

A novel mutation in the short-wavelength-sensitive cone pigment gene associated with a tritan color vision defect

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Abstract

Inherited tritan color vision deficiency is caused by defects in the function of the short-wavelength-sensitive (S) cones. This heterozygous group of disorders has an autosomal dominant pattern of inheritance. Amino acid variations of the S cone opsin are rare and all that have been identified thus far are associated with inherited tritan color vision defects. Here we report the identification of a 30-year-old male who made errors on standard color vision tests consistent with the presence of a mild tritan color vision deficiency. We tested the hypothesis that his color vision impairment was due to a mutation in the S cone photopigment gene. He was found to be heterozygous for a mutation that caused the amino acid proline to be substituted in place of a highly conserved leucine at amino acid position 56 in the S cone opsin. This mutation was absent in 564 S cone photopigment genes from 282 subjects who did not make tritan errors. Thus, we conclude that this mutation disrupts the normal function of S cones.

Keywords: Inherited tritanopia, S-cone opsin mutation, Photopigment, Color vision

Introduction

The molecular genetic basis for normal color vision and for the common inherited color vision deficiencies has been elucidated in recent decades. Genes encoding the long- (L) and middle-wavelength-(M) sensitive cone opsins are arrayed in tandem on the X-chromosome, and the gene for the short-wavelength-(S) sensitive cone opsin is on chromosome 7 (Nathans et al., 1986*b*). Each adult L or M cone photoreceptor expresses a single opsin gene from the X-chromosome array (Wang et al., 1999; Smallwood et al., 2002; Neitz et al., 2003). Males have one X-chromosome and females have two; however like males, females express an opsin gene from only one X-chromosome in each L or M photoreceptor as a consequence of X-inactivation. In contrast, each S cone expresses both of the autosomally encoded S opsin genes.

Inherited color vision deficiencies arise through mutations, deletions, and rearrangements of the genes that encode the L, M, and S opsins. By far the most common color vision defects are red–green defects, which affect about one in 12 Caucasian males and one in 230 females. In contrast, inherited tritan deficiencies are rare; however, obtaining a reliable estimate has suffered from the lack of good diagnostic criteria (Went & Pronk, 1985), and a large degree of phenotypic variability (Cole et al., 1966; Neuhann et al., 1976; Went & Pronk, 1985). Early estimates placed the frequency

of affected individuals at between one person in 13,000 to one in 65,000 (Wright, 1952; Kalmus, 1955), but more recently frequencies of one in 500 have been estimated from screenings done with more sensitive testing instruments (van Norren & Went, 1981; Went & Pronk, 1985).

Red–green and tritan color vision defects differ in the underlying genetic mechanisms. Red–green color vision deficiencies, which include protan and deutan defects, are associated with the absence of L or M cone contribution to vision, respectively. People with normal color vision most often have an L opsin gene followed by one or more M opsin genes on the X-chromosome. With rare exception, only the first two genes in the array, one L and one M, are expressed, and their expression is mutually exclusive (Hagstrom et al., 2000), thereby creating separate populations of L and M cones. Rearrangements of the X-chromosome opsin gene locus that produce arrays with a single visual pigment gene, or arrays in which the first two genes specify opsins with similar spectral properties, are the most common causes of protan and deutan defects (Nathans et al., 1986*a*; Neitz et al., 1995, 1999; Sharpe et al., 1998; Jagla et al., 2002). A relatively less common cause is the presence of an inactivating mutation in the first or second gene that prevents expression of a functional photopigment (Winderickx et al., 1992; Jagla et al., 2002; Neitz et al., 2004).

In contrast, the only known causes of inherited tritan color vision deficiencies are nucleotide replacements within the S opsin gene that lead to amino acid substitutions that are predicted to interfere with the structure and function of the photopigment (Weitz et al., 1992*a,b*), which, in turn, lead to a reduction or loss

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of S cone function. Tritan defects are autosomal dominant with incomplete penetrance (Wright, 1952; Padmos et al., 1978), so heterozygotes, who presumably have S cones containing both normal and mutant pigments, are often affected. However, it is uncertain whether inherited tritan color vision defects are congenital in the strict definition of the word, that is, present at birth. It may be that some individuals who carry a gene for a tritan defect and test as normal will manifest the color vision deficiency later in life.

Variation in the S opsin gene is rare (Weitz et al., 1992a; Shimmin et al., 1997, 1998), and all nucleotide substitutions that alter the amino acid sequence of the S pigment have been associated with tritan defects (Weitz et al., 1992a,b). In the present study, we identified an individual whose performance on standard color vision tests was consistent with a mild tritan color vision defect. We tested the hypothesis that his color vision impairment was due to mutations in the S pigment. The subject was found to be heterozygous for a novel mutation that substituted the amino acid proline for a highly conserved leucine residue in the S cone pigment. The mutation was absent from 282 control subjects.

Materials and methods

Color vision testing

Mass screening for color vision deficiencies was performed using the Neitz Test of Color Vision on 296 males and 238 females ranging in age from 21 years old to 31 years old. Only one subject (identification number 4041) incorrectly identified the colored symbols in both of the tritan panels. He made no other errors. He was later retested with the Neitz Test of Color Vision (Neitz & Neitz, 2001) as well as a battery of other standard color vision tests including the AO-HRR 1955 edition and Richmond-HRR 2002 edition pseudoisochromatic plates, the Farnsworth-Munsell (FM) 100 Hue, Farnsworth D15, and Lanthony's Desaturated D15 Hue tests. All tests were performed twice, with and without a Verilux Full Spectrum easel lamp. Color vision testing in control subjects was done with these same color vision tests except that some control subjects were given only a subset of the color vision tests, and some were also tested on the Neitz anomaloscope.

Subjects

Subject 4041, a 30-year-old male in good health, was the study subject suspected of having an inherited tritan color vision deficiency. Control subjects were 83 males and 3 females with con-

genital red-green color vision deficiencies and 126 males and 70 females with normal color vision. Experiments involving human subjects were conducted in accordance with the principles embodied in the declaration of Helsinki, and were approved by the Institutional Review Board at the Medical College of Wisconsin.

DNA isolation and genetic analyses

Whole blood was obtained from each subject. Genomic DNA was extracted from blood using standard methods. First, for the tritan subject (#4041) the five exons that comprise the amino acid coding region of the S opsin genes were amplified in the polymerase chain reaction (PCR), and sequenced using the primer pairs listed in Table 1. Each exon was amplified in a hotstart reaction using the XL-PCR kit and AmpliWax Gems (Applied Biosystems, Foster City, CA). Each reaction contained 200 μ M each of dATP, dCTP, dTTP and dGTP, 1.4 mM Mg(OAc)₂, 300 nM of each primer, and 1 ng of genomic DNA. The final reaction volume was 100 μ l. Thermal cycling conditions were one cycle of 94°C for 5 min, followed by 37 cycles of 94°C for 45 s, 58°C for 45 s, and 72°C for 45 s, followed by one cycle of 72°C for 7 min.

Amplified DNA was purified and sequenced directly using the ABI Prism BigDye Terminator sequencing kit, version 3.0 (Applied Biosystems). Sequencing primers were the same ones that were used for amplification. Sequencing reactions were analyzed on an ABI Prism 310 Genetic Analyzer.

Subject 4041 was heterozygous for a nucleotide polymorphism in codon 56 in exon 1 of the S opsin gene. Mutation screening was performed to determine whether the same polymorphism occurred in a control population. For 282 control subjects who did not make tritan errors, we used denaturing high-performance liquid chromatography (dHPLC) to detect heteroduplex formation as a rapid and efficient mutation screening method. Heteroduplexes form whenever two nonidentical strands of DNA are combined. A 249-base pair segment spanning exon 1 of the S cone photopigment gene was amplified from genomic DNA from each subject with primers Sx1mAA56F and Sx1mAA56R (see Table 1) in touchdown PCR. Each reaction consisted of 50 μ l, containing 1 mM MgCl₂, 300 nM of each primer, and 0.25 μ l of a 9:1 (volume:volume) mixture of AmpliTaq Gold DNA polymerase (Applied Biosystems) and Pfu DNA polymerase (Stratagene, La Jolla, CA). The thermal cycling conditions used for touchdown PCR were 1 cycle of 95°C for 10 min, followed by 14 cycles of 94°C for 20 s, annealing temperature for 1 min, and 72°C for 1 min, where the annealing temperature began at 62°C and was reduced by 0.5°C per cycle thereafter. This was followed by 20 cycles of 94°C for 20 s, 55°C

Table 1. PCR primers for S cone photopigment gene

Primer pair	Amplicon	Name	Sequence	Location
1	exon 1	B-31-11Fwd	5' AAGAGGACTCAGAGGAGGGTGTG	31 bp upstream of exon 1
		B377-358R	5' CTAACCCCTTTTCCCTGC	intron 1, 20 bp downstream of exon 1
2	exon 3	B1048-69F	5' GGAGAACAACAATCCAGGCATCT	intron 2, 69 bp upstream of exon 3
		B1320-1298	5' TCTTCCCTGACTATCAAATGCCA	intron 3, 29 bp downstream of exon 3
3	exon 4	B1870-90F	5' AGATGTGATGCTTTCCGTGCT	intron 3, 30 bp upstream of exon 4
		B2170-45R	5' GGACCATAGGAATGTGAATAAAGAGC	intron 4, 23 bp downstream of exon 4
4	dHPLC	Sx1mAA56F	5' GATGGGCCTCAGTACCACAT	71 bp into exon 1
		Sx1mAA56R	5' GCGACCGAAGACGAAGTATC	61 bp from 3' end of exon 1

for 1 min, and 72°C for 1 min. Reactions were incubated at 72°C for 10 min. To maximize the formation of heteroduplexes, if present, an aliquot of amplified DNA from each subject was incubated at 95°C for 5 min then cooled at the rate of 0.5°C per 20 s to 48°C. The samples were then assayed for the presence of heteroduplexes using the WAVE-MD dHPLC System (Transgenomic, Omaha, NE) at a column temperature of 62.3°C. Heteroduplexes are expected for any subject who is heterozygous for a nucleotide difference in the amplified region. For all subjects whose DNA showed heteroduplexes, the amplicons were sequenced on the ABI Prism 310 with primers Sx1mAA56F and Sx1mAA56R. Direct sequencing of exon 1 was also done for 18 control subjects who gave ambiguous results in the heteroduplex analysis.

Subjects homozygous for 4041's mutation would not have shown heteroduplexes in the above assay. Thus, each control sample was also analyzed for heteroduplex formation with the wild-type and with the mutant allele from subject 4041. We cloned the mutant and wild-type alleles from subject 4041 using standard methods. Wild-type and mutant alleles were separately mixed with exon 1 amplified from each control subject's S opsin genes and analyzed for heteroduplexes, as described above. This analysis served to demonstrate that exon 1 of the S opsin gene is not highly polymorphic, and to validate the heteroduplex method of detecting polymorphism.

Results

The Neitz Test of Color Vision, which contains six panels that test for protan and deutan defects and two panels that test for tritan defects, was used to screen for color vision deficiencies in 296 males and 238 females. The test was administered in a large lecture hall with incandescent illumination (average illuminance = 150 Lux, with a range of approximately 50–250 Lux depending on location in room). One subject (identification number 4041) did not correctly identify either of the tritan stimuli. No other subject made the same pattern of errors. Subject 4041 was retested with the Neitz Test of Color Vision under cool white fluorescent illumination (520 Lux) and under a Verilux Full Spectrum easel lamp that approximates illuminant C (color temperature = 7600°K; 900 Lux). Under these higher illumination levels 4041 did not make any errors on the Neitz test. The Richmond-HRR Test 2002 edition was administered under the same fluorescent and Verilux illumination conditions. Subject 4041 was unable to identify the most desaturated tritan symbol in the test (the triangle on plate 2). He made this error on two administrations of the test. He performed correctly on all of the other tritan stimuli. He did not correctly identify the protan and deutan stimuli on plate 7 when the test was administered under the Verilux Full Spectrum easel lamp, but he correctly identified them under standard fluorescent room lighting. He made no errors on the American Optical HRR 1955 edition. Subject 4041 made a pattern of errors on the FM100 Hue test that was consistent with a mild tritan defect (Fig. 1). The FM100 was administered twice; his scores on the first and second trials were 110 and 84, respectively. The error score range for individuals with normal color vision, ages 30–39 years, is 0–80 (95% CI) (Kinnear & Sahraie, 2002). When his error scores were divided into those falling on the red–green axis and those falling on the blue–yellow axis, he showed significantly more errors on the blue–yellow axis: partial error score of 3.43 (caps 1–12, 34–54, and 76–84), compared with a red–green axis partial error score of 2.85 (caps 13–33 and 55–75) (Smith et al., 1985). This yields a *P*-value of 0.0008 ($t(82) = 3.49$). Subject 4041 made tritan errors on one of two

administrations of Lanthony's Desaturated D15 test, with the cap order 1, 2, 3, 5, 4, 6, 7, 15, 14, 13, 12, 11, 8, 9, 10. On the Farnsworth Panel D15 test, his performance was perfect on the first administration, but he reversed the first two caps upon retesting. Taken together, subject 4041's performance suggested that he suffered from a mild inherited tritan color vision deficiency.

We hypothesized that subject 4041 had an inherited tritan color vision deficiency that was caused by a defect in the S cone photopigment gene. To test this hypothesis, we PCR amplified and sequenced the coding regions of his S opsin genes, which are comprised of five exons. Three mutations have been previously reported to be associated with tritanopia (Weitz et al., 1992*a,b*), none of which were found in subject 4041. Instead, he was heterozygous for a single nucleotide change that caused the amino acid proline to be substituted for leucine at position 56 (L56P) of the S cone photopigment molecule (Figs. 2 and 3). This was the only mutation found in subject 4041. In addition, he does have a silent polymorphism at amino acid 122 (silent polymorphisms have changes in nucleotide but code for the same amino acid in the protein). This polymorphism has also been seen by Shimmin et al. (1998).

To determine whether the L56P missense mutation was present in control subjects who did not make tritan errors, we used dHPLC to screen for heteroduplex formation in PCR amplified DNA spanning codon 56 in the S opsin gene in genomic DNA from 282 control subjects, representing 564 S cone photopigment genes. Subjects who are heterozygous for a polymorphism in the amplified region are expected to show homoduplexes and heteroduplexes, whereas subjects who are homozygous across the region will yield only homoduplexes (Fig. 4). Heteroduplexes were observed for four of the 282 control subjects (Fig. 4, middle panel). DNA sequence analysis revealed that all four subjects were heterozygous for an A to C nucleotide substitution in the third position of codon 74. This is a silent substitution that does not alter the amino acid sequence.

Any subject who was homozygous for 4041's mutation would only show homoduplexes. Thus, we combined an aliquot of amplified DNA from each control subject with an aliquot of amplified DNA from 4041's mutant allele. All subjects showed heteroduplexes, indicating that none were homozygous for 4041's mutation. In addition, an aliquot of amplified DNA from each control subject was mixed with the corresponding amplified segment from subject 4041's wild-type allele. Heteroduplexes were detected only for the four control subjects who were heterozygous for the codon 74 polymorphism, indicating that all but these four subjects are homozygous for the wild-type allele. As a control, the region subjected to mutation screening was sequenced for 18 of the control subjects, and all were found to be identical to subject 4041's wild-type allele.

Discussion

We have identified a novel missense mutation in the S opsin gene that is associated with a tritan color vision deficiency. The evidence that subject 4041 has a tritan color vision deficiency is two-fold. First, he made a pattern of errors on four different tests of color vision that people who have normal color vision or a red–green color vision defect did not make. He did not reliably see tritan stimuli in the Neitz Test of Color Vision or in the Richmond-HRR 2002 edition, and on the Lanthony's Desaturated D15 and the FM100 Hue tests his performance was consistent with a mild tritan defect. Second, subject 4041 was heterozygous for a mutation in

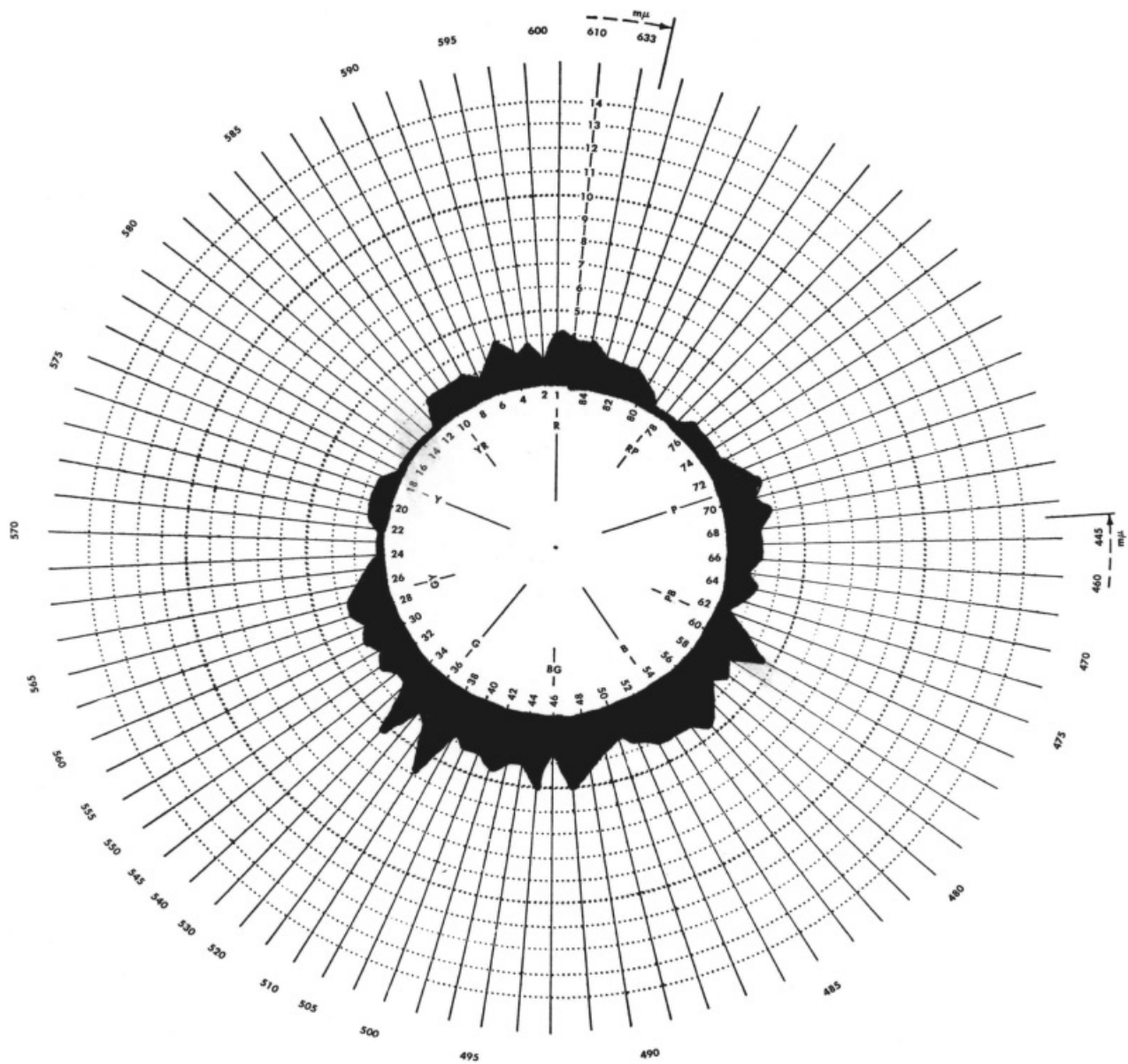


Fig. 1. Results of the Farnsworth-Munsell 100 Hue test for subject 4041. Shown are the average errors of the two testings. The partial error score along the blue-yellow axis is significantly greater than the partial error score along the red-green axis (see text).

the S cone opsin gene that substitutes a proline for a highly conserved leucine residue (L56P) in the first transmembrane helix of the S pigment. This mutation was absent from the 564 S opsin genes from control subjects that we examined. The results of the heteroduplex analysis demonstrated that exon 1 of the S opsin gene is not generally polymorphic, lending support to the idea that the mutation found in subject 4041 is the cause of his color vision deficit. In addition, the discovery of a second nucleotide substitution that does not alter the amino acid sequence demonstrates the sensitivity of heteroduplex analysis as a mutation screening method in our hands. The probability that the L56P mutation is not associated with the color vision loss in this subject is 0.0035 (Fisher's exact test).

In addition, leucine 56 is highly conserved among all members of the vertebrate rhodopsin family. There are 212 vertebrate rhodopsin sequences in the G-protein coupled receptor database (ww-

w.gpcr.org), of which 88% have leucine at the position corresponding to 56 in the human S opsin, 9% have isoleucine, and 4% have valine. None have proline. Of the 47 S-opsin gene sequences in the GenBank database that were of the same class of opsin as the human S opsin (OPN1SW or SWS1), all specified leucine at amino acid position 56. The organisms represented by these S opsin sequences include fish, birds, reptiles, amphibians, and mammals. Kinks caused by introducing a proline residue into an alpha-helix is a well-documented cause of disrupted protein function (Pakula & Sauer, 1989), and it is very likely that the proline substitution causes misfolding of the S cone photopigment and S cone dysfunction in subject 4041.

Investigation of the molecular mechanisms that underlie vision loss associated with S opsin mutations have been neglected in comparison to those associated with mutations in L and M opsin. Amino acid substitutions that shift the spectrum of the L and M

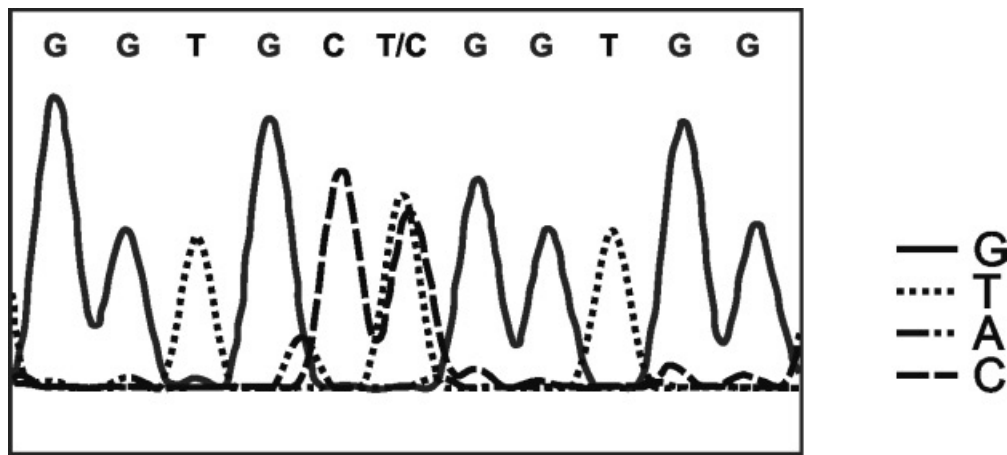


Fig. 2. Sequencing electropherogram showing that subject 4041 is heterozygous at the second nucleotide position of codon 56 of the S cone photopigment gene. A thymine (T) and cytosine (C) residue are both present in the second position of codon 56, so that the wild-type copy of the gene (CTG) encodes leucine and the mutant copy (CCG) encodes proline.

pigments are a major cause of red–green color vision deficiency. It is not clear how shifting the spectrum of the S opsin might similarly lead to tritan defects. In any case, the proline substitution described here is unlikely to produce a spectral shift.

The extensive work on the involvement of mutations in rhodopsin as a cause of autosomal dominant retinitis pigmentosa

(adRP) might, by analogy, provide insight into the molecular defect in inherited tritanopia. The form of autosomal dominant retinitis pigmentosa associated with rhodopsin mutations is a degenerative disease that shows variability in the age at onset and in the rate of progression. People with adRP often do not complain of impaired vision until late childhood. There is no variation in the

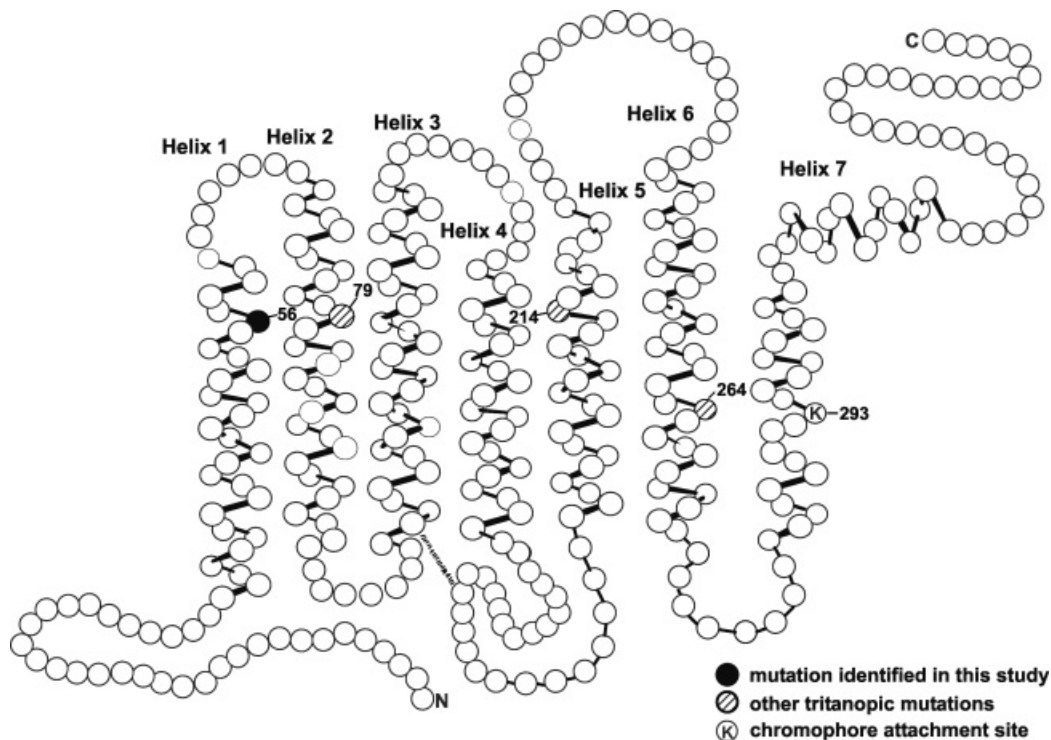


Fig. 3. Model of transmembrane topography of the S opsin. Circles represent amino acids. The seven transmembrane alpha helical segments are labeled. The lysine (K) at position 293 is the residue to which the chromophore is bound. The hatched circles show the locations of the three previously reported amino acid substitutions associated with tritan color vision defects: G79R, S214P, and P264S. The black circle in transmembrane helix I indicates the location of the L56P substitution found in the male in this study who made tritan errors in standard color vision tests (subject 4041).

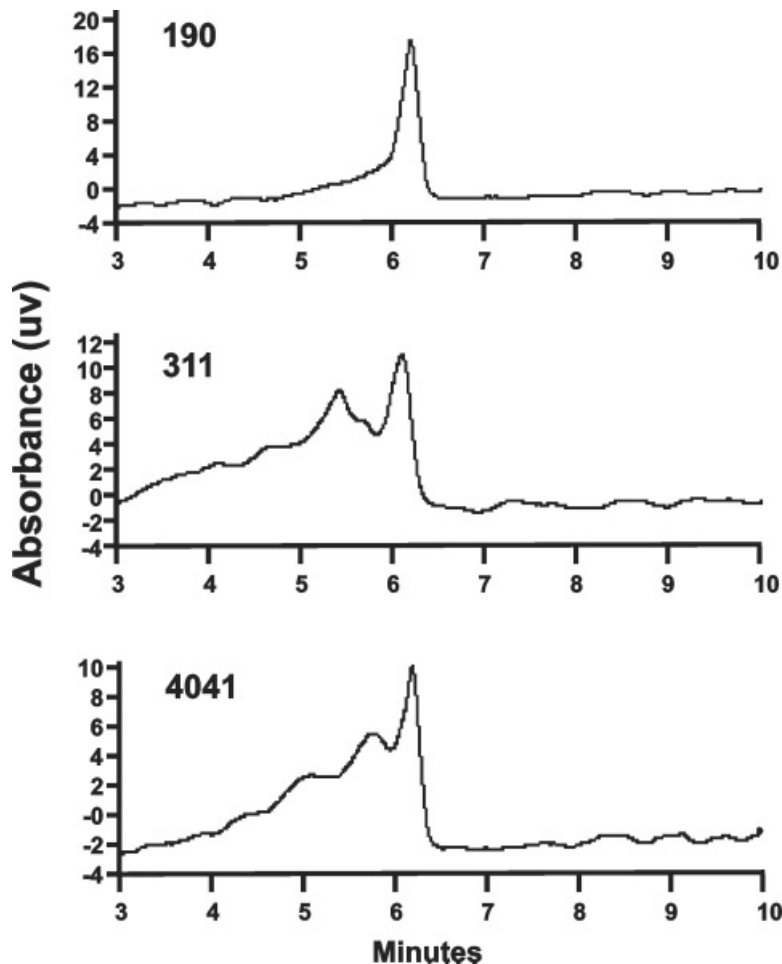


Fig. 4. Chromatograms showing heteroduplex analysis by dHPLC. *Top panel:* chromatogram from a control subject (subject #190) who is homozygous for the wild-type sequence. The homoduplex peak appears just after 6 min. *Middle panel:* chromatogram from a control subject (subject #311) showing heteroduplexes caused by heterozygosity for a silent nucleotide polymorphism. The homoduplex peak appears just after 6 min, the heteroduplex peaks are broad and appear between 5 and 6 min. *Bottom panel:* chromatogram from subject 4041 showing heteroduplexes caused by a missense mutation in codon 56. The homoduplex peak appears just after 6 min, the heteroduplex peaks appear between 5 and 6 min.

amino acid sequence of rhodopsin associated with normal vision; all known variant forms of human rhodopsin are associated with defective vision (Sung et al., 1991b). There is accumulating evidence to suggest that adRP-causing mutations interfere with processing, translocation to the outer segment, or degradation of wild-type rhodopsin, thereby impairing normal visual function (Dryja et al., 1991; Sung et al., 1991a; Mendes et al., 2005). Given the highly conserved nature of the photopigment molecules, and the close evolutionary relationship between rhodopsin and the S cone opsin (Nathans et al., 1986b), it is likely that dominant mutations in the S cone opsin cause tritanopia by similar mechanisms. If so, it might be expected that inherited tritanopia would be a degenerative disease, although the degeneration may more subtle compared to adRP because S cones are few in comparison to rods. In addition, it may be that inherited tritanopia is not congenital (i.e. present at birth) as protan and deutan defects are. Indeed, by analogy to adRP, inherited tritanopia may have a variable age at onset and this might account for the “incomplete penetrance” of the disorder.

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References

- COLE, B.L., HENRY, G.H. & NATHANS, J. (1966). Phenotypical variations of tritanopia. *Vision Research* **6**, 301–313.
- DRYJA, T.P., HAHN, L.B., COWLEY, G.S., MCGEE, T.L. & BERSON, E.L. (1991). Mutation spectrum of the rhodopsin gene among patients with autosomal dominant retinitis pigmentosa. *Proceedings of the National Academy of Sciences of the U.S.A.* **88**, 6481–6485.
- HAGSTROM, S.A., NEITZ, M. & NEITZ, J. (2000). Cone pigment gene expression in individual photoreceptors and the chromatic topography of the retina. *Journal of the Optical Society of America A* **17**, 527–537.
- JAGLA, W.M., JÄGLE, H., HAYASHI, T., SHARPE, L.T. & DEEB, S.S. (2002). The molecular basis of dichromatic color vision in males with multiple red and green visual pigment genes. *Human Molecular Genetics* **11**, 23–32.
- KALMUS, H. (1955). The familial distribution of congenital tritanopia. *Annals of Human Genetics* **20**, 39–56.
- KINNEAR, P.R. & SAHRAIE, A. (2002). New Farnsworth-Munsell 100 Hue Test normal of normal observers for each year of age 5–22 and for age decades 30–70. *British Journal of Ophthalmology* **86**, 1408–1411.
- MENDES, H.F., VAN DER SPUY, J., CHAPPLE, J.P. & CHEETHAM, M.E. (2005). Mechanisms of cell death in rhodopsin retinitis pigmentosa: Implications for therapy. *Trends in Molecular Medicine* **11**, 177–185.
- NATHANS, J., PANTANIDA, T.P., EDDY, R.L., SHOWS, T.B. & HOGNESS, D.S. (1986a). Molecular genetics of inherited variation in human color vision. *Science* **232**, 203–210.
- NATHANS, J., THOMAS, D. & HOGNESS, D.S. (1986b). Molecular genetics of human color vision: The genes encoding blue, green, and red pigments. *Science* **232**, 193–202.
- NEITZ, M., BOLLINGER, K. & NEITZ, J. (2003). Middle wavelength sensitive photopigment gene expression is absent in deuteranomalous colour

- vision. In *Normal and Defective Colour Vision*, eds. MOLLON, J.D., KNOBLAUCH, K. & POKORNY, J., pp. 318–327. Oxford, UK: Oxford University Press.
- NEITZ, M., CARROLL, J., RENNER, A., KNAU, H., WERNER, J.S. & NEITZ, J. (2004). Variety of genotypes in males diagnosed as dichromatic on a conventional clinical anomaloscope. *Visual Neuroscience* **21**, 205–216.
- NEITZ, M. & NEITZ, J. (2001). A new test for mass screening of school age children for red–green color vision defects. *Color Research and Application* **26**, S239–S249.
- NEITZ, J., NEITZ, M., HE, J.C. & SHEVELL, S.K. (1999). Trichromatic color vision with only two spectrally distinct photopigments. *Nature Neuroscience* **2**, 884–888.
- NEITZ, M., NEITZ, J. & JACOBS, G.H. (1995). Genetic basis of photopigment variations in human dichromats. *Vision Research* **35**, 2095–2103.
- NEUHANN, T., KALMUS, H. & JAEGER, W. (1976). Ophthalmological findings in the tritans, described by Wright and Kalmus. *Modern Problems in Ophthalmology* **17**, 135–142.
- PADOMOS, P., VAN NORREN, D. & FAJER, J.W. (1978). Blue cone function in a family with an inherited tritan defect, tested with electroretinography and psychophysics. *Investigative Ophthalmology and Visual Science* **17**, 436–441.
- PAKULA, A.A. & SAUER, R.T. (1989). Genetic analysis of protein stability and function. *Annual Review of Genetics* **23**, 289–310.
- SHARPE, L.T., STOCKMAN, A., JÄGLE, H., KNAU, H., KLAUSEN, G., REITNER, A. & NATHANS, J. (1998). Red, green, and red–green hybrid pigments in the human retina: Correlations between deduced protein sequences and psychophysically measured spectral sensitivities. *Journal of Neuroscience* **18**, 10053–10069.
- SHIMMIN, L.C., MAI, P. & LI, W.H. (1997). Sequences and evolution of human and squirrel monkey blue opsin genes. *Journal of Molecular Evolution* **44**, 378–382.
- SHIMMIN, L.C., MILLER, J., TRAN, H.N. & LI, W.H. (1998). Contrasting levels of DNA polymorphism at the autosomal and X-linked visual color pigment loci in humans and squirrel monkeys. *Molecular Biology of Evolution* **15**, 449–455.
- SMALLWOOD, P.M., WANG, Y. & NATHANS, J. (2002). Role of a locus control region in the mutually exclusive expression of human red and green cone pigment genes. *Proceedings of the National Academy of Sciences of the U.S.A.* **99**, 1008–1011.
- SMITH, V.C., POKORNY, J. & PASS, A.S. (1985). Color-axis determination on the Farnsworth-Munsell 100-Hue Test. *American Journal of Ophthalmology* **100**, 176–182.
- SUNG, C.H., DAVENPORT, C.M., HENNESSEY, J.C., MAUMENEE, I.R., JACOBSON, S.G., HECKENLIVELY, J.R., NOWAKOWSKI, R. & FISHMAN, G. (1991a). Rhodopsin mutations in autosomal dominant retinitis pigmentosa. *Proceedings of the National Academy of Sciences of the U.S.A.* **88**, 6481–6485.
- SUNG, C.H., SCHNEIDER, B.G., AGARWAL, N., PAPERMASTER, D.S. & NATHANS, J. (1991b). Functional heterogeneity of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa. *Proceedings of the National Academy of Sciences of the U.S.A.* **88**, 8840–8844.
- VAN NORREN, D. & WENT, L.N. (1981). New test for the detection of tritan defects evaluated in two surveys. *Vision Research* **21**, 1303–1306.
- WANG, Y., SMALLWOOD, P.M., COWAN, M., BLESCH, D., LAWLER, A. & NATHANS, J. (1999). Mutually exclusive expression of human red and green visual pigment-reporter transgenes occurs at high frequency in murine cone photoreceptors. *Proceedings of the National Academy of Sciences of the U.S.A.* **96**, 5251–5256.
- WEITZ, C.J., MIYAKE, Y., SHINZATO, K., MONTAG, E., ZRENNER, E., WENT, L.N. & NATHANS, J. (1992a). Human tritanopia associated with two amino acid substitutions in the blue sensitive opsin. *American Journal of Human Genetics* **50**, 498–507.
- WEITZ, C.J., WENT, L.N. & NATHANS, J. (1992b). Human tritanopia associated with a third amino acid substitution in the blue sensitive visual pigment. *American Journal of Human Genetics* **51**, 444–446.
- WENT, L.N. & PRONK, N. (1985). The genetics of tritan disturbances. *Human Genetics* **69**, 255–262.
- WINDERICKX, J., SANOCKI, E., LINDSEY, D.T., TELLER, D.Y., MOTULSKY, A.G. & DEEB, S.S. (1992). Defective colour vision associated with a missense mutation in the human green visual pigment gene. *Nature Genetics* **1**, 251–256.
- WRIGHT, W.D. (1952). The characteristics of tritanopia. *Journal of the Optical Society of America* **42**, 509–521.