

# Rhodopsin mutations in Chinese patients with retinitis pigmentosa

Wai Man Chan, Kwun Yan Yeung, Chi Pui Pang, Larry Baum, Tung Ching Lau, Alvin Kwan Ho Kwok, Dennis Shun Chiu Lam

## 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%).

(Br J Ophthalmol 2001;85:1046-1048)

Mutations in the gene encoding rhodopsin (*RHO*) lead to retinal degeneration in species from *Drosophila*<sup>1</sup> to humans. *RHO* mutations cause autosomal dominant retinitis pigmentosa (ADRP), although a few mutations cause recessive RP or congenital night blindness,<sup>2-6</sup> and account for about 25% of ADRP in Europe<sup>7,8</sup> and North America,<sup>9-11</sup> and less in Japan.<sup>12,13</sup> 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.<sup>14</sup>

## 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,

Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, China

W M Chan  
K Y Yeung  
C P Pang  
L Baum  
T C Lau  
A K H Kwok  
D S C Lam

Correspondence to:  
Dr C P Pang, Hong Kong Eye Hospital 3/F, 147K Argyle Street, Kowloon, Hong Kong  
cppang@cuhk.edu.hk

Accepted for publication  
12 March 2001

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	ctg	ggt	gac	gat	gag	gcc	tct	gct	acc	gtg	tcc	aag	acg	gag	acg	agc	cag	gtg	gcc	ccg	gcc	tag
P	P	L	G	D	D	E	A	S	A	T	V	S	K	T	E	T	S	Q	V	A	P	A	
				-	-	-	*	*	*	*	-	*	*										
1 bp Deletion	cac	tgg	gtg	acg	atg	agg	cct	ctg	cta	ccg	tgt	cca	aga	cgg	aga	cga	gcc	agg	tgg	ccc	ccg	cct	aag
H	W	V	T	M	R	P	L	L	P	C	P	R	R	R	R	A	R	R	W	P	R	P	K
	+		*		+							+	+	+	+					+			+
	acc	tgc	cta	gga	ctc	tgt	ggc	cga	cta	tag													
	T	C	L	G	L	C	G	R	L	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.<sup>15-17</sup> 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,<sup>18</sup> 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.<sup>19-22</sup> Mutations in these amino acids cause severe ADRP.<sup>23-25</sup>

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, Val104Ile<sup>26</sup> and Phe220Leu.<sup>9</sup> Sung *et al*<sup>27</sup> 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.<sup>28</sup> 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.<sup>29</sup> There, the most frequent mutation, Pro23His, accounts for one third of rhodopsin induced cases but probably arose in a single ancestor.<sup>9</sup> It occurs nowhere else. Excluding Pro23His, the proportion of ADRP families with rhodopsin mutations is 16-18%,<sup>7,9</sup> or about 6-7% of all RP cases, consistent with Japanese studies.<sup>12,13</sup> 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.

Sponsor: Industrial Support Fund, Li Ka Shing Foundation, Hong Kong.

- Colley NJ, Cassill JA, Baker EK, *et al*. Defective intracellular transport is the molecular basis of rhodopsin-dependent dominant retinal degeneration. *Proc Natl Acad Sci USA* 1995;92:3070-4.
- Humphries P, Kenna P, Farrar GJ, *et al*. On the molecular genetics of retinitis pigmentosa. *Science* 1992;256:804-8.
- Rosenfeld PJ, Cowley GS, McGee TL, *et al*. A null mutation in the rhodopsin gene causes rod photoreceptor dysfunction and autosomal recessive retinitis pigmentosa. *Nat Genet* 1992;1:209-13.
- Dryja TP, Berson EL, Rao VR, *et al*. Heterozygous missense mutation in the rhodopsin gene as a cause of congenital stationary night blindness. *Nat Genet* 1993;4:280-3.
- Sieving PA, Richards JE, Bingham EL, *et al*. Dominant congenital complete nyctalopia and gly90-to-asp rhodopsin mutation. *Invest Ophthalmol Vis Sci* 1992;33:(Abstract 1397).
- Al-Jandal N, Farrar GJ, Kiang AS, *et al*. A novel mutation within the rhodopsin gene (thr94 to ile) causing autosomal dominant congenital stationary night blindness. *Hum Mutat* 1999;13:75-81.
- Bunge S, Wedemann H, David D, *et al*. Molecular analysis and genetic mapping of the rhodopsin gene in families with autosomal dominant retinitis pigmentosa. *Genomics* 1993;17:230-3.
- Inglehearn CF, Keen TJ, Bashir R, *et al*. A completed screen for mutations of the rhodopsin gene in a panel of patients with autosomal dominant retinitis pigmentosa. *Hum Mol Genet* 1992;1:41-5.
- Dryja TP, McEvoy JA, McGee TL, *et al*. Novel rhodopsin mutations Gly114Val and Gln184Pro in dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2000;41:3124-7.
- Sung CH, Schneider BG, Agarwal N, *et al*. Functional heterogeneity of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa. *Proc Natl Acad Sci USA* 1991;88:8840-4.
- Macke JP, Davenport CM, Jacobson SG, *et al*. Identification of novel rhodopsin mutations responsible for retinitis pigmentosa: implications for the structure and function of rhodopsin. *Am J Hum Genet* 1993;53:80-9.
- Shiono T, Hotta Y, Noro M, *et al*. Clinical features of Japanese family with autosomal dominant retinitis pigmentosa caused by point mutation in codon 347 of rhodopsin gene. *Jpn J Ophthalmol* 1992;36:69-75.
- Saga M, Mashima Y, Akeo K, *et al*. A mutation in codon 181 (Glu→Lys) of the rhodopsin gene in a Japanese family. *Ophthalmol Genet* 1994;15:61-7.
- Nathans J, Hogness DS. Isolation and nucleotide sequence of the gene encoding human rhodopsin. *Proc Natl Acad Sci USA* 1984;81:4851-5.
- Sibley DR, Benovic JL, Caron MG, *et al*. Regulation of transmembrane signaling by receptor phosphorylation. *Cell* 1987;48:913-22.
- Ohguro H, Van Hooser JP, Milam AH, *et al*. Rhodopsin phosphorylation and dephosphorylation in vivo. *J Biol Chem* 1995;270:14259-62.
- McDowell JH, Robinson PR, Miller RL, *et al*. Only phosphorylated residues in the carboxyl terminal region of rhodopsin will activate arrestin. *Invest Ophthalmol Vis Sci* 2000;41:S608.
- Horn M, Humphries P, Kunisch M, *et al*. Deletions in exon 5 of the human rhodopsin gene causing a shift in the reading frame and autosomal dominant retinitis pigmentosa. *Hum Genet* 1992;90:255-7.

- 19 Deretic D, Schmerl S, Hargrave PA, et al. Regulation of sorting and post-Golgi trafficking of rhodopsin by its C-terminal sequence QVS(A)PA. *Proc Natl Acad Sci USA* 1998;**95**:10620–5.
- 20 Sung CH, Makino C, Baylor D, et al. A rhodopsin gene mutation responsible for autosomal dominant retinitis pigmentosa results in a protein that is defective in localization to the photoreceptor outer segment. *J Neurosci* 1994;**14**:5818–33.
- 21 Chuang JZ, Sung CH. The cytoplasmic tail of rhodopsin acts as a novel apical sorting signal in polarized MDCK cells. *J Cell Biol* 1998;**142**:1245–56.
- 22 Green ES, Menz MD, LaVail MM, et al. Characterization of rhodopsin mis-sorting and constitutive activation in a transgenic rat model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 2000;**41**:1546–53.
- 23 Berson EL, Rosner B, Sandberg MA, et al. Ocular findings in patients with autosomal dominant retinitis pigmentosa and rhodopsin, proline-347-leucine. *Am J Ophthalmol* 1991;**111**:614–23.
- 24 Sandberg MA, Weigel-DiFranco C, Dryja TP, et al. Clinical expression correlates with location of rhodopsin mutation in dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 1995;**36**:1934–42.
- 25 Macke JP, Hennessey JC, Nathans J, et al. Rhodopsin mutation proline347-to-alanine in a family with autosomal dominant retinitis pigmentosa indicates an important role for proline at position 347. *Hum Mol Genet* 1995;**4**:775–6.
- 26 Macke JP, Davenport CM, Jacobson SG, et al. Identification of novel rhodopsin mutations responsible for retinitis pigmentosa: implications for the structure and function of rhodopsin. *Am J Hum Genet* 1993;**53**:80–9.
- 27 Sung CH, Davenport CM, Hennessey JC, et al. Rhodopsin mutations in autosomal dominant retinitis pigmentosa. *Proc Natl Acad Sci USA* 1991;**88**:6481–5.
- 28 Horn F, Weare J, Beukers MW, et al. GPCRDB: an information system for G protein-coupled receptors. *Nuc Acids Res* 1998;**26**:277–81.
- 29 Berson EL. Retinitis pigmentosa: Unfolding its mystery. *Proc Natl Acad Sci USA* 1996;**93**:4526–8.
- 30 Dryja TP, Hahn LB, Cowley GS, et al. Mutation spectrum of the rhodopsin gene among patients with autosomal dominant retinitis pigmentosa. *Proc Natl Acad Sci USA* 1991;**88**:9370–4.
- 31 Kawano H, Hotta Y, Fujiki K, et al. A study on the rhodopsin gene in Japanese retinitis pigmentosa—screening of mutation by restriction endonucleases and frequencies of DNA polymorphisms. (Abstract) *Nippon Ganka Gakkai* 1995;**99**:1151–7.
- 32 Dryja TP, McGee TL, Hahn LB, et al. Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. *N Engl J Med* 1990;**323**:1302–7.

### Contributors please note:

Communications from **all countries except the UK and Republic of Ireland** should be sent to Professor C Hoyt, Editor, *British Journal of Ophthalmology*, University of California, Department of Ophthalmology, 10 Kirkham Street, K 301, San Francisco, CA 94143-0730, USA (tel: 001 415 502-6871; fax: 001 415 514-1521).

Manuscripts from the **UK and the Republic of Ireland** should be sent to Professor Andrew Dick, UK Editor, *British Journal of Ophthalmology*, Division of Ophthalmology, University of Bristol, Lower Maudlin Street, Bristol BS1 2LX (tel: +44 (0) 0117 929-4496; fax: +44 (0)117 929-4607).



## Rhodopsin mutations in Chinese patients with retinitis pigmentosa

Wai Man Chan, Kwun Yan Yeung, Chi Pui Pang, et al.

*Br J Ophthalmol* 2001 85: 1046-1048

doi: 10.1136/bjo.85.9.1046

---

Updated information and services can be found at:

<http://bjo.bmj.com/content/85/9/1046.full.html>

---

### References

*These include:*

This article cites 29 articles, 16 of which can be accessed free at:

<http://bjo.bmj.com/content/85/9/1046.full.html#ref-list-1>

Article cited in:

<http://bjo.bmj.com/content/85/9/1046.full.html#related-urls>

### Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

---

### Topic Collections

Articles on similar topics can be found in the following collections

[Retina](#) (1217 articles)

[Eye \(globe\)](#) (543 articles)

---

### Notes

---

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>