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Molecular Genetics of Color Vision and Color Vision Defects

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Color is an extremely important component of the information that we gather with our eyes. Most of us use color so automatically that we fail to appreciate how important it is in our daily activities. It serves as a nonlinguistic code that gives us instant information about the world around us. From observing color, for example, we can find the bee sting on an infant's arm even before it begins to swell by looking for the little spot where the infant's skin is red. We know when fruit is ripe; the ripe banana is yellow not green. We know when meat is cooked because it is no longer red. When watching a football game, we can instantly keep track of the players on opposing teams from the colors of their uniforms. Using color, we know from a distance which car is ours in the parking lot—it is the blue one—and whether we will need to stop at the distant traffic light, even at night, when we cannot see the relative positions of red and green lights.

In the human eye, there are 2 types of photoreceptor cell—rods and cones—that serve different functions. Rods mediate vision at low light levels and thus serve vision only under conditions, such as at night, when little light is available. In contrast, cone photoreceptors mediate vision under light levels encountered in daily life. Most of our daily activities are performed in daylight and at room light levels that are above those where rods contribute significantly to vision. Thus, under most normal conditions, our vision is based on cone photoreceptors. A component of cone-based vision is the capacity to see in color, which requires multiple classes of cone photoreceptor.

Rhodopsin and cone pigments are the light-sensitive molecules in rods and cones, respectively. These are collectively termed *photopigments* or *visual pigments* and are composed of 2 parts—a protein component termed *opsin* and the chromophore 11-*cis*-retinal. Human visual pigments share the same chromophore; however, the opsins differ between rods and cones and between different types of cones. The first step in vision is the absorption of a photon, which causes 11-*cis*-retinal to undergo a conformational change. The protein opsin functions as a receptor molecule that is activated by the change in retinal from its 11-*cis* to all-*trans* form. The activated opsin, in turn, triggers a series of biochemical events within the photoreceptor, which ultimately results in the transmission of a neural signal.

All visual pigments are believed to have evolved from a single common ancestor, and they have a great deal in common structurally and functionally. The visual pigments are members of the G protein–coupled receptor superfamily. Other members of this superfamily include receptors for odor and taste, neurotransmitter receptors, and hormone receptors.

The molecular genetics of rhodopsin are relatively simple. Rhodopsin is encoded by a single gene on chromosome 3, and that gene is expressed in all rod photoreceptors. In contrast, it has been long understood that the organization of the visual pigment genes for human color vision would have to be complex enough to accommodate the production of 3 opsin types in 3 spectral classes of cone. From the inheritance of color vision defects, it was expected that an autosomal gene

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would encode the blue cone opsin, and the other 2 genes—one for the red and another for the green cone opsin—would be on the X chromosome.

The molecular genetics of color vision has turned out to be much more complex than originally suspected. This complexity derives in part from the fact that red and green opsin genes are adjacent to one another and they are about 98% identical. It seems that during human evolution, because of their close proximity and high similarity, the red and green genes were subject to frequent homologous recombination. This, perhaps in conjunction with relaxed natural selection against color vision defects in civilized humans, has given rise to a great deal of variability in the red and green photopigment genes. The rearrangements have included duplications of the red and green genes so that most people have extra pigment genes. Individual X chromosomes contain variable numbers of red and green genes, arranged in a tandemly repeated array. Nevertheless, in the face of the unanticipated complexity, much progress has been made toward understanding the relationship between color vision genotype and phenotype. During the past dozen years, consideration of the results from molecular genetics combined with those from physiology and psychophysics has brought about a revolution in how we think about the biological basis of color vision. One purpose of this article is to review current thinking about the underpinnings of human color vision and to contrast the current views with those held 15 years ago.

Work on the biological basis of color vision and color blindness has provided numerous practical benefits. We have gained an appreciation of the incredible variety that occurs in human color vision among people categorized with color vision defects and among those with normal color vision. Color blindness can serve as a model for understanding other inherited disorders that affect vision. Understanding the basis for color vision defects may ultimately lead to a treatment, and an immediate goal of recent work is to develop a genetic diagnostic test to detect inherited color vision defects and to determine their type and severity. The advantages of a genetic color vision test include that it theoretically could be used for all age groups, including young children, and that it could distinguish inherited from acquired defects. Potentially, a genetic-based color vision test could be developed that would be easily administered, inherently objective, reliable, and valid, and could serve as a universal standard.

COLOR VISION TERMINOLOGY

Human color vision is normally trichromatic, and requires at least 3 cone photopigments: 1 from each of 3 well-separated spectral classes. The 3 classes of pigment differ in their relative spectral sensitivities, and are commonly referred to as the blue, green, and red cone pigments. However, using color names for the photopigments can be misleading. Calling the 3 photopigment classes short-, middle-, and long-wavelength sensitive, abbreviated S, M, and L, can minimize confusion. Many people who work in the field of color vision prefer these labels. For the remainder of this article, when referring to the cones and the cone pigments, we will use S, M, and L instead of blue, green, and red.

CONGENITAL RED-GREEN COLOR VISION DEFECTS

Until recently, color vision defects were said to be caused either by the alteration or by the loss of one kind of cone pigment. Herein we will promote the idea that the concept of an altered pigment as the cause is no longer useful within the framework of current understanding. Losses in color vision can be best understood by considering what is missing to cause the perceptual loss. As we will explain, in people with less severe color vision defects, the degree of color vision that remains can be understood by considering what remains after the loss. Within this framework, almost all red-green color vision defects can be explained as being caused by the absence of one class of cone photopigment (**Figure 1**). The class of defects characterized by the absence of M cones is termed with the prefix *deutan*, while those defects characterized by the absence of L cones are termed with the prefix *protan*. S cone defects are given terms with the prefix *tritan*. Inherited protan and deutan defects, which are collectively termed *red-green color vision defects* (or deficiencies), are common, affecting about 8% to 10% of men in the United States. In contrast, congenital tritan defects are rare, affecting less than 1 in 10 000 people.

The inherited color vision defects in which one pigment class is absent are not usually associated with any other vision loss. Rare conditions do exist, however, in which more than 1 class of cone is absent; severe losses in visual acuity are associated with these conditions, and they are called achromatopsias because color vision is essentially absent. Incomplete achromatopsia (blue cone monochromacy) is characterized by the loss of L and M cone function, and complete achromatopsia (rod monochromacy) is characterized by the absence of function of all 3 cone types.

Dichromacy

The most severe of the common inherited red-green color vision defects are the dichromacies. Dichromats base color vision on just 2 pigments (in 2 types of cones). Protanopes have lost L pigments, and deuteranopes have lost M pigments. Each of these types of dichromacy occurs at a rate of about 1% in white men. Dichromacy is much rarer in women, but this should be not misinterpreted to mean that it does not ever occur in them. About 1 in 4000 women are dichromats.

In most cases, the direct cause of the color vision loss in dichromacy is the loss of the genes that encode one class of cone photopigment. For protanopes (who have no L cone function), it is the loss of L cone pigment genes that causes the color vision defect. There are rare exceptions, however. Two cases have been reported in which a protanope was found to have an apparently intact L pigment gene in addition to M pigment genes.[1,2] It is assumed that in these rare cases there is a genetic defect associated with the L opsin gene that interferes with the expression or function of the encoded L pigment.

Like the protanopes who have lost L genes, many deuteranopes, who lack M cone function, have lost all

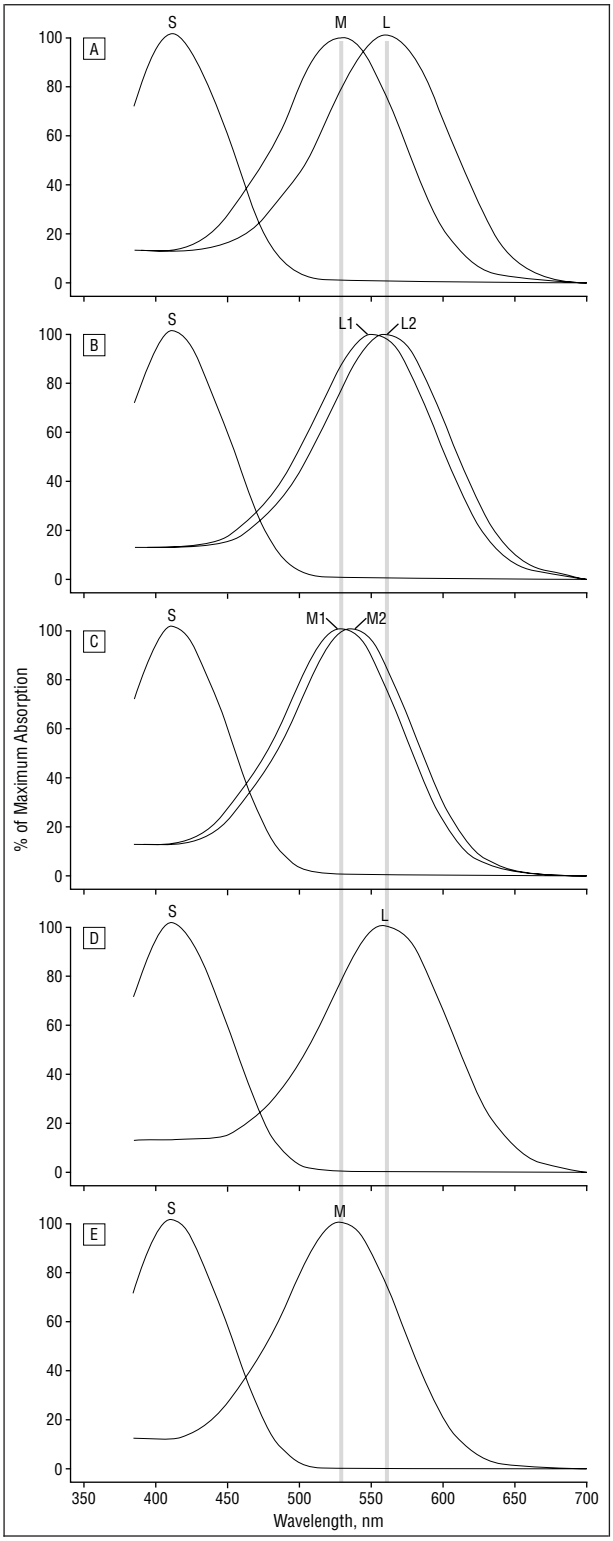

Figure 1. *Photopigments underlying normal and defective red-green color vision are illustrated. Normal trichromats have at least 1 each of the short-, middle-, and long-wavelength sensitive classes of pigments, identified as S, M, and L. Red-green color-deficient individuals are missing either all members of the L class or all members of the M class of pigment. The degree of color vision deficiency in persons with anomalous trichromacy depends on the magnitude of the spectral difference between the pigment subtypes. Dichromats have only 1 pigment in the L or M region of the spectrum. A, Normal trichromacy. The normal S, M, and L pigment spectra are shown. B, Anomalous trichromacy (deuteranomaly). Deuteranomalous trichromats have 2 slightly different L pigments. C, Anomalous trichromacy (protanomaly). Protanomalous trichromats have 2 slightly different M pigments. D, Dichromacy (deuteranopia). Deuteranopes have an L but no M pigment. E, Dichromacy (protanopia). Protanopes have an M but no L pigment. Vertical lines indicate 530 nm, near which M pigments cluster in peak sensitivity, and 560 nm, near which L pigments cluster in peak sensitivity.*

M opsin genes. There are exceptions for the deuteranopes as well. For example, we recently examined the cone pigment genes in 140 school-aged boys, each of whom had been diagnosed as having a color vision defect (unpublished data, 1999). In that group, 12 were diagnosed as having deuteranopia. Of the 12, 2 had grossly normal pigment genes, including intact M pigment genes. From the nucleotide sequences, it was determined that both boys had lost function of their M pigment genes as the result of a point mutation changing a cysteine to an argenine at amino acid position 203, a highly conserved site among G protein–coupled receptors. The cysteine is essential for formation of a functional visual pigment.[3] This same deleterious mutation had been found earlier to be associated with 2 other vision disorders, incomplete achromatopsia[4] and deuteranomaly.[5] The type of defect associated with this mutation (achromatopsia or deuteranomaly) is apparently determined by the remaining functional cone pigment genes that an individual has.

In summary, in most cases, the most severe red-green color vision defects, the dichromacies, are explained by the straightforward deletion of cone pigment genes. However, cases have been found in which loss of function comes from point mutations in the genes. In the few dichromats in whom a genetic defect has not been identified, it is assumed that the problem arises from an as yet unidentified deleterious mutation that interrupts photopigment expression or function.

Anomalous Trichromacy

Protanomaly. The milder forms of red-green color blindness are the anomalous trichromacies. There are 2 types—protanomaly and deuteranomaly—that parallel the 2 dichromatic types—protanopia and deuteranopia. Protanomalous trichromats are missing normal L photopigment just like the protanopes. However, as the term for their condition implies, they have trichromatic color vision. Their trichromacy is not based on L, M, and S pigments like in those with normal color vision. In the classic descriptions of this disorder, protanomalous trichromats were said to have normal S (blue) and M (green) pigment but an abnormal or "anomalous" L (red) pigment. From what we know, the concept of an anomalous L pigment in protanomaly no longer seems apt. The molecular genetics results indicate that protanomalous trichromats can be more accurately characterized as having lost all L pigments. They have an S pigment remaining and 2 M (or M-like) pigments that are usually conceived as differing by a small shift in spectral peak. The genetic basis for having 2 different M pigment genes is believed to arise from rearrangements within the normal tandem array of pigment genes. Most red-green color vision defects are believed to arise from gene rearrangements and, as we have explained, for most protan observers this "rearrangement" includes the deletion of all genes that could encode pigments falling into the nor-

mal L class. The rearrangements also create hybrid or "chimeric" genes in which some of the L gene sequences have been replaced by M gene sequences. These chimeric genes encode pigments with spectral properties that place them in the M class. However, for all protanomalous trichromats, there is more than one pigment gene left remaining in the X chromosome array, which includes one or more typical M pigment genes.

Chimeric Genes. We use the term *chimeric genes* to refer to the variant forms of the human L and M pigment genes. There is an extremely high degree of genetic polymorphism in the human L and M genes, in people with normal and with defective color vision. This variation can be explained as having arisen from shuffling of the L and M gene segments that has occurred in the process of human evolution. Thus, the genes in people with normal and defective color vision can be "chimeras" with different segments derived from what, in our early evolution, may have been original L and M genes. In light of our current understanding, this term, *chimera*, seems preferable to the alternatives, *hybrid* or *fusion* genes, that were the terms introduced by Nathans et al[1] to describe an early molecular-genetic concept of the genes underlying color anomaly. A replacement of the term fusion gene with chimeric can serve to reinforce a shift in understanding. From the early studies, it was assumed that all normal color vision was based on a stereotyped normal L pigment gene and a stereotyped M pigment gene. Hybrid genes were conceived of as arising from isolated gene rearrangement events that caused color vision defects. Evidence has accumulated during the past decade suggesting a history of extensive recombination between M and L genes.[5-10] Only those events leading to the loss of all genes encoding one class of pigment, a loss of their function or expression, lead to color vision defects. The other extensive variations in the pigment genes contribute to individual differences in normal color vision.

Historically, there has been debate about the relationship between genes and pigments in people with normal color vision compared with those with color vision defects. The realization that there is widespread variation in the sequences of the M and L genes in normal color vision allows for the possibility of considerable overlap between the variant M genes in normal color vision and the subtypes of M genes in the color defect protanomaly. However, there do appear to be differences in the distribution of variant M genes in those with normal color vision vs those with protanomaly such that some specific types of chimeric genes that occur in protanomalous trichromats have not been found in individuals with normal color vision.[11] The characterization of similarities and differences between the M genes in anomalous trichromats and in those with normal color vision is important for developing theories about the causes and interrelationships among color vision phenotypes, and it is important for the development of a genetic diagnostic test for color blindness.

Deuteranomaly. The most common type of anomalous trichromacy is deuteranomaly. In fact, it is the most common of all inherited color vision defects by a large margin. In the United States, it is estimated to affect 1 in 20 men. It is also estimated to affect many more women than any of the other red-green color vision deficiencies; about 3 in 1000 women are deuteranomalous, a rate about 25 times higher than that of any other color vision defect. Like other forms of normal and anomalous trichromacy, deuteranomaly is based on 3 pigments. It is based on the presence of the S cones plus 2 spectral subtypes of L cones. As a basis for 2 spectral types of L pigments, all persons with deuteranomaly have at least 2 different genes to encode L pigments. In this aspect, the correspondence between genotype and phenotype is perfect and thus can be used reliably in the genetic diagnosis of deuteranomaly.

In contrast, there is an aspect of deuteranomaly in which the relationship between genotype and phenotype is not clear at all, ie, most persons with deuteranomaly have M pigment genes and thus it is not understood why they do not have M cone function. This is probably the most important unanswered question concerning the molecular genetics of color vision defects. There is evidence that persons with deuteranomaly lack an M cone contribution to vision because they lack both functional M cones and expressed M photopigment. However, what causes this loss is uncertain. Several hypotheses have been forwarded to explain the loss of M function. One hypothesis is that only the first 2 pigment genes in the X chromosome array are expressed and that the second L pigment gene (that occurs in all persons with deuteranomaly) displaces the M pigment gene out of an "expressed" position.[5] Another hypothesis is that persons with deuteranomaly have L and M pigment expressed in the same cones.[1] However, there is strong evidence against each of these hypotheses.[10,12]

In summary, the red-green anomalous trichromacy can be explained as arising from the loss of one class of cone photopigment. Protanomalous trichromats lack pigments from the L class and persons with deuteranomaly lack pigments from the M class. In persons with protanomaly, L pigment genes are almost always missing. In about two thirds of deuteranomalous men, M genes are present and the reason for loss of function is not clear. Persons with protanomaly must always have at least 2 different M genes to encode 2 spectral subtypes of M pigment. One of those M genes can be a chimeric gene that is unlike the genes typically found in persons with normal color vision. Persons with deuteranomaly must have at least 2 different L genes to encode 2 spectral subtypes of L pigment. These characteristics allow one to design a genetic test that would, most of the time, distinguish between the 2 classes of anomalous trichromacy, distinguish the anomaly from the corresponding dichromacy, and distinguish anomaly from normal color vision.

Differences in Vision Between Persons With Color Defects and Persons With Normal Color Vision

It is a common misconception that red-green color blindness does not affect performance in daily tasks. In a survey conducted by Steward and Cole,[13] more than 75% of red-green color-blind individuals reported having diffi-

culties with daily tasks. Common everyday complaints among color-blind subjects include having difficulty navigating the highly color-coded World Wide Web on the computer Internet, seeing that someone has become sunburned or has a slight rash, reading color-coded maps, distinguishing traffic signals, and dressing in appropriately matching clothes.

The difference between normal color vision and dichromacy is large. The term *dichromacy* means, literally, 2 hues and derives from the fact that dichromats can match any color using mixtures of just 2 "primary" hues. However, the term *dichromat* is also appropriate because dichromats see only 2 hues. To them, objects are black, white, shades of gray, or 1 of 2 hues. In contrast, people with normal color vision see more than 100 different hues in addition to black, white, and gray. Dichromats confuse red with green, and they confuse, with red and green, all colors in the spectrum that fall between them, including yellow, orange, and brown. They see blue and violet as the same color, and blue-green is indistinguishable from white or gray. Magenta and its pastel counterpart pink also appear white or gray. The most severely affected anomalous trichromats have color vision that is similar to that of a dichromat. Mildly affected anomalous trichromats have more difficulty distinguishing between pastel colors than between the saturated versions of those same colors. They may see the difference between red and green, but cannot see the difference between more similar colors such as olive green and brown.

It is often said that the term *color blind* is a misnomer. However, it is difficult to find a more appropriate term for individuals who are unable to distinguish all but 2 of the more than 100 hues that are normally seen as different. Dichromats may say that they see many colors but have difficulty with certain shades. In truth, dichromats become adept at using brightness and saturation differences as visual cues, and they learn to call these differences "colors."

The 2 most devastating problems that can be encountered by color-blind people involve (1) career choices and (2) early education. For example, all too often it happens that a young man has planned his whole life to be a police officer only to find out at the age of 25 years, after years of hard work, that he can never attain his life's dream because of color blindness. There are many similar stories about people prevented from attending the Air Force Academy, being airline pilots, and entering other professions that require normal color vision. Great frustration is experienced by individuals trying to enter a field that has no formal color vision requirements, yet good color vision is required for success, as, for example, is true in chemistry and geology.

For young children, problems may arise at school because color blindness causes a form of visual miscommunication. Children live in a world of natural and human-made color coding. In the early grades, colors are used as tools of communication. Children are expected to learn to differentiate colors, know color names, and associate colors with specific meanings in their lives. Color codes are used as cues to teach reading and math. These methods can be extremely helpful for most children, but they can cause problems and frustration for children with color vision deficiencies. The most serious problems arise when color vision defects are misinterpreted as learning difficulties, inattentiveness, or laziness. Frustrations from inappropriate career choices can be minimized if a diagnosis is made early and career counseling is offered. Similarly, many of the potential problems in early education can be avoided with kindergarten or preschool diagnosis so that alternative strategies that do not rely on color coding can be used with color-deficient children.

A genetic test for color vision defects has potential for use in ameliorating 2 of the more salient problems caused by congenital color vision defects. A genetic test could be administered at preschool ages, making it ideally suited for use before early education. Because of the intellectual immaturity of preschool-aged children, their performance on color plate tests, such as the Ishihara tests for color deficiency, is difficult to evaluate. Also, a genetic test would be objective and could be standardized, making it useful for setting job requirement policies and for evaluating children against those policies at early ages.

Medical Implications of Color Blindness. To our knowledge, there is no known association between the inherited forms of color blindness and any other kind of blinding eye disorder. However, acquired color blindness is symptomatic of many blinding disorders, such as glaucoma, diabetic retinopathy, and macular degeneration. Acquired color blindness is also a symptom of exposure to certain toxic drugs and chemicals. In all cases, detection of the acquired color vision loss can be an important tool in diagnosis and treatment. However, a preexisting congenital color vision defect can make an accurate diagnosis difficult. A genetic test for congenital color vision defects would clearly be extremely valuable in diagnosing acquired color vision loss.

Inheritance Patterns. As previously explained, congenital red-green color vision defects are characterized by the absence of functional expression of either L or M pigment. The genes encoding these pigments lie on the X chromosome[1] and thus color vision defects resulting from mutations within the L and M genes are inherited as X-linked recessive traits. This accounts for the pronounced sex difference in the frequency of red-green color blindness.

Tritanomaly is inherited as an autosomal dominant defect, with incomplete penetrance. The defect has been shown to be caused by missense mutations in the S cone pigment gene,[14,15] which lies on autosome 7.[1] Complete achromatopsia is inherited as an autosomal recessive trait; a mutation in the gene encoding a subunit of a cone-specific cation channel has recently been shown to underlie some cases of this disease.[16] Additional genetic causes of this disease are also under investigation.[17]

SPECTRAL TUNING OF L AND M PIGMENTS

It has been long recognized that understanding congenital color blindness at a molecular level would require an understanding of how the protein component—opsin—tunes the absorption spectrum of the chromophore so that it is maximally sensitive to different wavelengths of light.

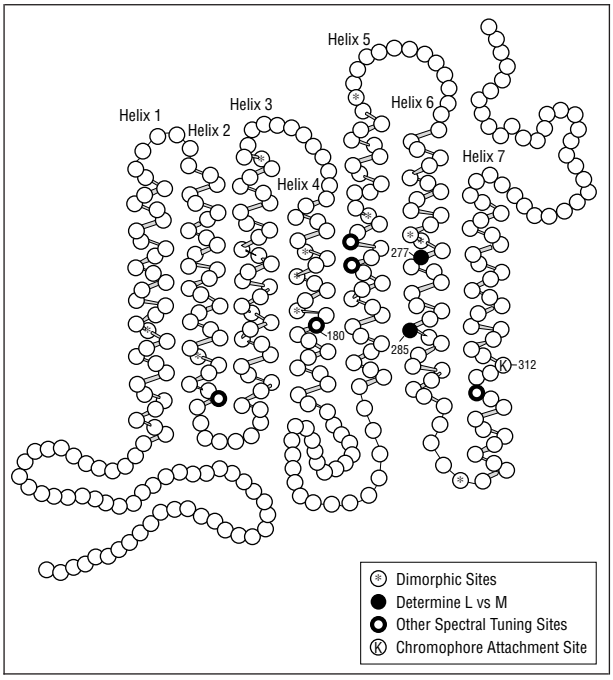

Figure 2. *Diagram of long-wavelength sensitive (L) or middle-wavelength sensitive (M) cone opsin. Amino acids are illustrated as "beads" along the protein strand. The 19 dimorphic sites that occur between and among M and L pigments are indicated.*

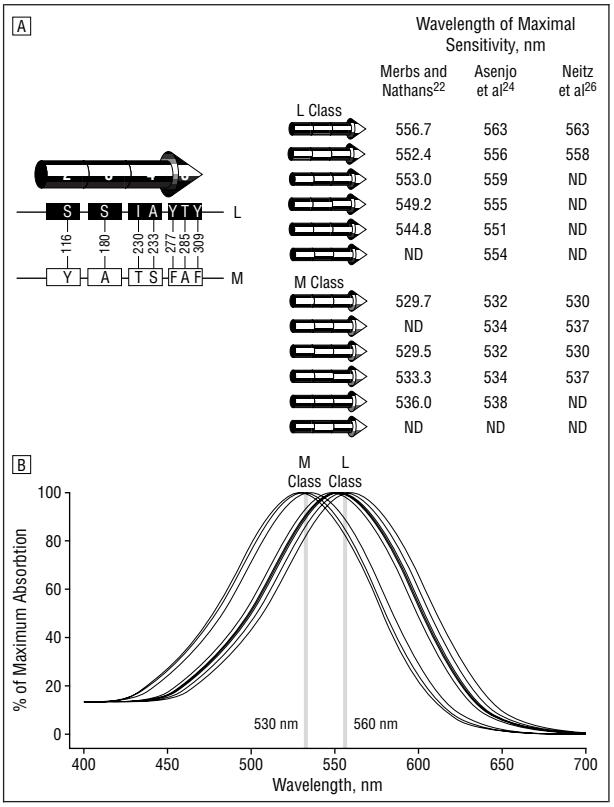

Figure 3. *Spectral tuning of long-wavelength sensitive (L) and middle-wavelength sensitive (M) pigments. A, Exons 2 to 5 of the genes and the 7 spectral tuning sites they encode are shown (left). Codon numbers or amino acid positions for the spectral tuning sites are given. Codons 277 and 285 encode amino acids that determine whether the specified pigment is L or M. Black and white boxes indicate that amino acids specified at the spectrally active position are those that shift the spectra long and short, respectively. Arrows representing exons 2 to 5 of genes for L-class and M-class pigments are similarly color coded. The wavelengths of maximal sensitivity, as measured in the indicated studies, are given. Since the measurements were made using different methods, the discrepancies in the absolute values for wavelengths of maximal sensitivity are not surprising. There is good agreement in the relative shifts produced by each substitution. S indicates serine; Y, tyrosine; A, alanine; I, isoleucine; T, threonine; F, phenylalanine; and ND, not determined. B, Spectral sensitivities of L and M pigments are plotted. The maximum wavelength values are from the data of Asenjo et al.[24] Because some of the amino acid substitutions do not shift the peak of M pigments, there are more potential spectral variants of L than of M pigments.*

In essence, the question has been what differences in the opsins make one cone photopigment absorb most strongly in the middle wavelengths (M) and another absorb in the long wavelengths (L). Moreover, what produces the more subtle spectral differences between the pigments underlying color anomaly? In the past 10 years, tremendous advances have been made in identifying the roles for specific amino acid differences in tuning the absorption spectra of the L and M pigments. This information has provided insight into the photopigment basis for normal and defective red-green color vision.

The opsin component of the visual pigment molecule has 7 transmembrane α helical segments, which form a hydrophobic pocket for the chromophore. There are 19 amino acid dimorphisms that occur among and between the human L and M pigments (**Figure 2**), but only a few of them produce a spectral shift. The effects of amino acid substitutions on the absorption spectra of the pigments have been investigated using various experimental approaches.[18-25] Merbs and Nathans[22] and Asenjo et al[24] carried out in vitro studies in which the spectral properties of chimeric pigments expressed in cell culture were measured spectrophotometrically. In an in vivo study,[26] electroretinographic flicker photometry was used to derive spectral sensitivity functions for L and M pigments in the retinas of human dichromats and the corresponding visual pigment genes were sequenced. Most recently, Sharpe et al[8] analyzed the M and L pigment genes of protanopes and deuteranopes and then examined the spectral sensitivities of the M pigments of protanopes and the L pigments of deuteranopes using psychophysical methods. The results of these studies are summarized in **Figure 3**, A. In vitro substitutions at 7 amino acid positions are required to shift the spectrum from that of the longest-absorbing L pigment to that of the shortest-absorbing M pigment.[24] The 7 substitutions known to be spectrally active are shown in Figure 3, A.

Together, the substitutions at positions 277 and 285 produce a spectral shift of 16 to 24 nm and thus can be thought of as separating the human X-encoded pigments into the 2 main classes—M and L. All the other amino acid changes produce relatively smaller changes in absorption spectrum. When these amino acids are changed, they produce spectral variations within the main classes. Of the substitutions that produce smaller spectral shifts, arguably the most important is the substitution of serine for alanine at position 180. This substitution shifts the absorption maximum in the range of 3 to 7 nm, depending on how it is measured and on whether the substitution is in an M pigment (with phenylalanine at position 277 and alanine at position 285) or an L pigment (with tyrosine at position 277 and threonine at position 285). Substitutions at the remaining spectrally ac-

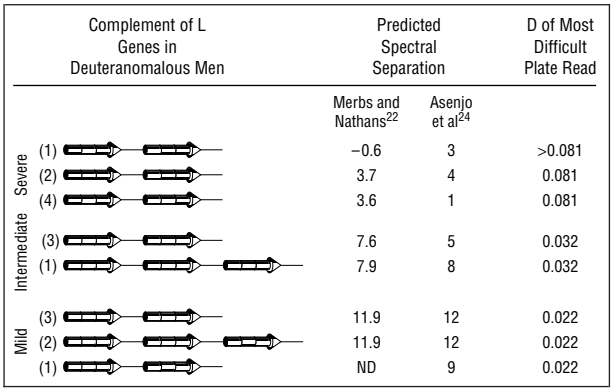

Figure 4. *A test of the spectral proximity hypothesis in deuteranomaly. The long-wavelength sensitive (L) genes underlying deuteranomaly in 16 men are drawn as arrows indicating exons 2 to 5 of the genes. The color coding is the same as that given for Figure 3, A. Of the 16 men studied, 7 were severely, 4 were intermediately, and 5 were mildly affected. The number of men with each L gene complement is indicated in parentheses. The predicted spectral separation between the L pigments for each gene complement was calculated using the values given in Figure 3, A. The D value is a measure of color vision behavior, and was obtained by measuring the colors in the designs of the Hardy, Rand, and Rittler pseudoisochromatic plates (American Optical Company, Southbridge, Mass) as specified by their coordinates in units of the Commission International de l'Eclairage* u′v′ *diagram.[33] Some of the subjects also had middle-wavelength sensitive genes, which are not shown. ND indicates not determined.*

tive positions (116, 230, 233, and 309) produce relatively small spectral shifts. Each of these appears to have a smaller effect when the substitution occurs in an M pigment vs an L pigment. The substitution at position 116 is reported to have a small effect on L pigments and no effect on M pigments.[24] The spectral sensitivity functions of the pigments are plotted in Figure 3, B. The absorption maxima form 2 clusters depending on the identities of amino acids at positions 277 and 285—the M class clusters near 530 nm and the L class clusters near 560 nm. There is a span of about 10 nm that separates the peak of the longest-absorbing M pigment from that of the shortest-absorbing L pigment.

In summary, whether a gene encodes an L or an M pigment can be predicted from the amino acids specified at positions 277 and 285 in the pigment. Five other spectrally active substitutions produce subtypes of L and subtypes of M pigments. These subtypes are associated with normal color vision and with color vision defects.[27]

INDIVIDUAL DIFFERENCES IN SEVERITY OF COLOR VISION DEFICIENCY

One of the most puzzling features of red-green color vision anomaly is the enormous range of severity. For example, people diagnosed as having deuteranomalous trichromacies can have color vision that ranges in severity from nearly dichromatic to nearly normal. These differences in severity pose a challenge for understanding the biological basis for color vision defects. They also pose practical challenges for assessment of color vision defects and for setting standards of performance in occupations that have color vision requirements.

The wealth of information about spectral tuning that has come from molecular biology results has provided the foundation for testing a long-favored hypothesis to explain the large range in severity in deuteranomaly. Although various versions of the hypothesis have been proposed,[28-32] the most modern version of it has been termed the *spectral proximity hypothesis*.[33] It proposes simply that color vision discrimination ability depends on the size of the difference between the absorption spectra of the pigments. For example, the normal L and M photopigments are separated by about 30 nm in spectral peak. It is the large difference in spectral absorption between L and M cones that is used to provide the excellent color discrimination that people with normal color vision experience in the red to green part of the spectrum. Individuals with deuteranomaly lack M cones, but they have 2 subtypes of L cones with pigments that are spectrally different enough to provide the basis for limited color vision in the red-green range. Theoretically, a deuteranomalous person with a large spectral difference between L pigment subtypes would have the basis for much better color vision than a person with 2 L pigments that were nearly identical. We tested the hypothesis that differences in spectral separation of the L pigment subtypes could explain the differences in color discrimination among 16 deuteranomalous men.[7] The results are summarized in **Figure 4**. For each subject, the L pigment gene sequences were determined, and the spectral separation between the encoded pigments was predicted using the scheme in Figure 3, A. Among the 16 deuteranomalous men, there was a nearly perfect correlation between color discrimination ability as measured by performance on the Hardy, Rand, and Rittler pseudoisochromatic plates (American Optical Company, Southbridge, Mass) and the magnitude of the spectral separation between the L pigments predicted from the genetic analysis. Together, the genetic and behavioral data define 3 categories of deuteranomaly—mild, severe, and intermediate. These results provide strong support for the spectral proximity hypothesis and demonstrate that the severity of deutan deficiency can be predicted with a genetic assay.

MOLECULAR GENETICS OF RED-GREEN COLOR VISION

As emphasized in this article, in the human population, there is variation in the number of visual pigment genes per X chromosome. This presumably derives from a history of recombination that has been promoted by the high degree of homology shared by the tandemly arranged L and M genes.[34] As illustrated in Figure 4, unequal homologous recombination can effectively contract and expand the size of the array. To our knowledge, there are no known disadvantages (or advantages) to having extra genes in addition to one L and one M. However, the reduction of genes to less than the required one L and one M leads to color vision defects. For example, intergenic recombination between the region downstream of the first gene in one array and the region downstream of all genes in a second array will produce an array with a single L pigment gene (**Figure 5**, A). Arrays with this structure are diagnostic of deuteranopia.[7,26]

Variability in the genes and their arrangements in normal color vision complicates our ability to understand the

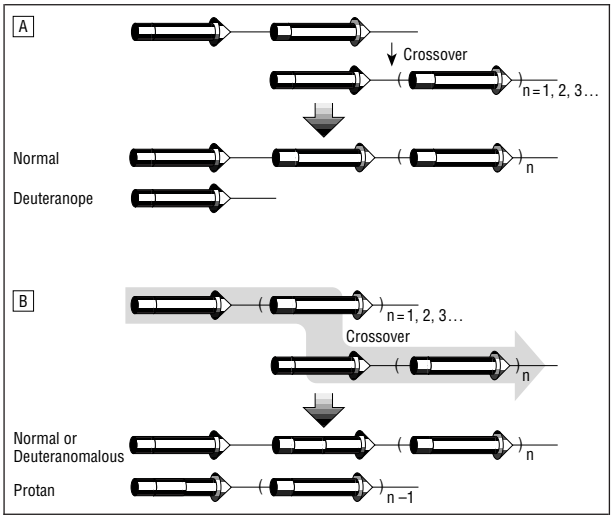

Figure 5. *Recombination mechanisms believed to underlie variation in the size of the X chromosome pigment gene arrays and to produce arrays underlying red-green color blindness. Black arrows indicate long-wavelength sensitive (L) genes; white arrows, middle-wavelength sensitive (M) genes. The top 2 arrays in A and B are hypothetical parental arrays underlying normal color vision, each with a single L gene followed by 1 or more M genes. A, Intergenic recombination is proposed to produce arrays underlying deuteranopia. B, Intragenic recombination produces arrays proposed to underlie deuteranomaly and protan defects. The first gene in the protan array specifies phenylalanine at position 277 and alanine at position 285 and thus encodes an M pigment. The second gene in the normal or deuteranomalous array specifies tyrosine at position 277 and threonine at position 285 and thus encodes an L pigment. Arrays with the genes shown in the array labeled normal or deuteranomalous have been found in men with both phenotypes; however, the order of the genes in the array in those men is not known.*

relationship between genotype and phenotype. However, there are consistencies among persons with normal color vision that make predicting phenotype from genotype simpler. One example is that the first gene (most upstream) in the array has been found to always encode an L pigment in persons with normal color vision.[35] One implication of this is that arrays with an M pigment gene in the first position are most likely to be ones from which an L pigment gene has been deleted. An example of a recombination event that would delete L-encoding sequences in the most upstream position is illustrated in Figure 5, B.[1,18,26] Thus, having an M gene in the first position is an excellent predictor of protan color vision defects. Female carriers of protan defects can also be identified using molecular biology, because they have one array with an M gene first and a second array with an L gene first.[35]

At the other extreme, gene arrays in which extra L genes have been added complicate understanding of genotype-phenotype relationships. An example of a gene rearrangement in which an L gene is added is shown in Figure 5, B. Arrays with multiple L genes, with and without M genes, were initially discovered in men with deuteranomalous color vision. L genes that occur in positions downstream of the first gene were termed *M-L hybrid genes*, and were proposed to cause deuteranomaly.[1] There are 2 issues that pertain to these genes. First, the presence of additional L pigment genes is required to explain why deuteranomalous trichromats have an extra dimension of color vision compared with deuteranopes. The red-green color vision of persons with deuteranomaly is based on having 2 L pigment subtypes, while deuteranopes are reduced to only a single L and no M pigments. Whether the extra L genes are simultaneously responsible for a loss of M pigment gene expression or function remains an open question.

The second issue is that because hybrid genes were initially identified in people with color vision defects[1] it was assumed that the presence of hybrid genes distinguishes defective from normal color vision. However, sequence analysis of the L and M genes in persons with normal color vision and in persons with deuteranomaly revealed that there is a high degree of "bimorphism" among and between the L and M genes and the encoded pigments.[5,7,9,36] Sequence analysis of the genes expressed in the eyes of 92 male donors presumed to have had normal color vision revealed genes encoding 19 distinct L pigment variants and 9 M pigment variants (S. Sjoberg, M. Neitz, PhD, and J. Neitz, unpublished data, 1999).[37] These observations are consistent with the idea, previously introduced, that the genes represent various mixtures of 2 ancestral genes, one L and one M. However, the present variety of mixtures is so great that the genes are better termed chimeras not hybrids.

The chimeric L genes originally proposed to cause deuteranomaly were later found to be commonly present in men with normal color vision as well.[6,9,37] To reconcile this finding with the theory that these L genes cause color blindness, Yamaguchi et al[38] hypothesized that these genes are expressed in men with deuteranomaly but not in men with normal color vision. They proposed a mechanism in which only the first 2 genes in an array are expressed, and in deuteranomalous men, M genes, if present, are displaced to nonexpressed positions. To test this hypothesis, Yamaguchi et al identified 3 male eye donors with putative normal color vision who had genes for 2 L pigments. In each case, one gene specified isoleucine, alanine, and methionine at amino acid positions 230, 233, and 236, respectively, and the second gene specified threonine, serine, and valine at these respective positions. Expression of the L gene specifying threonine at position 230, serine at position 233, and valine at position 236 was not detected in total retinal messenger RNA (mRNA) from the 3 donors. This was taken as evidence that expression of the second L gene causes color blindness, and only 2 genes in an array are expressed. There is no doubt that examples can be found in which pigment genes are not expressed. However, a recent systematic characterization of the visual pigment genes expressed in retinas from men with normal color vision does not support the hypothesis that strictly 2 genes from the array are expressed.

Sjoberg et al[10] directly sequenced the X-linked visual pigment genes expressed in a punch of retina 6 mm in diameter and centered on the fovea from 92 male eye donors. Each donor was presumed to have had normal color vision because in each retinal punch both L and M pigment mRNA was detected. Direct DNA sequence analysis of the expressed genes revealed that about 8% of men with normal color vision expressed 2 distinct L pigment genes in addition to M genes. Thus, the presence of an extra L pigment gene in those with deuteranomaly is not alone sufficient to explain the loss of M cone contribution to deuteranomaly.

In the same study, Sjoberg et al[10] also reported that, in their total male eye donor population, which included men with putative normal color vision and color-blind men, 5.3% of the eyes did not show detectable expression of M pigments but did show expression of L pigments. This frequency is consistent with the population frequency of deutan color vision defects (6%). This study suggests the further insight that it is, indeed, the absence of expressed M pigment that causes deuteranomaly. It is the cause of that loss that is, in many cases, not known.

There is much about the expression of L and M pigment genes in the retina that is not well understood. It is clear that the expression patterns of the individual genes in an array are complex. For example, individual genes in an array are expressed at different levels. The first gene in the array is expressed in the most cones, usually in more cones than all the other genes combined.[10,11] Furthermore, the relative amounts of L vs M pigment mRNA vary as a function of retinal eccentricity.[39,40] There are clear cases in which more than just 2 of the genes in an array are expressed,[10] and there are clear cases in which some of the genes in an array are not expressed.[11,38,41] The question of what controls the expression of the visual pigment genes in the array on the X chromosome is an intriguing one, and one that remains open.

TOWARD A GENETIC DIAGNOSTIC TEST FOR RED-GREEN COLOR BLINDNESS

Printed pseudoisochromatic plates are the most widely used tests for color vision defects. Of these, the Ishihara tests for color deficiency have become a standard. The relative strengths that a genetic test might offer can be illustrated by comparing how such a test might perform compared with the Ishihara tests. There are at least 3 diagnostic functions that a test for color vision deficiency should perform. It should be able to discriminate people with normal color vision from those with color vision defects. It also should discriminate between protan and deutan defects, and acquired from congenital defects. The Ishihara tests do not perform satisfactorily for any of these diagnostic functions. Nearly half of the people who have normal color vision make errors on the Ishihara tests. Thus, distinguishing between normal and color-defective vision requires judgments about whether the number and types of errors constitute a color vision defect. Differences in instructions given by the examiner, lighting differences, and slight differences in the color printing in different test editions affect the number of misreadings that can be allowed to consider a person to have normal color vision. What this means in practice is that the test can be used to accurately detect color vision defects but only when administered by an experienced and knowledgeable practitioner. The number designs of the Ishihara test and even the pathway designs intended for evaluation of nonverbal adults are difficult for children and thus the test is not effective for detecting color vision defects in children younger than 7 or 8 years.

Distinguishing between protan and deutan defects can be an important aspect of color vision diagnosis. Ishihara results correctly predict protan vs deutan for about 50% of persons with color defects. Most people for whom the test is not predictive either make no errors on the protan-deutan diagnostic plates or miss the protan and deutan symbols.

A major limitation of the Ishihara tests is that most persons with color defects, dichromats and anomalous trichromats, fail to see the correct symbol in almost all the test plates and thus it is not possible to estimate the severity of color vision deficiency. Finally, the Ishihara tests cannot distinguish between congenital and acquired color vision deficiencies. In summary, even though the Ishihara tests are the best screening instruments that are widely available, they have serious limitations in the detection of color vision defects and particularly in assaying the type and severity of the defect.

A genetic test based on available technology has many potential strengths. In most cases, it could distinguish between congenital and acquired color vision problems. A genetic test would be extremely reliable in differentiating protan from deutan defects because almost all protan subjects are missing L pigment genes.

As previously noted, the most severe forms of the common red-green color vision deficiencies (the dichromacies) are commonly associated with X-linked pigment gene arrays that contain a single gene.[1,7,26,42] These dichromats can be distinguished from anomalous trichromats with perfect accuracy. Differences in severity within anomalous trichromacy are also highly correlated with the gene structures.[7] The correlation does not appear to be perfect,[43] but it is quite high and it may improve as our knowledge of structure and function continues to improve for the photopigment genes.

Two obvious advantages for detection of color vision problems are that a genetic test would be objective and could be used for any age, which could be a particular advantage for use with young children. A problem for the use of genetic information in detection is that arrays that contain L and M genes are found in men with normal and deuteranomalous color vision.[1,7,44] However, normal color arrays generally have more M than L genes, whereas deuteranomalous arrays usually have more L than M genes[1,6,34,45] and many more L genes than are found in men with normal color vision.[46] Deuteranomalous arrays with more M than L genes appear to be uncommon. A genetic assay to estimate the relative ratio of L/M genes could be used to predict whether an individual is more likely to be deuteranomalous or to have normal color vision. However, some persons with normal color vision would be misdiagnosed as having deuteranomaly if this were used as the only information. For the immediate future, considering our present state of understanding, perhaps the best test for color vision defects would be a combination of visual and genetic tests. The visual component could be simple, enough so that it would be appropriate for use with young children. The visual component would have an important function in detection of color vision problems. A follow-up genetic test would be used to provide confirmation of a congenital defect and to provide information about the type and severity of the defect. The results from such a combined test would be unmatched in detecting and typing color vision deficiencies.

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