Compound Heterozygosity of Two Novel Truncation Mutations in *RP1* Causing Autosomal Recessive Retinitis Pigmentosa

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Purpose. To evaluate the phenotypic effects of two novel frameshift mutations in the *RP1* gene in a Chinese pedigree of autosomal recessive retinitis pigmentosa (ARRP).

Methods. Family members of a proband with ARRP were screened for *RP1*, *RHO*, *NR2E3*, and *NRL* mutations by direct sequencing. Detected *RP1* mutations were genotyped in 225 control subjects. Since one family member with the *RP1* deletion mutation in exon 2 was found to have age-related macular degeneration (AMD) but not RP, exons 2 and 3 of *RP1* were screened in 120 patients with exudative AMD. Major AMD-associated SNPs in the *HTRA1* and *CFH* genes were also investigated.

Results. Two novel frameshift mutations in *RP1*, c.5_6delGT and c.4941_4942insT, were identified in the pedigree. They were absent in 225 control subjects. Family members who were compound heterozygous for the nonsense mutations had early-onset and severe RP, whereas those with only one mutation did not have RP. No mutations in *RHO*, *NR2E3*, and *NRL* were identified in the pedigree. Subject I:2 with AMD carried both at-risk genotypes at *HTRA1* rs11200638 and *CFH* rs800292. No mutation in *RP1* exons 2 and 3 was identified in 120 AMD patients.

Conclusions. This report is the first to associate ARRP with compound heterozygous nonsense mutations in *RP1*. Identification of the nonsense-mediated mRNA decay (NMD)–sensitive mutation c.5_6delGT provided further genetic evidence that haploinsufficiency of RP1 is not responsible for RP. The authors propose four classes of truncation mutations in the *RP1* gene with different effects on the etiology of RP. (*Invest Ophthalmol Vis Sci.* 2010;51:2236–2242) DOI:10.1167/iovs.09-4437

Retinitis pigmentosa (RP, MIM 268000; Mendelian Inheritance in Man; National Center for Biotechnology Information, Bethesda, MD) is a group of hereditary retinal diseases characterized by progressive degeneration of rod and cone photoreceptors. The classic triad of ocular changes includes optic disc pallor, attenuated retinal vessels, and diffuse pigmentary changes over the retina. Symptoms usually start with night blindness during adolescence, followed by peripheral visual loss in young adulthood, and finally central visual loss and even total blindness after mid life as the disease progresses.[1,2] In most RP patients, there is no effective prevention or treatment. RP occurs across ethnic groups and is one of the major causes of severe and irreversible visual disability in individuals younger than 60 years, with a worldwide prevalence of ~1:4000 to 1:6000.[3–10]

RP is genetically heterogeneous, with different modes of inheritance: autosomal dominant (ADRP), autosomal recessive (ARRP), and X-linked (XLRP). Approximately half of all RP patients have no known family history or segregation pattern and are therefore classified as having simplex RP.[1] So far, more than 40 genes have been identified to cause ADRP, ARRP, and XLRP (RetNet, the Retinal Information Network, http://www.sph.uth.tmc.edu/RetNet; provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX). Among these genes, four (*RHO*, *RP1*, *NR2E3*, and *NRL*) have been implicated in both ADRP and ARRP. *RHO* (rhodopsin, MIM 180380) mutations account for approximately 25% of ADRP and 1% of ARRP.[2] Mutations in *NR2E3* (nuclear receptor subfamily 2 group E member 3, MIM 604485) and *NRL* (neural retina leucine zipper, MIM 162080) each account for approximately 1% of RP cases.[2,11] The reported mutations in *RHO*, *NR2E3*, and *NRL* are predominantly missense changes resulting in dysfunctions in the mutant proteins. Therefore, these mutations most likely have dominant negative or gain-of-function effects on the molecular mechanism of RP. In contrast, mutations in the *RP1* gene (retinitis pigmentosa 1, MIM 603937) account for approximately 5.5% of ADRP and 1% of ARRP.[2] They are predominantly truncation mutations resulting in premature termination codons.[12–30] Only a few *RP1* missense variants (e.g., p.Thr373Ile for ARRP,[23] and p.Asp984Gly for ADRP[26]) have been identified as disease-causing.[14–17,19,20,23,25] Many *RP1* missense variants are considered to be benign or have uncertain pathogenicity.[27–30] So far, the effects of *RP1* truncation mutations in the mechanism of RP remain uncertain.

RP1 is a photoreceptor-specific protein localized at the connecting cilia of photoreceptors and may assist in the maintenance of cilial structure or transport down the photoreceptors.[12,31] Berson et al.[19] suggested that retinal degenerations caused by *RP1* nonsense mutations are probably due to deleterious properties of truncated RP1 proteins and not haploinsufficiency. Moreover, the identification of several loss-of-function truncation mutations in ARRP (i.e., p.Glu488Stop[24]) also suggest that haploinsufficiency of RP1 does not cause RP. On the other hand, based on an *Rp1-myc* mutant mouse model, Liu et al.[32] suggested that *RP1* disease in humans is caused by haploinsufficiency with decreased expression of functional RP1 protein. As the *RP1* truncation mutations identified to date are located in the last exon,[12–30] the mutant genes are expected to be translated into truncated RP1 proteins, because

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TABLE 1. Demographic and Clinical Features and Genotypes of *RP1* Mutations and AMD-Associated SNPs in the Family with ARRP

				RE (SE, D)		BCVA		*RP1* Mutations		AMD-Associated SNP		
Subject	Sex	Age	Phenotype	OD	OS	OD	OS	c.5_6delGT	c.4941_4942insT	*HTRA1* (−625G>A)	*CFH* V62I	*CFH* Y402H
I:2	F	72	AMD	+0.5	+1.25	20/100	20/50	Del	Wild	AA	GG	TT
II:2	M	54	Myopia	−0.25	−3.0	20/25	20/30	Wild	Ins	AA	GG	TT
II:5	F	48	RP	N/A	−2.5	LP	20/100	Del	Ins	AG	GG	TT
II:6	F	46	RP	−4.75	−3.5	20/100	HM (5cm)	Del	Ins	AA	GA	TT
III:1	M	24	Myopia	−6.5	−5.75	20/20	20/20	Wild	Wild	AA	GG	TC
III:2	F	15	Myopia	−3.25	−2.75	20/20	20/20	Wild	Ins	AG	GA	TT
III:3	M	11	Myopia	−1.0	−1.0	20/20	20/20	Del	Wild	AG	GA	TT

Demographic and clinical features of the subjects recruited from the family and the genotyping results of *RP1* frameshift mutations and the three AMD-associated SNPs in *HTRA1* and *CFH* are shown in the table.

these premature-termination, codon-containing transcripts are insensitive to nonsense-mediated mRNA decay (NMD). NMD is an mRNA surveillance mechanism found in all eukaryotic organisms that leads to a degradation of the transcripts with introns in the 3′ untranslated region and subsequently prevents the synthesis of truncated proteins that may have toxic effects such as dominant negative interactions.[33–37] However, there is still no direct genetic evidence from humans to support haploinsufficiency of RP1 as a cause of RP. In this study, we report two novel frameshift mutations in *RP1* coexisting in a proband from a Chinese pedigree with classic ARRP, with one being NMD sensitive, providing evidence of depletion of RP1 as a cause of RP. As one family member (I:2) who carries the NMD-sensitive mutation was found to be free of RP but had age-related macular degeneration (AMD), we therefore investigated whether NMD-sensitive mutations in *RP1* are involved in sporadic AMD and also evaluated two major AMD-associated genes: HtrA serine protease 1 (*HTRA1*, MIM 602194) and complement factor H (*CFH*, MIM 134370) in the pedigree, to dissect the genetic causes of RP and AMD phenotypes in the subjects.

MATERIALS AND METHODS

All study subjects, including patients and controls, were recruited from the Hong Kong Eye Hospital. The study protocol was approved by the Ethics Committee on Human Research of the Chinese University of Hong Kong. Informed consent was obtained from all study subjects. All procedures used conformed to the tenets of the Declaration of Helsinki.

Clinical Assessment of the Pedigree

The proband (II:5) of the Hong Kong Chinese family has been attending our clinic since 2001. She was invited for our genetic study and underwent ophthalmic examinations of fundus photography, slit lamp biomicroscopy, and full-field electroretinography (ERG). After two *RP1* mutations were identified in the proband, her family members were invited for a complete ophthalmic examination in January 2009. Their detailed clinical features are documented in Table 1. Venous blood samples were collected for genetic analysis.

Control Subjects and Patients with Exudative AMD

A total of 225 unrelated Chinese subjects, aged between 60 and 99 years, were recruited as control subjects and were given complete ophthalmic examinations. All control subjects had no sign of RP or other major eye diseases except senile cataract. We also recruited 120 unrelated patients with exudative AMD randomly selected from our previously reported AMD cohort,[38,39] for the genotyping of AMD-associated polymorphisms.

Mutation Screening

Genomic DNA of all study subjects was extracted from whole blood (QIAamp DNA Blood kit; Qiagen, Valencia, CA). For the proband and family members, coding sequences of four RP genes (*RHO*, *RP1*, *NR2E3*, and *NRL*) were screened by polymerase chain reaction (PCR) followed by direct DNA sequencing with dye-termination chemistry (BigDye Terminator Cycle Sequencing Kit, ver. 3.1; Applied Biosystems, Inc. [ABI], Foster City, CA) on an automated DNA sequencer (3130XL; ABI), according to the manufacturer's instructions. Detected sequence variations were further screened in 225 control subjects by PCR and direct DNA sequencing. Since one family member, I:2, was found to have AMD and an NMD-sensitive frameshift mutation in exon 2 of *RP1*, we investigated the occurrence of NMD-sensitive frameshift mutations in AMD by screening *RP1* exons 2 and 3 in 120 patients with exudative AMD. We also investigated major AMD-associated SNPs in *HTRA1* (−625G>A) and *CFH* (p.Val62Ile and p.Tyr402His) in the pedigree by direct sequencing, as described herein and in previous studies.[38,39] The primers used to genotype the *RP1* frameshift mutations are shown in Table 2. More detailed information on the primers and PCR conditions used in this study is shown in Supplementary Table S1, http://www.iovs.org/cgi/content/full/51/4/2236/DC1.

TABLE 2. Primer Sequence and Thermal Cycling Condition for Genotyping the *RP1* Truncation Mutations

Target	Primer	Primer Sequence (5′-3′)	Ta (°C)	$MgCl_2$ (mM)
c.5_6delGT (exon 2)	Forward	ATGTATTCGCTATGGTGCTGTGATTCT	63	1.0
	Reverse	CTGCCGTGGGAACATAGGTAGGACTC		
c.4941_4942insT (exon 4)	Forward	CAGTGACAGTGAGCAGCCATATAAAACATC	65	1.0
	Reverse	TGCCTTTGTCAATCAGTACTCCTTCGT		

Ta, Annealing temperature.

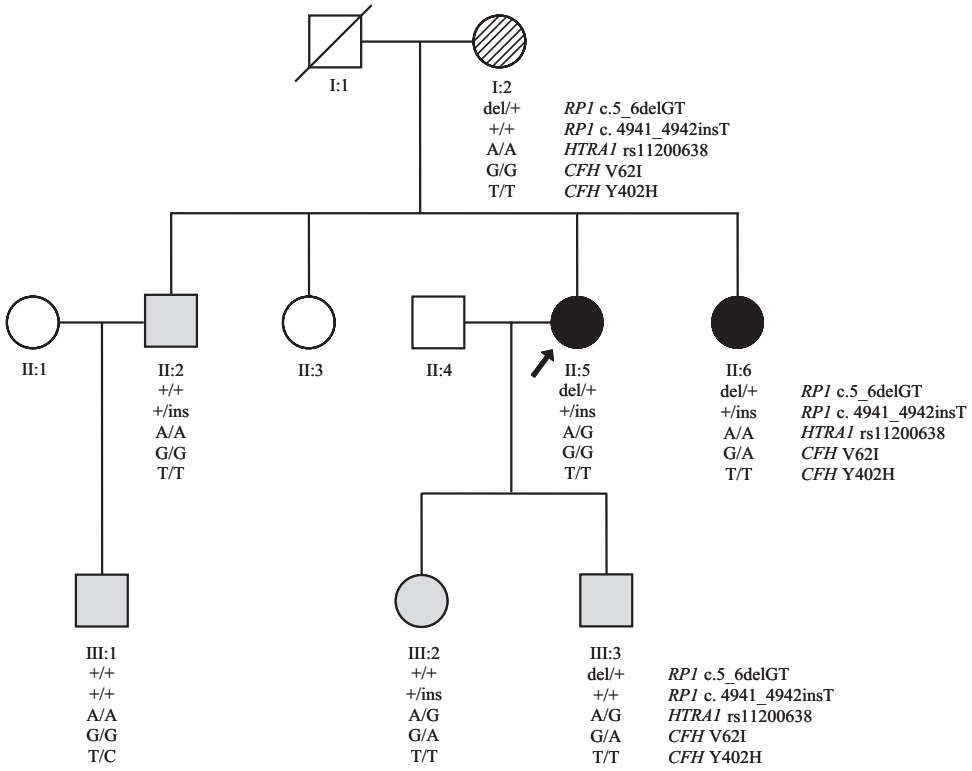

FIGURE 1. The Chinese pedigree showing *RP1* mutations and AMD-associated SNPs. *Squares*: males; *circles*: females; *black symbols*: individuals affected with RP; *gray symbols*: individuals with myopia; *hash-marked symbols*: individual affected with intermediate AMD; *symbol with diagonal line*: deceased family member; *arrow*: the proband; +, wild-type.

RESULTS

Clinical Manifestations of Members of the Pedigree

In this three-generation pedigree, seven members consented to participate in this study (Fig. 1). The proband (II:5) and her younger sister (II:6) had diagnoses of nonsyndromic RP. Fundus examination of the proband showed typical RP features, including waxy pallor of the optic discs, attenuated retinal arterioles, diffuse bone spicule pigmentary changes over the retina, and atrophic changes at the macula (Fig. 2A). Both scotopic and photopic full-field ERGs of the proband showed nonrecordable waveforms (data not shown). Chronologically, the proband experienced night blindness from the age of 7 years with reduced visual acuity from the age of 12 years. She developed significant peripheral visual field loss in her early 30s. Her best corrected visual acuity (BCVA) had been reduced to light perception (LP) in the right eye and 20/100 in the left eye since the age of 41 years. In addition to RP-associated retinal changes, slit lamp examination also demonstrated posterior subcapsular cataract. The proband's younger sister (II:6) had similar symptoms, with night blindness since the age of 12 years and visual field constriction plus significant visual loss at ~20 years. Her BCVA at the last examination was 20/100 in the right eye and hand movement (HM) at 5 cm in the left eye.

Other members of the family did not have clinical signs and symptoms suggestive of RP (Table 1; Figs. 2B, 2C). However, slit lamp biomicroscopy of the proband's 72-year-old mother (subject I:2) showed signs of intermediate AMD with multiple medium-size drusen.[40] OCT demonstrated serous pigment epithelial detachment (PED) in the right eye consistent with AMD (Fig. 2D). Other subjects (II:2, III:1, III:2, and III:3) were free of any major eye diseases except for mild to moderate myopia. Full-field ERG was also performed in subject III:2. The parameters of the scotopic, photopic, and 30-Hz flicker ERGs were within normal limits (data not shown).

Mutation Screening and SNP Genotyping Results

Six *RP1* sequence variants were identified in the proband, and all were heterozygous: c.5_6delGT (p.Ser2ArgfsX16, exon 2), c.2615G>A (p.Arg872His, rs444772, exon 4), c.4941_4942insT (p.Pro1648SerfsX13, exon 4), c.5008G>A (p.Ala1670Thr, rs446227, exon 4), c.5071T>C (p.Ser1691Pro, rs414352, exon 4), and c.5175A>G (p.Gln1725Gln, rs441800, exon 4). The two frameshift mutations were novel, and the others were common polymorphisms registered in the dbSNP database (www.ncbi.nlm.nih.gov/projects/SNP; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD). Sequence chromatograms of the *RP1* frameshift mutations are shown in Figure 3. No sequence variant was detected in the other three genes (*RHO*, *NR2E3*, and *NRL*). In the pedigree, affected RP subjects II:5 and II:6 carried both of the frameshift mutations, whereas the unaffected subjects carried one mutation each, except subject III:1, who did not have either (Table 1). Neither of the two frameshift mutations was detected in 225 unrelated control subjects. Screening of exons 2 and 3 of *RP1* in the 120 patients with exudative AMD revealed no NMD-sensitive truncation mutations. Finally, genotyping of the *HTRA1* and *CFH* SNPs showed that the subject with AMD (I:2) carried the at-risk AA genotype at the *HTRA1* rs11200638 (−625G>A) and the at-risk GG genotype and nonrisk TT genotype at the *CFH* SNPs p.Val62Ile (c.184G>A) and p.Tyr402His (c.1204T>C), respectively (Table 1).

DISCUSSION

In this study, we found two novel *RP1* mutations in an ARRP family. Further genotype and phenotype investigations revealed compound heterozygous frameshift mutations in *RP1* resulting in ARRP in this family. In subjects with both of the *RP1* frameshift mutations, c.5_6delGT and c.4941_4942insT, RP developed at an early age. Their vision deteriorated rapidly, with severe visual loss by the age of 40 years. In contrast,

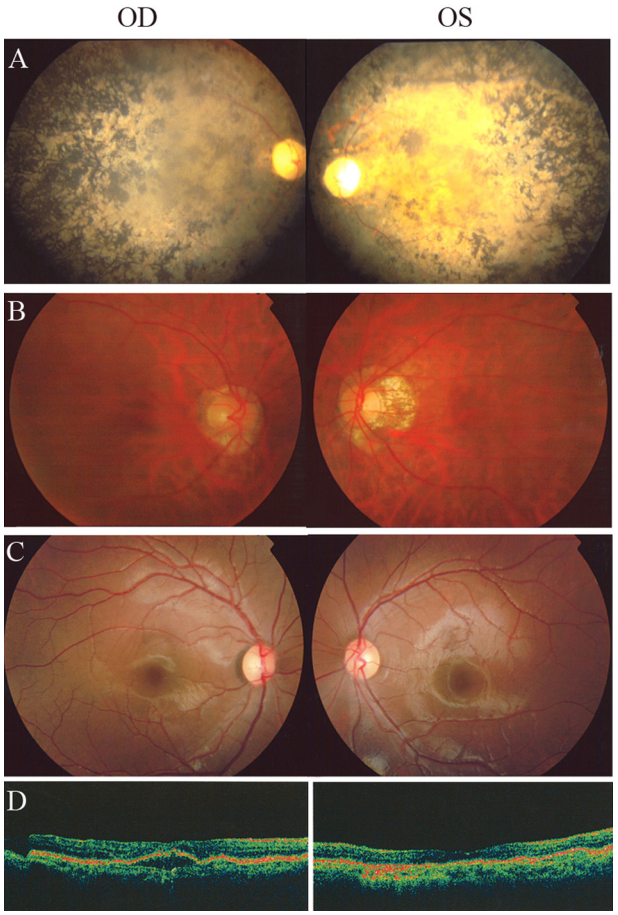

FIGURE 2. Fundus photographs of (**A**) the proband (II: 5) who was affected with RP and compound heterozygous *RP1* nonsense mutations; (**B**) subject II:2 who was unaffected and a carrier of the insertion mutation; (**C**) subject III:3 who was unaffected and a carrier of the deletion mutation; and (**D**) optical coherence tomography image of individual (I:2) with nonexudative AMD, showing serous pigment epithelial detachment with no evidence of subretinal fluid in the right eye and pigment epithelial atrophy in the left eye.

individuals who carried only one *RP1* mutation did not develop the identifiable fundus changes of RP, even at older ages (I:2 and II:2). To our knowledge, this is the first report on compound heterozygous frameshift *RP1* mutations as the genetic cause of RP. In addition, AMD and RP coexisted across different generations in this pedigree. The proband's mother (I:2) was found to have intermediate AMD, whereas the proband and her sister had RP. The coexistence of AMD and RP has only been reported in three unrelated families from the United States.[41] This study presented the first report of such coexistence in ethnic Chinese.

In this family, we found that loss of function of RP1, rather than haploinsufficiency (i.e., inactivation of only one copy of the gene), was responsible for RP. The novel 2-bp deletion identified in exon 2, c.5_6delGT, is predicted to cause a frame shift and result in a truncated protein denoted as p.Ser2ArgfsX16. Notably, this mutation is located just next to the start codon. Thus, if translated, the 16-residue mutant peptide (MRYPFYWFFHHSSYVFX) may truncate the 2156-residue RP1 protein by >99% and is expected to be nonfunctional. It may be degraded by the ubiquitin system.[42,43] More likely, however, expression of this truncated protein is disrupted by the mechanism of nonsense-mediated mRNA decay. We predicted the transcript with the c.5_6delGT mutation to be sensitive to NMD, because this mutation may introduce a premature termination codon into the first exon of *RP1* mRNA with intronic structure downstream. Premature termination codons close to the 5′ end usually provoke NMD.[33,44,45] Therefore, we presumed the mutated *RP1* mRNA to be degraded as a result of NMD, leading to little production of the mutant protein by the photoreceptor cells, which express *RP1*.[14,32] Therefore, the unaffected family members who carried only the c.5_6delGT mutation are likely to have normal RP1 proteins translated from the copy of the wild-type gene. So far, there is no evidence to show that the production of RP1 protein is reduced due to depletion of one copy of the *RP1* gene in humans. However, in an *RP1* transgenic mouse model, Gao et al.[46] showed that haploinsufficiency of the RP1 protein occurred in $Rp1^{+/-}$ mice, as they expressed approximately 50% less RP1 protein than did $Rp1^{+/+}$ mice. Moreover, ERGs of $Rp1^{+/-}$ mice were significantly reduced compared with $Rp1^{+/+}$ mice. A similar haploinsufficiency of RP1 protein may therefore also occur in $RP1^{+/-}$ humans, given no complementary or rescue mechanisms. In our study, subjects with only the NMD-sensitive mutation (I:2 and III:3) did not develop RP, including subject I:2, who was 72 years old. Thus, our findings support the conclusion that haploinsufficiency of RP1 is insufficient to cause RP in humans. Accordingly, we propose that half the normal amount of the normal RP1 protein is sufficient to sustain proper functioning of the photoreceptor cells.

The 1-bp insertion mutation (c.4941_4942insT) identified in this study reveals a new property in *RP1* truncation mutations. This mutation, which is NMD-insensitive, is predicted to cause a frame shift and result in a truncated protein denoted as p.Pro1648SerfsX13. In our study family, two subjects who were heterozygous for this mutation did not have any of the changes of RP, even past middle age. Therefore, this mutation does not seem to have a dominant negative effect. However, the mutant protein (p.Pro1648SerfsX13) does not seem to have a normal RP1 function either, as shown by the fact that carriers with compound heterozygosity for the c.5_6delGT and p.Pro1648SerfsX13 mutations developed RP at a young age, indicating that the truncated protein does not function as normally as the wild-type RP1 protein. Therefore, we hypothesize that this insertion mutation also leads to a loss of function of RP1.

So far, at least 33 truncation mutations have been identified in *RP1* (Fig. 4), all of which are located on exon 4. The mutant transcripts are presumably insensitive to NMD due to absence of a downstream intron.[12–30] Liu et al.[32] detected mutant RP1 mRNA in lymphoblasts of patients heterozygous or homozygous for p.Arg677Stop, suggesting that the mutant mRNAs have escaped NMD with subsequent production of truncated RP1 proteins. Among the truncation mutations, 28 have been implicated in ADRP. This group of mutations clustered in a relatively constricted region of mutation hot spots between p.500 and p.1053.[12–22,25–30] Since our findings showed that haploinsufficiency of RP1 is not enough to cause RP, these mutations may play a role in the etiology of ADRP through a dominant negative mechanism or a gain-of-function effect. It is possible that truncation mutations in this region disrupt the functional domains and result in a production of mutant proteins that are cytotoxic to the photoreceptors. The amino acid sequence between codons p.486 and p.635 shares some homology with the *Drosophila melanogaster* protein BIF (encoded by the gene bifocal, *bif*), which is essential for normal photoreceptor morphogenesis.[12] Of interest, except for p.Met500SerfsX33, all the previously identified truncation mutations for ADRP truncated the RP1 protein after this domain. The loss of the C-terminal half to one third of the RP1 protein may expose this domain and result in deleterious effects, suggesting that at least part of the C-terminal amino acid sequence

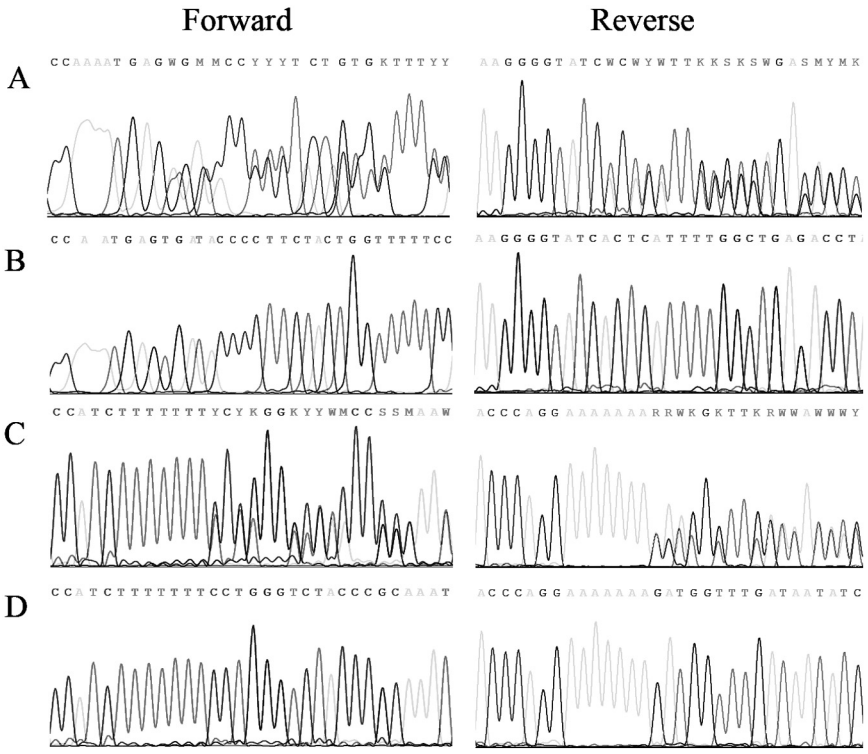

FIGURE 3. The forward (*left*) and reverse (*right*) sequence chromatograms of (**A**) the proband II:5, showing the 2-bp deletion in exon 2, c.5_6delGT, resulting in a frameshift, p.Ser2ArgfsX16; (**B**) unaffected individual III:1, showing a wild-type sequence; (**C**) the proband II:5, showing the 1-bp insertion in exon 4, c.4941_4942insT, resulting in a frameshift, p.Pro1648SerfsX13; and (**D**) unaffected individual III:1, showing a wild-type sequence.

is necessary for suppressing the toxic effects of this region. Apart from the ADRP mutations, three truncation mutations (p.Glu488Stop, p.Arg1519GlufsX1, and p.Asn1751IlefsX3), which were predicted to truncate the protein before the BIF domain or within the C-terminal domain,[24] caused ARRP. Heterozygous carriers were disease free.[23,24] Therefore, it has been suggested that truncation of RP1 protein before the BIF motif or within the C-terminal portion of the protein will cause loss of RP1 function and result in RP only in individuals homozygous for the mutations.[24] However, at the C-terminal

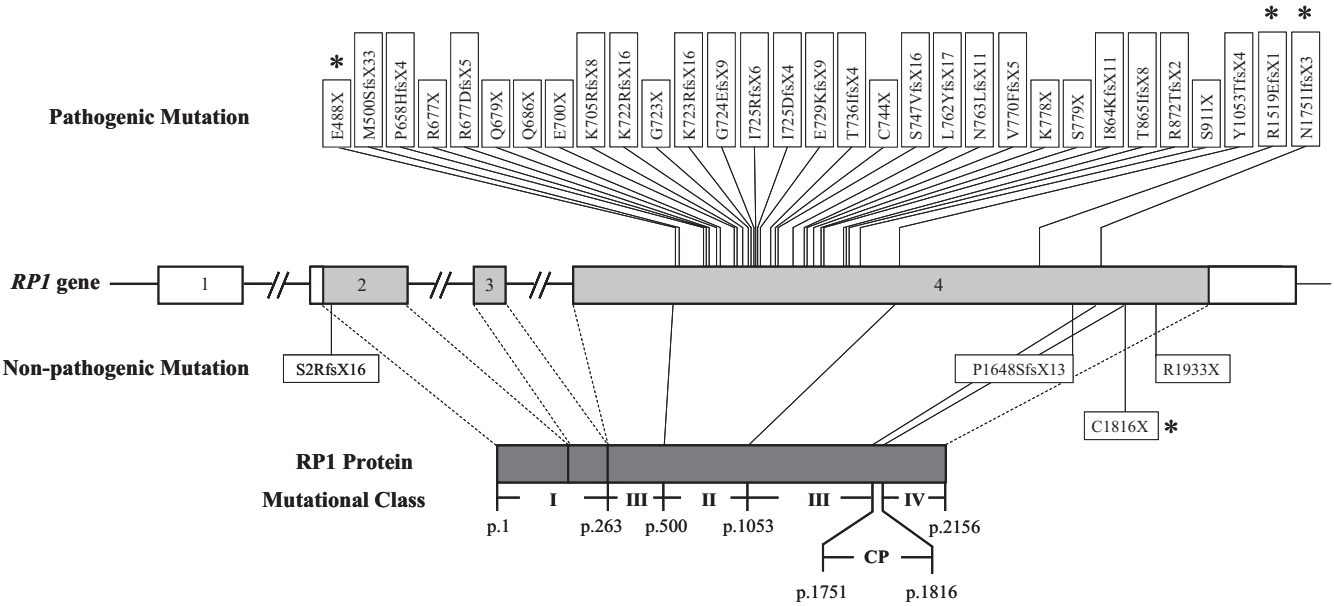

FIGURE 4. The *RP1* gene showing the locations of pathogenic and nonpathogenic truncation mutations found by our group and others.[12–30] Some mutations were renamed at the protein level,[47] according to the deletion or insertion found in the original studies by using a reference sequence from Ensembl (ENST00000220676).[48] Regarding the nonsense mutations, this figure could be considered an updated version of Figure 4 in the report of Berson et al.[19] The mutations above the schematic gene are pathogenic mutations that are responsible for ADRP or ARRP. Mutations in the shorter rectangles were nonsense point mutations and those in the longer rectangles were frameshift mutations. Those under the schematic gene are nonpathogenic. The mutations identified in the present study are also shown under the gene, although they also cause disease when they coexist. The lower part of the diagram is the schematic RP1 protein with four classes of truncation mutations that are defined in this study. The region between p.1751 and p.1816 was expected to have a critical position (CP) that could distinguish functional and nonfunctional RP1 truncated proteins. *Homozygous genotype.

region, another truncation mutation (p.Cys1816Stop) has been found to be nonpathogenic for RP, even in homozygous carriers.[25] These findings indicate that the truncation of less than 340 C-terminal amino acids causes no significant damage to the photoreceptors. Moreover, within the 65-residue region that spans p.1751 to p.1816, there may be a critical position or functional domain, which, if truncated, could result in a nonfunctional RP1 protein. Although no functional domain is known to exist in this region, it is highly likely that a critical position is present.

According to our findings, we propose four classes of truncation mutations in the *RP1* gene with implications for RP (Fig. 4): Class I mutations are NMD-sensitive truncations located in exons 2 and 3. The region is relatively small (263 amino acids) and is not a mutation hot spot. As shown in our study, class I mutations do not cause RP in humans due to loss-of-expression caused by NMD. Class II mutations are NMD-insensitive truncations located in a region spanning approximately p.500 to p.1053 in exon 4. Mutations in this region represent the majority of pathologic *RP1* truncation mutations. The mutant proteins are expected to impose distinct deleterious (dominant negative) effects on the photoreceptors, resulting in cell death and leading to ADRP. Class III involves NMD-insensitive truncation mutations located in the regions p.264 to p.499 and p.1054 to p.1751. The truncated proteins are expected to be loss-of-function and will cause RP in individuals homozygous or compound heterozygous for the mutations. The p.Pro1648SerfsX13 mutation detected in this present study belongs to this class. Class IV includes NMD-insensitive truncations located after p.1816 in exon 4. The resulting truncated proteins are expected to possess normal RP1 functions and are not pathogenic, even in homozygotes.

In our pedigree, the c.5_6delGT mutation was presumed to cause loss of expression due to NMD, and the p. Pro1648SerfsX13-truncated protein was presumed to cause loss of function. Therefore, the two RP subjects in our pedigree lacked functional RP1 protein in the photoreceptors and developed RP at an early age with severe visual symptoms. Such RP phenotypes caused by compound heterozygosity resembled that of the $Rp1^{-/-}$ mice as described by Gao et al.[46] These deletions are very rare, as we did not detect any other frameshift or nonsense mutations in exons 2 and 3 in 230 RP patients, 225 control subjects, and 120 AMD patients. The occurrence of compound heterozygous mutations is even more unlikely.

On the detection of AMD in subject I:2, who carried the c.5_6delGT mutation, we hypothesized that a reduced amount of RP1 caused by NMD-sensitive mutations in exons 2 or 3 of the gene may be implicated in late-onset retinal degeneration as AMD rather than RP, which is early onset. However, we did not detect any mutations in exons 2 and 3 in 120 patients with exudative AMD, suggesting a lack of correlation between the dosage of RP1 and AMD. To date, more than 13 genes have been found to be associated with AMD and nearly 40 for RP. Only one gene, *ABCA4*, has been implicated in both AMD and ARRP (RetNet 2009). As *RP1* mutations have been identified as the cause of RP in our pedigree, it is therefore unlikely that the *ABCA4* gene is the concurrent cause of RP and AMD in this pedigree. Instead, we evaluated the major AMD-associated polymorphisms in *HTRA1* and *CFH*, to dissect the genetic factors underlying the RP and AMD phenotypes in the pedigree. Intriguingly, the AMD subject (I:2) was found to carry both the homozygous at-risk genotypes at *HTRA1* rs11200638 (AA) and *CFH* p.Val62Ile (GG). Based on our previous estimation,[38] the subject may have a 23-fold increased risk of AMD than do individuals carrying homozygous nonrisk genotypes. Therefore, the AMD phenotype in this subject was likely due to advanced age and the *HTRA1* and *CFH* at-risk alleles. However, whether haploinsufficiency of RP1 may impose modifying effects to the genetic susceptibility of AMD remains to be investigated. Apart from the AMD subject, the RP subjects also carried the at-risk genotypes for both SNPs. Although it is unlikely that either *HTRA1* or *CFH* is associated with disease onset of RP, there is still a possibility that the at-risk alleles have modifier effects that lead to an accelerated progression of RP.

In summary, we have for the first time identified compound heterozygous truncation mutations in the *RP1* gene as the genetic cause of ARRP. Identification of the c.5_6delGT mutation, which is NMD-sensitive and does not lead to RP separately, provides further evidence that haploinsufficiency of RP1 is not responsible for RP. We also propose that four classes of *RP1* truncation mutations have different effects on the etiology of RP.

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