Rhodopsin mutations in Chinese patients with retinitis pigmentosa

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Abstract

Aim—To determine the pattern of rhodopsin mutations in Chinese retinitis pigmentosa (RP) patients.

Methods—The rhodopsin gene was examined in 101 RP patients and 190 controls from Hong Kong.

Results—Three coding changes were identified: Pro347Leu, Ala299Ser, and 5211delC. Each protein sequence alteration was found in one patient. Ala299Ser also existed in two controls.

Conclusion—The C-terminal nonsense mutation may cause mis-sorting of rhodopsin protein. The finding of controls with Ala299Ser suggests this is only the third missense alteration reported that does not cause RP. The expected frequency of rhodopsin mutations in RP is <7% (2/101=2.0%, 95% confidence interval: 0.2%–7.0%).

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Mutations in the gene encoding rhodopsin (*RHO*) lead to retinal degeneration in species from *Drosophila*¹ to humans. *RHO* mutations cause autosomal dominant retinitis pigmentosa (ADRP), although a few mutations cause recessive RP or congenital night blindness,^{2–6} and account for about 25% of ADRP in Europe^{7,8} and North America,^{9–11} and less in Japan.^{12,13} To investigate the frequency of rhodopsin mutations in Chinese patients with retinitis pigmentosa (RP), and to discover new mutations that may shed more light on rhodopsin's function, we examined the entire coding region and exon splice sites of rhodopsin for sequence changes in 101 RP patients and 190 controls who were all unrelated Hong Kong residents.

Methods

Unrelated RP patients were identified by fundus examination, assisted by visual field and electrodiagnostic tests at the Prince of Wales Hospital or Hong Kong Eye Hospital in Hong Kong. Control subjects were visitors to

the above hospitals who were over 60 years old and had no major eye disease except cataract. All subjects were ethnic Chinese. Family members of some RP patients also participated. A blood sample was taken after informed consent was given for participation in genetic studies. Approval was given by the ethics committee for human research of the Chinese University of Hong Kong.

We examined the five coding exons and splice sites of *RHO* in 101 Chinese RP patients and 190 controls from Hong Kong. DNA was extracted from blood, and sequence alterations were detected using polymerase chain reaction (PCR), conformation sensitive gel electrophoresis (CSGE), and DNA sequencing. Sequences were compared with the published human *RHO* sequence.¹⁴

Results

Of nine nucleotide changes, three alter the protein sequence (Table 1). One was a novel nonsense mutation, 5211delC, predicted to alter the C-terminal sequence by replacing the negatively charged final 22 amino acids containing six phosphorylation sites by a 32 amino acid positively charged sequence with only two Thr. This mutation occurred in one patient, a 53 year old woman whose earliest symptom was night blindness at age 30. She had a visual acuity of right eye 0.5/200 and left eye 5/200, waxy optic discs, attenuated arterioles, mottled retinal pigment epithelium (RPE), and scattered bone spicule pigmentation. Her younger daughter, who also carried the mutation, was 26 years old with no RP symptoms. However, her RPE was also mottled. Three other relatives were genotypically and phenotypically normal. Two relatives with RP were not available for study.

The commonly reported rhodopsin mutation Pro347Leu was found in one patient, a 55 year old woman. Her earliest symptom was night blindness at the age of 17. She had an acute attack of angle closure glaucoma in her right eye in 1996 which was aborted by laser peripheral iridotomy. Final vision in her right eye dropped to light perception only whereas the left eye held at the level of 20/70. The right optic disc was extremely pale with a cup/disc ratio (C/D) of 0.8 and optic atrophy subsequent to the acute glaucoma, whereas the left had mild pallor with a C/D of 0.2. Her left visual field was constricted. Fundal examination revealed bone spicule pigments and attenuated vessels. The Pro347Leu mutation was detected in three of her children, aged 33, 28, and 17. All had night blindness at 17. They had visual acuity of 20/30, pinkish optic discs,

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Table 1 Sequence alterations identified in *RHO* in our study

Codon change	Nucleotide change	Exon	Frequency in patients	Frequency in controls	References
5' UTR	A269G	1	1/101	1/190	27, 30
Thr62Thr	ACC480ACT	1	1/101	0/190	Novel
Leu259Leu	ATC4175ATT	4	1/101	0/190	Novel
Ala299Ser	GCC4293TCC	4	1/101	2/190	Novel
Intron 4	G5145A	Intron 4	35/101	58/190	27, 31
From 327	5211delC	5	1/101	0/190	Novel
Thr340Thr	ACG5251ACA	5	1/101	0/190	Novel
Pro347Leu	CCG5271CTG	5	1/101	0/190	32
3' UTR	C5321A	5	11/101	20/190	27, 31

Codons	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	
Wild type	cca P	ctg L	ggt G	gac D	gat D	gag E	gcc A	tct S	gct A	acc T	gtg V	tcc S	aag K	acg T	gag E	acg T	agc S	cag Q	gtg V	gcc A	cgg P	gcc A	tag
				-	-	-	★		★		★		★	-	★	★							
1 bp Deletion	cac H	tgg W	gtg V	acg T	atg M	agg R	cct P	ctg L	cta L	ccg P	tgt C	cca P	aga R	cgg R	aga R	cga R	gcc A	agg R	tgg W	ccc P	cgg R	cct P	aag K
	+			★		+							+	+	+	+		+			+		+
	acc T	tgc C	cta L	gga G	ctc L	tgt C	ggc G	cga R	cta L	tag X													
	★							+															

Figure 1 Nucleotide and amino acid sequences of wild type and 5211delC mutant rhodopsin C-terminus. ★Potential phosphorylation sites; +Positively charged residue; -, negatively charged residue.

bone spicule pigments, constricted visual field, and poor scotopic and photopic response in electroretinogram (ERG) in both eyes. The one child without the mutation had no symptoms of RP at age 34.

The remaining protein sequence alteration, Ala299Ser, was found in one patient. Two control subjects also had Ala299Ser.

Discussion

The novel nonsense mutation 5211delC should radically alter and extend the carboxyl terminus (Fig 1). Three pathogenic mechanisms are possible:

- (1) Loss of two negatively and addition of nine positively charged residues alters the charge of the carboxyl terminus, possibly affecting the structure and function of rhodopsin.
- (2) Loss of all six phosphorylatable residues at the C-terminus may prevent activation of arrestin.¹⁵⁻¹⁷ This may kill the cell by overstimulating the phototransduction pathway, analogous to constant light induced cell death. But ADRP also resulted from a 1 bp deletion closer to the C-terminus, at codon 340,¹⁸ which may not affect phosphorylation since only three of six phosphorylatable residues would be removed.
- (3) Loss of the highly conserved C-terminal sequence QVAPA, which controls rhodopsin transport, may misdirect rhodopsin to incorrect cellular locations.¹⁹⁻²² Mutations in these amino acids cause severe ADRP.²³⁻²⁵

Despite the altered C-terminus, patients did not develop RP early in life. RPE mottling was found at age 26, with onset of night blindness around 30. Thus, there does not seem to be an additive effect of the above possible pathogenic mechanisms.

The patients with the most commonly reported rhodopsin mutation, Pro347Leu, all had relatively early onset at 17. This mutation was found in one of 101 index patients, consistent with a frequency of 3.6% among American ADRP families.

Neutral amino acid substitutions in rhodopsin are rare. So far, only two non-pathogenic missense changes have been reported, Val104Ile²⁶ and Phe220Leu.⁹ Sung *et al*²⁷ screened *RHO* on 322 chromosomes and found 39 ADRP mutations but no amino acid substitutions unrelated to ADRP. In our study, Ala299Ser was found in a patient and two controls. Ala299 is not well conserved; among 65 vertebrate opsins, only 27 have Ala at this position, while the others all have serine.²⁸ Serine

can be thought of as a hydroxylated version of alanine. These observations may help to explain why this missense change is well tolerated in humans. However, changing residue 299 to amino acids besides Ser might still cause RP.

RHO mutations represent the most common known cause of RP, occurring in 10% of cases and over 25% of ADRP in North America.²⁹ There, the most frequent mutation, Pro23His, accounts for one third of rhodopsin induced cases but probably arose in a single ancestor.⁹ It occurs nowhere else. Excluding Pro23His, the proportion of ADRP families with rhodopsin mutations is 16–18%,^{7,9} or about 6–7% of all RP cases, consistent with Japanese studies.^{12,13} The proportion of *RHO* mutations in Chinese RP patients was not previously reported, but the current study measures it as less than 7% (2/101 = 2.0%, 95% confidence interval 0.2%–7.0%). This might be lower than in North America, but more patients must be screened to confirm this trend.

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