# A PHOTORECEPTOR GENE MUTATION IN AN INDIGENOUS BLACK AFRICAN FAMILY WITH RETINITIS PIGMENTOSA IDENTIFIED USING A RAPID SCREENING APPROACH FOR COMMON RHODOPSIN MUTATIONS

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Hereditary retinal degenerations may be subdivided into those affecting predominantly the central (macular) or peripheral regions of the retina. Retinitis pigmentosa (RP) affects the photoreceptors; death of the rod cells is followed by a progressive loss of cone cells, resulting in relatively early loss of peripheral vision and progressive constriction of the visual fields. A mutation in the gene encoding the photoreceptor protein, rhodopsin, was the first molecular defect identified as a potential cause of inherited retinal degeneration (RD). In the study reported here, simple tests for rhodopsin involvement in 194 southern African patients with a history of retinal degeneration, including 14 black African patients, were performed. Two RP patients were identified with disease-causing mutations in the rhodopsin gene: one from a black African family in which a codon 347 mutation resulted in a Pro-Leu substitution, and one in a family of Caucasian origin where a codon 58 alteration resulted in a Thr-Arg substitution. This is the first report of a disease-causing rhodopsin mutation in an indigenous black African family with retinitis pigmentosa.

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Retinitis pigmentosa (RP) is a group of hereditary retinal degenerative (RD) disorders characterised by poor night vision and progressive constricted visual fields resulting in partial or total blindness. The reported frequency of RP is about 1 in 3 000;[1] however, in southern Africa no accurate incidence of the condition is known. From clinical records it appears that in southern Africa the condition is inherited as an autosomal dominant condition in 32% of families, as an autosomal recessive trait in 11% of families, and in an X-linked recessive manner in 4% of families.[2]

In 1989, McWilliam *et al.*[3] reported the localisation of the first autosomal dominant retinitis pigmentosa (ADRP) locus on chromosome 3q21.[3] Subsequent screening of the obvious candidate genes in that region revealed many patients with RP who had mutations in the rhodopsin gene.[4] Of particular note was the observation that one single base-change (in codon 23) correlated with the disease in about 12% of ADRP patients in the USA. This mutation has not been reported at such a high frequency in other populations and it is believed to represent a founder effect for RP in the USA, with origins in the UK.[5] So far, the reported frequencies of rhodopsin mutations in ADRP patients throughout the world range from 20% to 31%, while in the UK the figure appears to be as high as 50%.[6] On the basis of these figures it could therefore be anticipated that among the 194 patients with retinal degeneration investigated in this study, of whom 45 have a definite ADRP pattern of inheritance, at least 9 might have a mutation in the rhodopsin gene.[6]

## RETINITIS PIGMENTOSA IN SOUTHERN AFRICA

In 1993, exclusion of linkage to the D3S47 locus on 3q in a large southern African family of British origin was reported.[7] The ADRP locus (RP13) in this kindred was subsequently mapped to 17p13.3.[8,9] In 1995, Bardien *et al.*[10] reported a second novel southern African RP locus (RP17) at 17q24 in a family of German origin, thus indicating that the gene pool for retinal degeneration in southern Africa is possibly different from that in other parts of the world.

Although more than 80 different mutations in the rhodopsin gene have been identified in RP patients from all over the world,[1] only 2 mutations have been reported in different populations: the Pro-347-Leu amino acid substitution has been reported in the USA, UK, Germany and Japan, and the substituted Thr-58-Arg has been reported in the USA and UK. It has therefore been suggested that these might possibly be mutation hotspots in the rhodopsin gene.[6] Both these mutations are in CpG dinucleotides, which is why they are probably hotspots. Screening AD families as well as simplex cases of RP using a simple polymerase chain reaction (PCR)-based assay involving amplification of 2 exons followed by a restriction enzyme digest was considered to be a worthwhile rapid-screening approach. Such a screen of 120 patients in the UK revealed about 6% of ADRP patients with mutations in codon 58 and 347 of the rhodopsin gene.[6]

## RAPID SCREEN FOR COMMON RHODOPSIN MUTATIONS

In the study reported here a total of 194 southern African patients with RD were screened for the codon 58 and 347

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mutations known to create or destroy a DdeI or MspI restriction enzyme site, respectively. Of the patients studied, 45 were from ADRP families and 19 were classified as simplex cases. Of these southern African families with a history of RD, 14 were of ethnic black African origin, 5 of Asian origin and 26 of mixed ancestry (San, Khoi-Khoi, West African Negro, Madagascar, Javanese and western European origin).

One patient from an ethnic black African ADRP family (RPD45.1) was found to carry the codon 347 mutation (CCG-CTG) that destroys a MspI restriction site. One ADRP patient of Caucasian origin (RPD 199.1) was found to have the codon 58 mutation (ACG-AGG) that creates a DdeI restriction site. The respective extended family members available for study were then examined with the same rapid-screen approach.

Following a MspI restriction enzyme digest of the PCR product of a rhodopsin exon 5 amplification, the altered larger fragment (220bp) was also identified in the affected parent of the index patient (RPD45.1). In the other ADRP family (RPD199) the index patient's affected daughter also carried the same mutation and this mutation was absent in a clinically unaffected sibling. Subsequent sequence analysis of the PCR product confirmed the same base change that results in the predicted amino acid change in each case: Pro-347-Leu (RPD 45) and Thr-58-Arg (RPD199).

Apart from a novel potential disease-causing mutation that was recently reported in exon 1 of the rhodopsin gene in one southern African ADRP family,[11] single-stranded confirmation polymorphism (SSCP) analysis of all 5 exons of the rhodopsin gene has been performed, and to date no other disease-causing mutations have been identified in our patient cohort of 194 individuals (unpublished data).

## Discussion

This study supports the findings of Tarttelin *et al.*[6] that ADRP families can be assessed rapidly and efficiently for two common rhodopsin disease-causing mutations (codon 58 and 347); this is the first report of a disease-causing rhodopsin mutation in an indigenous black African family with RP. It should also be noted that correlations have been documented between specific rhodopsin mutations and the severity of RP in certain patients. In some cases the Pro-23-His mutation is less severe and the Pro-347-Leu mutation more severe than the average clinical phenotype observed in patients with the autosomal dominant form of RP.[12,13]

Undoubtedly many genes that cause inherited retinal degeneration will be identified within the next few years. The most important question now is: 'What will this mean to the patient?' In the short term it will mean accurate diagnosis, prognosis and management. As has been demonstrated here and reported recently,[11] it is already possible to diagnose the presymptomatic gene carrier in southern African families in which a rhodopsin mutation has been identified as the disease-causing gene. In addition, it is also possible to predict the clinical course of the condition in certain of these forms of RP.

In the long term, it should be possible to design specialised therapies based on prior knowledge of the biological defect. It has been proposed that such therapy should be relatively easy in RD as direct access to the back of the retina and the sub-retinal space should make it possible to direct the gene therapy and place the therapeutic agent in direct contact with the target tissue. One constraint is that RD affects the retina and the cells of the mature retina do not divide, so the use of viral vectors that integrate into replicating DNA is precluded. However, there have already been reports of successful uptake of vectors and expression of the altered protein in the photoreceptor cells of affected RP animals.[14]

Clearly, within the next decade effective gene therapy for some forms of RD in humans will be possible, but once again these will be mutation-specific, so it is essential to identify the retinal disease-causing genes in each and every southern African RD patient.

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