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Color-deficient cone mosaics associated with Xq28 opsin mutations: A stop codon versus gene deletions

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ABSTRACT

Our understanding of the etiology of red–green color vision defects is evolving. While missense mutations within the long- (L-) and middle-wavelength sensitive (M-) photopigments and gross rearrangements within the L/M-opsin gene array are commonly associated with red–green defects, recent work using adaptive optics retinal imaging has shown that different genotypes can have distinct consequences for the cone mosaic. Here we examined the cone mosaic in red–green color deficient individuals with multiple X-chromosome opsin genes that encode L opsin, as well as individuals with a single X-chromosome opsin gene that encodes L opsin and a single patient with a novel premature termination codon in his M-opsin gene and a normal L-opsin gene. We observed no difference in cone density between normal trichomats and multiple or single-gene deutans. In addition, we demonstrate different phenotypic effects of a nonsense mutation versus the previously described deleterious polymorphism, (LIAVA), both of which differ from multiple and single-gene deutans. Our results help refine the relationship between opsin genotype and cone photoreceptor mosaic phenotype.

1. Introduction

Unequal homologous recombination produces rearrangements of the array of long- (L) and middle-wavelength sensitive (M-) cone opsin genes on the X-chromosome; these rearrangements are the most common cause of inherited red–green color vision deficiency (Drummond-Borg, Deeb, & Motolsky, 1989; Nathans, Thomas, & Hogness, 1986). In individuals with inherited red–green color vision defects there is tremendous variation in both the arrangement of cone opsin genes and in the associated color vision phenotype (Bollinger, Bialozynski, Neitz, & Neitz, 2001; Deeb et al., 1992; Jagla, Jägle, Hayashi, Sharpe, & Deeb, 2002; Nathans et al., 1986; Neitz et al., 2004; Sharpe et al., 1998; Ueyama et al., 2004). Over the past 25 years great strides have been made in understanding how the nature of the gene rearrangements affects phenotype in terms of color vision behavior. Recently, high-resolution imaging of the cone mosaic in the living human eye using adaptive optics has made it possible to address the question of how different genetic rearrangements affect the retinal phenotype at the cellular level.

The most common color vision deficiencies are deutan defects, which are characterized by a loss of M-cone function and often associated with X-chromosome opsin gene arrays in which the first two head-to-tail genes at the 5′-end of the array encode L opsin. If the encoded opsins form pigments that differ in spectral sensitivity, the resulting behavioral phenotype is deuteranomalous trichromacy, but if they form pigments of identical spectra the behavioral phenotype is deuteranopia. In arrays with more than two opsin genes, only the first two are usually expressed, so the additional downstream genes will not affect the color vision phenotype (Hayashi, Motulsky, & Deeb, 1999; Neitz, Bollinger, & Neitz, 2003). Deuteranopic defects will also occur when all but one of the X-chromosome opsin genes is deleted, leaving an array that contains one functional L-opsin gene (Nathans et al., 1986; Neitz et al., 2004). It is unknown whether or how the cellular phenotype varies between single- and multiple-gene deutans.

More rarely, mutations that ultimately render the opsin or photopigment non-functional have also been identified as a cause of inherited red–green color vision deficiencies. These mutations fall into two categories. The first category encompasses a variety of missense mutations that are the result of random mutations that

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introduce a nucleotide change that creates a deleterious amino acid substitution (Gardner et al., 2010; Neitz et al., 2004; Ueyama et al., 2002; Winderickx et al., 1992). The most common of these is the replacement of the highly conserved cysteine residue at position 203 with arginine (C203R) (Bollinger, Bialozynski, Jagla et al., 2002; Neitz & Neitz, 2001; Neitz et al., 2004; Winderickx et al., 1992). The second category has only recently been recognized, and includes deleterious combinations of amino acids at dimorphic positions that distinguish ancestral L from M opsins. These combinations appear to have arisen via recombination events that mix L and M genes together, producing new alleles that encode pigments that ultimately lead to photoreceptor dysfunction. Three such combinations involving amino acids encoded by exon 3 are leucine 153, isoleucine 171, alanine 174, valine 178, and alanine 180, abbreviated "LIAVA" using the single letter amino acid code (Carroll, Neitz, Hofer, Neitz, & Williams, 2004; Neitz et al., 2004), leucine 153, isoleucine 171, alanine 174, valine 178, and serine 180, abbreviated "LIAVS" (Mizrahi-Meissonnier, Merin, Banin, & Sharon, 2010), and leucine 153, valine 171, alanine 174, valine 178, and alanine 180, abbreviated "LVAVA" (McClements, Neitz, Moore, & Hunt, 2010). Adaptive optics imaging has been used to investigate the effect of the C203R mutation and the LIAVA polymorphism on the architecture of the cone mosaic in terms of cone density and cone packing in individuals with a red–green color vision defect (Carroll et al., 2004, 2009; Torti et al., 2009). For two individuals with the C203R mutation and one individual with the LIAVA polymorphism, adaptive optics revealed reduced cone densities compared to normal trichromats (Carroll et al., 2009; Torti et al., 2009). For the LIAVA polymorphism, the appearance of the cone mosaic suggested that the LIAVA-encoding M-opsin gene was expressed in a subset of cones that became nonfunctional at some point after foveal cone migration had completed, leaving areas where no cones were visible in the adaptive optics images (Carroll et al., 2004). In contrast, the appearance of the retinas with the C203R mutation suggests that cones were lost prior to the completion of foveal cone migration. This would result in a contiguous cone mosaic, albeit of overall reduced density (Carroll et al., 2009). Understanding how additional mutations affect the health of the cone photoreceptor mosaic at the cellular level would help establish a more comprehensive understanding of the etiology of red–green color vision defects.

We identified a patient with a novel missense mutation in his M-opsin gene at codon 149 that changes it from coding for the amino acid tryptophan (W) to a translation termination signal (X). There is no known equivalent rhodopsin mutation at this location. This mutation adds to the growing number of missense mutations reported in the L/M opsins; at least eleven other missense mutations have been reported: C203R (Winderickx et al., 1992), R247X and P307L (Nathans et al., 1993), N94K, R330Q, and G338E (Ueyama et al., 2002), P231L (Ueyama et al., 2004), P187L (Neitz et al., 2004), W90X (Wissinger, Papke, Tippmann, & Kohl, 2006), V120M (Mizrahi-Meissonnier et al., 2010) and W177R (Gardner et al., 2010). Of these missense mutations only W90X, reported by Wissinger et al. (2006) in a single patient with blue cone monochromacy, results in a translation termination signal. Here we used adaptive optics imaging to evaluate the cone mosaic in our patient with the M-opsin gene mutation in codon 149 (W149X). In addition, we assessed cone density and cone mosaic regularity for 9 other deutans, four with multiple-gene arrays and five with single-gene arrays. Individuals with multiple-gene arrays would be expected to have cone mosaics with similar density and regularity as mosaics from normal trichromats because, just as for a normal trichromat, the first two genes in the array are expressed in separate populations of cones (Bollinger, Sjoberg, Neitz, & Neitz, 2004). X-chromosome opsin gene arrays with a single gene can be considered as equivalent to an opsin gene knockout. A single transcriptional enhancer is shared by all opsin genes in the array, ensuring mutually exclusive expression (Smallwood, Wang, & Nathans, 2002), so it is anticipated that all of the cones destined to be L or M cones would express the available X-chromosome opsin gene in a single-gene deutan. If true, the cone mosaics of single-gene deutans might be expected to be indistinguishable from those of normal trichromats and of multiple-gene deutans. However, if cones that would have normally expressed the second gene in the array do not express any opsin gene, then we might expect a reduction in cone density owing to the absence of opsin (Carroll et al., 2010). In the case of the W149X mutation, the mutant opsin gene should be transcribed, at least initially; however, mammalian cells have "surveillance" mechanisms that maintain tight quality control on the biogenesis of messenger RNA (mRNA), including one that promotes rapid decay of mRNAs containing a premature translation termination codon in all but the last exon. This ultimately leads to down regulation of transcription of the mutant gene (Silva & Romano, 2009). Thus, the W149X mutant cone opsin would be expected to give rise to cones that neither contain a photopigment nor elaborate an outer segment.

Our imaging results revealed no significant difference in cone density or regularity between the single-gene deutans and the multiple-gene deutans or normal trichromats, providing the first direct evidence that individuals whose opsin gene arrays have been reduced to a single gene have complete retinal cone mosaics. In addition, the W149X mutation was associated with a reduced cone density, consistent with cone opsin playing an important structural role for the cone photoreceptor.

2. Methods

2.1. Subjects

Individuals with normal color vision as well as individuals with color vision defects were recruited by advertisement and most were of Western European ancestry. Ten deutans (nine males, one female; ranging in age from 10 to 55, with a mean of 29 years) and 27 normal trichromats (18 females, nine males; ranging in age from 10 to 55, with a mean of 26 years) were identified and recruited for adaptive optics retinal imaging. A complete ophthalmic exam was performed on the individual whose deutan color vision defect was caused by a novel opsin mutation, subject 6643 (age 40 years), which included visual acuity measurement, autofluorescence imaging, and dilated funduscopic exam. This examination was unremarkable and subject 6643 had a best-corrected visual acuity of 20/20. Axial length was measured on all subjects using a Zeiss IOLMaster (Carl Zeiss Meditec, Dublin, CA) for calibration of adaptive optics images. All research on human subjects followed the tenets of the Declaration of Helsinki and was approved by IRBs at the Medical College of Wisconsin (genetics, color-vision testing, and adaptive optics imaging) and the University of Rochester (color-vision testing and adaptive optics imaging). Informed consent was obtained from all participating adults as well as from parents of participating minors, after explanation of the nature and possible consequences of the study.

2.2. Color-vision testing

Color vision was assessed using the Neitz Test of Color Vision (Neitz & Neitz, 2001) and those whose performance indicated a red–green deficit were examined further using a variety of color vision tests including the AO-HRR, the Farnsworth-Munsell 100-Hue Test, the Lanthony's Desturated D-15, and Rayleigh color match on a Nagel Model 1 anomaloscope (Schmidt Haensch). Individuals who manifested a protan defect on all tests were excluded from the current study.