ruiz RS, Blanton SH, Northrup H, Gire AI, Seaman R, Duzkale H, Spellicy CJ, Zhu J, Shankar SP, Daiger SP (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. doi:10.1167/iovs.05-1443

Sung CH, Davenport CM, Hennessey JC, Maumenee IH, Jacobson SG, Heckenlively JR, Nowakowski R, Fishman G, Gouras P, Nathans J (1991) Rhodopsin mutations in autosomal dominant retinitis pigmentosa. Proc Natl Acad Sci USA 88:6481–6485

Tanackovic G, Ransijn A, Thibault P, Abou Elela S, Klinck R, Berson EL, Chabot B, Rivolta C (2011) PRPF mutations are associated with generalized defects in spliceosome formation and pre-mRNA splicing in patients with retinitis pigmentosa. Hum Mol Genet 20:2116–2130. doi:10.1093/hmg/ddr094

Vaclavik V, Gaillard MC, Tiab L, Schorderet DF, Munier FL (2010) Variable phenotypic expressivity in a Swiss family with autosomal dominant retinitis pigmentosa due to a T494M mutation in the PRPF3 gene. Mol Vis 16:467–475

Vervoort R, Lennon A, Bird AC, Tulloch B, Axton R, Miano MG, Meindl A, Meitinger T, Ciccodicola A, Wright AF (2000)

Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa. Nat Genet 25:462–466. doi:10.1038/78182

Wang H, den Hollander AI, Moayedi Y, Abulimiti A, Li Y, Collin RW, Hoyng CB, Lopez I, Abboud EB, Al-Rajhi AA, Bray M, Lewis RA, Lupski JR, Mardon G, Koenekoop RK, Chen R (2009) Mutations in SPATA7 cause Leber congenital amaurosis and juvenile retinitis pigmentosa. Am J Hum Genet 84:380–387. doi:10.1016/j.ajhg.2009.02.005

Wang Q, Wang P, Li S, Xiao X, Jia X, Guo X, Kong QP, Yao YG, Zhang Q (2010) Mitochondrial DNA haplogroup distribution in Chaoshanese with and without myopia. Mol Vis 16:303–309

Wang XT, Mion B, Aherne A, Engel PC (2011) Molecular recruitment as a basis for negative dominant inheritance? propagation of misfolding in oligomers of IMPDH1, the mutated enzyme in the RP10 form of retinitis pigmentosa. Biochim Biophys Acta 1812:1472–1476. doi:10.1016/j.bbadis.2011.07.006

Xiao X, Mai G, Li S, Guo X, Zhang Q (2011) Identification of CYP4V2 mutation in 21 families and overview of mutation spectrum in Bietti crystalline corneoretinal dystrophy. Biochem Biophys Res Commun 409:181–186. doi:10.1016/j.bbrc.2011.04.112

Xu W, Dai H, Lu T, Zhang X, Dong B, Li Y (2011) Seven novel mutations in the long isoform of the USH2A gene in Chinese families with nonsyndromic retinitis pigmentosa and Usher syndrome Type II. Mol Vis 17:1537–1552

Zhang Q, Li S, Guo X, Guo L, Xiao X, Jia X, Kuang Z (2001) Screening for CRX gene mutations in Chinese patients with Leber congenital amaurosis and mutational phenotype. Ophthalmic Genet 22:89–96

Zhang Q, Li S, Xiao X, Jia X, Guo X (2007) The 208delG mutation in FSCN2 does not associate with retinal degeneration in Chinese individuals. Invest Ophthalmol Vis Sci 48:530–533. doi:10.1167/iovs.06-0669

Ziviello C, Simonelli F, Testa F, Anastasi M, Marzoli SB, Falsini B, Ghiglione D, Macaluso C, Manitto MP, Garre C, Ciccodicola A, Rinaldi E, Banfi S (2005) Molecular genetics of autosomal dominant retinitis pigmentosa (ADRP): a comprehensive study of 43 Italian families. J Med Genet 42:e47