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Molecular Basis of Abnormal Red-Green Color Vision: A Family with Three Types of Color Vision Defects

M. Drummond-Borg, S. Deeb, and A. G. Motulsky

Departments of Medicine and Genetics, and Center for Inherited Diseases, University of Washington, Seattle.

Summary

The molecular nature of three different types of X-linked color-vision defects, protanomaly, deuteranomaly, and protanopia, in a large 3-generation family was determined. In the protanomalous and protanopic males the normal red pigment gene was replaced by a 5′ red–3′ green fusion gene. The protanomalous male had more red pigment DNA in his fusion gene than did the more severely affected protanopic individual. The deuteranomalous individual had four green pigment genes and one 5′ green-3′ red fusion gene. These results extend those of Nathans et al., who proposed that most red-green color-vision defects arise as a result of unequal crossing-over between the red and green pigment genes. The various data suggest that differences in severity of color-vision defects associated with fusion genes are caused by differences in crossover sites between the red and green pigment genes. Currently used molecular methodology is not sufficiently sensitive to define these fusion points accurately, and the specific color-vision defect within the deutan or protan class cannot be predicted. The DNA patterns for color-vision genes of female heterozygotes have not previously been described. Patterns of heterozygotes may not be distinguishable from those of normals. However, a definite assignment of the various color pigment gene arrays could be carried out by family study. Two compound heterozygotes for color-vision defects who tested as normal by anomaloscopy were found to carry abnormal fusion genes. In addition, a normal red pigment gene was present on one chromosome and at least one normal green pigment gene was present on the other. Thus, the presence of normal green and red pigment genes insured normal color vision. The presence of fusion genes did not grossly influence color perception in these individuals. A third compound heterozygote had decreased luminosity for yellow in one eye only.

Introduction

X-linked red-green color-vision anomalies affect about 8% of the male Caucasian population and have been subdivided phenotypically into six groups defined as severe, moderate, or mild alterations in either red or green color perception (table 1). This classification has been supported by genetic studies (see Pokorny et al. 1979) that suggest allelism of characteristic mutations affecting either the red-sensitive pigment gene (protan anomalies) or the green-sensitive pigment gene (deutan anomalies). The color-vision locus has been localized near the end of the long arm of the X chromosome (Xq28) (Purrello et al. 1984). The nature of mutations affecting color vision remained unknown until Nathans et al. (1986*a*, 1986*b*) isolated clones of the red and green pigment genes and showed that males with normal color vision have one red pigment gene and one or more green pigment genes arranged in tandem. Red-green color-vision anomalies usually resulted from unequal crossing-over between the red and green pigment genes, leading to pigment gene deletions or to fusion (hybrid) genes that consisted of portions of both red and green pigment genes (such as 5′ red-3′ green or 5′ green-3′ red). A high frequency of such crossovers was postulated to occur because of the marked degree (98%) of homology between the nucleotide coding sequences of the red and green pigment genes. Several different kinds of pigment gene configurations were found in color-defective

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Address for correspondence and reprints: Dr. Arno G. Motulsky, Division of Medical Genetics, RG-25, University of Washington, Seattle, WA 98195.

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individuals, even within the same deutan or protan subclass. This heterogeneity suggests that a strict categorization into only one mild and only one moderate type of deuteranomaly or protanomaly (table 1) is unlikely to apply.

We previously studied a large kindred with three different types of X-linked color-vision anomalies (Thuline et al. 1969) (fig. 1). To extend our knowledge of the molecular basis of color-vision defects and to correlate genotypes and phenotypes for color vision, we determined the molecular genotypes (male and female) in family members of this kindred.

Methods

A family (fig. 1) previously reported by Thuline et al. (1969) had been extensively studied using anomaloscopy, Ishihara, and Hardy-Rand-Ritter plates to determine the nature of their color vision anomalies.

In anomaloscopy the subject matches a yellow field with a mixture of green and red light. The range of acceptable matches determines whether a subject has severe or mild deuteranomaly (green color-vision defects) or severe or mild protanomaly (red color-vision defects). See the legend to figure 5 for quantitation of anomaloscopy results. Members of a fourth generation, born since 1969, were also studied. Samples (15 ml) of heparinized blood were obtained from available family members for DNA analysis. The DNA specimens of an unselected population of 90 Caucasian males who had normal color-vision genes on molecular analysis were used to determine the distribution of the density ratios of the red and green pigment gene fragments (full studies to be published).

DNA Preparation

DNA was extracted from peripheral blood according to the method of Poncz et al. (1983). Cells were pelleted, washed three times with PBS (2.68 mM KCl, 1.47 mM KH_2PO_4, 137 mM NaCl, 8.06 mM $Na_2HPO_4 \cdot 7H_2O$), and resuspended in hemolysis buffer (0.89 mM NH_4HCO_3, 0.131 M NH_4Cl). The white cells were sedimented by centrifugation and resuspended in 1 ml PBS. Ten milliliters of lysis buffer (proteinase K 100 μg/ml, SDS 0.5%, NaCl 10 mM, EDTA 10 mM, Tris pH 8 10 mM) were added; the suspension was incubated at 37 C overnight, then extracted twice with phenol:chloroform (1:1 mix) and once with chloroform. The DNA was precipitated with 1 M ammonium acetate and 2.5 vol cold 95% ethanol overnight. The pellets were dissolved in TE buffer (10 mM Tris pH 7.4, 1 mM EDTA), reprecipitated with 1 M ammonium acetate and 2.5 vol cold 95% ethanol, dried, and dissolved in TE buffer.

Genomic Restriction-Endonuclease Analyses

Samples (10 μg) of DNA were digested to completion with *Eco*RI, *Rsa*I, or *Eco*RI/*Bam*HI according to the specifications of the manufacturer (Bethesda Research Laboratories). Samples were electrophoresed on agarose gels: 0.6% agarose gel for 20 h at 30 V for *Eco*RI fragments, 1.2% agarose gel for 18 h at 15 V for *Rsa*I, and 1.0% agarose gel for 18 h at 25 V for *Bam*HI/*Eco*RI digest. DNA was then transferred to nitrocellulose paper according to the method of Southern (1975).

Probes for the color-vision pigment genes were supplied by Dr. J. Nathans. A probe from the 5′ end of the cDNA of the red pigment gene encompassing exon 1 and part of exon 2 (probe A) was used to probe the *Eco*RI and *Eco*RI/*Bam*HI digests for fragments at the 5′ end of the red and green genes (fig. 2). A probe from the 3′ end of the genomic DNA of the green gene encompassing the 3′ end of the fourth intron (probe B) was used to probe the *Rsa*I digests to detect fragments at the 3′ end of the red and green genes (Nathans et al. 1986*b*) (fig. 2). The probes were labeled with [^{32}P]dCTP (New England Nuclear) by random prim-

Table 1

Types of X-linked Color-Vision Defects

Degree of Impairment	Defective Red Color Perception	Defective Green Color Perception
Severe	Protanopia[a]	Deuteranopia[b]
Moderate	Protanomaly[c]	Deuteranomaly[c]
Mild	Mild protanomaly[c]	Mild deuteranomaly[c]

[a] Dichromats, missing red color-vision perception.
[b] Dichromats, missing green color-vision perception.
[c] Trichromats, anomalous color perception.

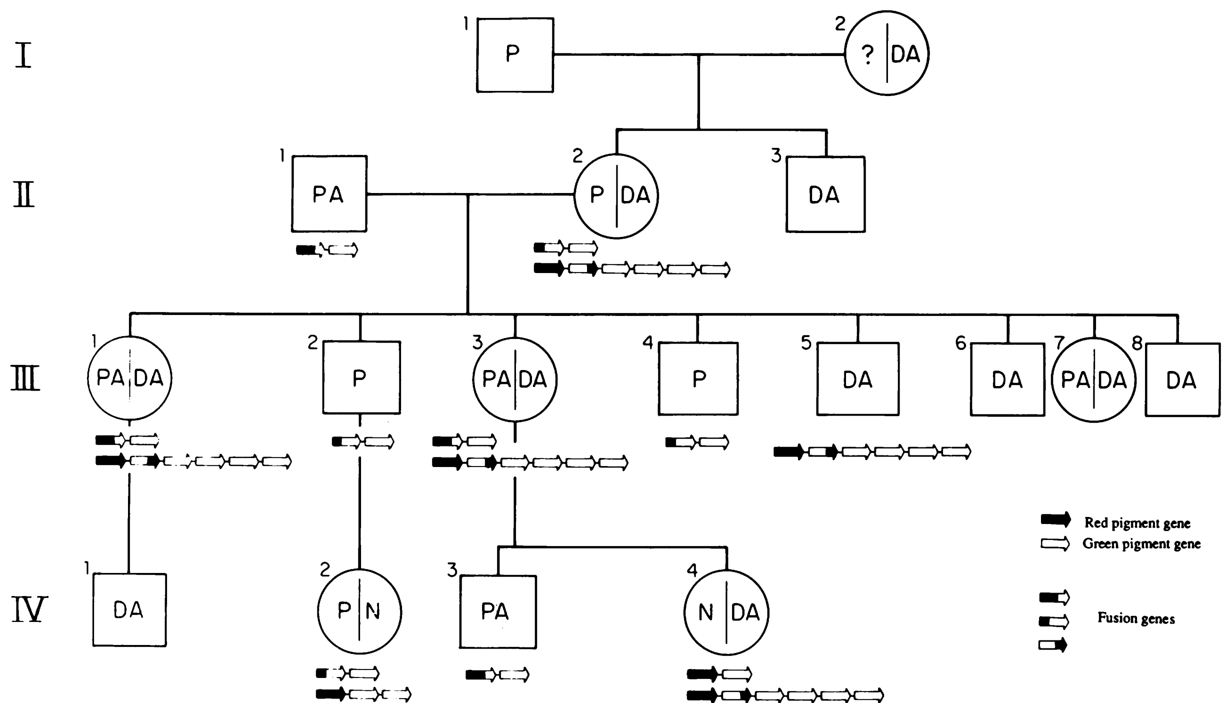

Figure 1 Family pedigree with color-vision phenotypes and genotypes. P = protanopia; PA = severe protanomaly; DA = mild deuteranomaly; N = normal color-vision gene.

ing (Boehringer Mannheim Labelling Kit) according to the procedure suggested by the manufacturers.

The Southern blots were prehybridized at 42 C overnight with 50% formamide, 4.6 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate), 5 × Denhardt's solution (1 × Denhardt's = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA), 0.1% SDS, 100 g denatured salmon sperm DNA/ml, and then were hybridized overnight at 42 C with 50% formamide, 4.6 × SSC, 5 × Denhardt's solution, 0.1% SDS, 100 g denatured salmon sperm DNA/ml, 10% dextran sulfate, and labeled probe (5 × 10^6 cpm/blot). The blots were washed twice in 0.1% SDS and 0.5 × SSC at 65 C for 10 min and 90 min. They were dried and exposed to preflashed x-ray film at −70 C for 1–7 days.

Densitometry

Densitometry of the autoradiograms was done using a Quick Scan Jr. densitometer (Helena Laboratories). Absorbance readings within the linear range of response were used to calculate ratios of the various bands.

Interpretation of Southern Blot Patterns

The *Eco*RI digests, when hybridized with probe A (see above), showed two bands—a slow-moving band from the 5′ end of the red pigment gene (A_r) and a fast-moving band from the 5′ end of the green pigment gene (A_g) (figs 2, 3). After hybridization of the *Rsa*I digests with probe B (see above), a fast-moving band, D_r (from the 3′ end of the red pigment gene), and a slow-moving band, D_g (from the 3′ end of the green pigment gene),

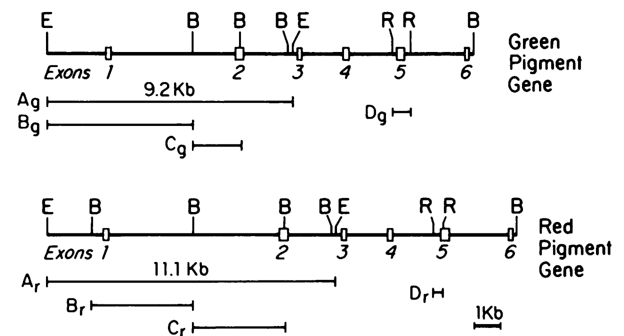

Figure 2 Visual pigment genes. E = *Eco*RI cleavage site; B = *Bam*HI cleavage site; R = *Rsa*I cleavage site. A_r, B_r, C_r, and D_r = red pigment gene fragments; A_g, B_g, C_g, and D_g = green pigment gene fragments. A, B, and C fragments were detected by a 300-bp cDNA probe encompassing exon 1 and part of exon 2. D fragments were detected by a 350-bp genomic DNA probe from the 3′ end of the fourth intron.

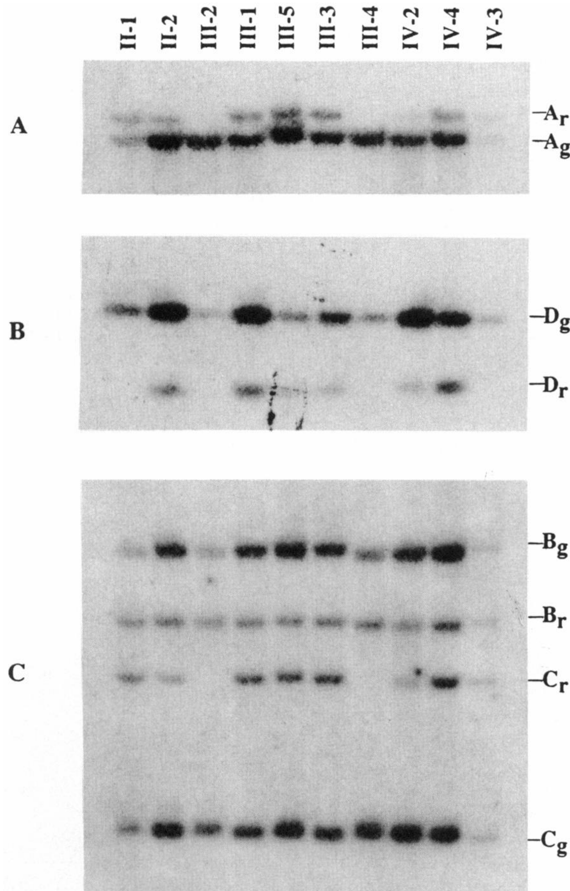

Figure 3 Southern blots of family members. *A*, *Eco*RI digest: A_r = 5′ fragment from red pigment gene; A_g = 5′ fragment from green pigment gene. *B*, *Rsa*I digest: D_g = 3′ fragment from green pigment gene; D_r = 3′ fragment from red pigment gene. *C*, *Bam*HI/*Eco*RI digest: B_g = 5′ fragment from A_g (green pigment gene fragment); B_r = 5′ fragment from A_r (red pigment gene fragment); C_r = 3′ fragment from A_r (red pigment gene fragment); C_g = 3′ fragment from A_g (green pigment gene fragment).

were observed. The ratios A_g:A_r and D_g:D_r were determined from densitometric measurements. Males with normal color vision had A_g:A_r and D_g:D_r ratios of 1:1, 2:1, 3:1, and 4:1 (Nathans et al. 1986*b;* Drummond-Borg et al. 1987), demonstrating the presence of one red pigment gene and one or more green pigment genes.

Gene fragments B_g, C_g, B_r and C_r (fig. 2) were found when genomic DNA was double digested with *Eco*RI and *Bam*HI and hybridized with probe A. Since B_g and C_g, and B_r and C_r were derived from the pigment gene fragments A_g and A_r, respectively (Nathans et al. 1986*b*), the fragment ratios B_g:B_r and C_g:C_r in males with normal color vision were expected to be identical to their respective A_g:A_r and D_g:D_r ratios.

As DNA fragments of different sizes usually did not transfer and hybridize equally to give equivalent signals on autoradiography, means and SDs of all the density ratios were calculated from males with normal color-vision pigment gene arrays (table 2). The assumption that all the individuals with equivalent A_g:A_r and D_g:D_r had normal arrays and thus normal color vision may not be totally correct, since more complex patterns may also give similar ratios. This is unlikely on statistical grounds. These values were used as standards for the determination of abnormal gene arrangements, i.e., complete gene deletions or fusion (hybrid) genes comprising pieces of both red and green genes. The hybrid genes were assumed to be full-length genes, since they were most likely generated by homologous recombination. Thus, the total number of 5′ gene fragments (A_g + A_r) equaled the total number of 3′ gene fragments (D_g + D_r). With this constraint, the number of copies of each gene fragment (A_g, A_r, D_g, and D_r) could be calculated from the density ratios (A_g:A_r and D_g:D_r) and the gene arrangement could be inferred. The number of copies of each gene fragment (B_g, B_r, C_g, and C_r) were calculated from the B_g:B_r and C_g:C_r density ratios on the assumption that the total number of B_g and B_r (as well as C_g and C_r) gene fragments equaled the total number of A_g and A_r gene fragments. Examples of abnormal gene arrangements and their density ratios from Nathans et al. (1986*a*) are shown in figure 4. There was some overlap of the ranges of density ratios for multiple copies of green genes, and an exact number for these genes could not always be unambiguously determined. However, the family data provided additional information which made it possible to assign a specific gene array to a given individual.

Results

The previously reported family (Thuline et al. 1969) in figure 1 and table 3 included a man with severe protanomaly (II-1) married to a woman (II-2) with normal color vision who was a compound heterozygote for protanopia and mild deuteranomaly. Among their eight children, two males (III-2 and III-4) had protanopia and three males (III-5, III-6, and III-8) had mild deuteranomaly. All three daughters (III-1, III-3, and III-7) were compound heterozygotes for severe protanomaly and mild deuteranomaly. Among the offspring of the fourth generation (not previously reported), there was one mild deuteranomalous male (IV-1), one severely protanomalous male (IV-3), one obligatory female heterozygote for protanopia (IV-2), and one obligatory fe-

Table 2

Ratio of Green:Red Gene Fragments in Family Members and Normal Males

A. Family Members

	Color-Vision Defect	A_g:A_r	B_g:B_r	C_g:C_r	D_g:D_r
Males:					
II-1	Protanomaly (severe)	1.07	1.53	0.75	∞
IV-3	Protanomaly (severe)	1.00	1.58	1.17	∞
III-2	Protanopia	∞	1.04	∞	∞
III-4	Protanopia	∞	2.32	∞	∞
III-5	Deuteranomaly (mild)	4.40	4.26	1.81	1.90
Females:					
II-2	Compound heterozygote for mild deuteranomaly and protanopia	8.30	4.40	7.30	2.85
III-1	Compound heterozygote for mild deuteranomaly and severe protanomaly	2.90	2.63	1.28	2.90
III-3	Compound heterozygote for mild deuteranomaly and severe protanomaly	3.05	3.39	1.53	4.00
IV-2	Simple heterozygote for protanopia	4.27	2.42	3.47	4.50
IV-4	Simple heterozygote for mild deuteranomaly	2.80	3.32	2.04	1.80

B. Normal Males (in Mean ± SD)[a]

Green:Red Pigment Gene Ratio	A_g:A_r	B_g:B_r	C_g:C_r	D_g:D_r
1:1	1.04 ± .14 (20)	1.64 ± .81 (5)	.63 ± .4 (5)	1.21 ± .36 (20)
2:1	2.0 ± .21 (47)	3.7 ± 1.02 (9)	1.2 ± .35 (9)	2.62 ± .66 (47)
3:1	3.04 ± .42 (19)	4.4 ± 1.65 (6)	2.09 ± .62 (6)	3.11 ± .89 (19)
4:1	3.99 ± .44 (4)	6.56 ± 1.88 (2)	3.13 ± 1.23 (2)	5.33 ± 1.12 (4)

[a] Numbers in parentheses are number of individuals studied.

male heterozygote for either mild deuteranomaly or severe protanomaly (IV-4), since her mother was a compound heterozygote for these defects.

We were able to study the red and green color-vision pigment genes of males with the three different color-vision defects: protanopia, severe protanomaly, and mild deuteranomaly. Several simple and compound female heterozygotes were also investigated. Ratios of red and green fragments generated by digesting genomic DNA with *Eco*RI, *Rsa*I, and with both *Bam*HI and *Eco*RI (figs. 2, 3) were determined following quantitation by densitometry (table 2). Gene arrays for color-vision pigment genes were assigned as explained under Methods.

Males

Severe protanomaly—II-1 and IV-3.—The color pigment gene fragment analysis for II-1 showed an A_g:A_r ratio of 1.07, which was consistent with the presence of one copy each of the 5′ ends of the red and green genes. This was confirmed by the B_g:B_R ratio of 1.53 and C_g:C_r ratio of 0.75, which were within the ranges of 1:1 ratios for these fragments (table 2). The D_g:D_r ratio of infinity was due to the absence of the D_r band, caused by the absence of the 3′ end of the red pigment gene.

All these findings were best explained by the absence of an intact red pigment gene, the presence of an intact green pigment gene, and a 5′red-3′green pigment fusion gene (see pigment gene symbols in fig. 1). The DNA analysis of IV-3 showed ratios similar to those observed in his maternal grandfather (II-1), except for the C_g:C_r ratio; this ratio was 1.17, compared with the 0.75 found

Genotype	Expected Ratios Ag/Ar	Dg/Dr	Phenotype
	1	1	Normal
	2	2	Normal
	3	3	Normal
	0	0	Deuteranopia
	1	0	Deuteranopia
	2	0.5	Severe Deuteranomaly
	3	1	Severe Deuteranomaly
	2	0	Mild Deuteranomaly
	0	inf	Protanopia
	1	inf	Protanopia
	2	inf	Protanopia
	inf (Bg/Br=1 Cg/Cr=inf)	inf	Protanopia
	2	inf	Mild Protanomaly
	4	inf	Severe Protanomaly

Figure 4 Color-vision pigment genotypes and phenotypes in defective color vision (from Nathans et al. 1986*a*, 1986*b*). See legend of fig. 1 for explanation of gene symbols and legend of fig. 2 for definition of A_g, B_g, C_g, D_g, A_r, B_r, C_r, D_r. inf = Infinite ratios.

in II-1. However, these values do fall within the 95th percentile range for a 1:1 C_g:C_r ratio. These findings were interpreted to reflect the same gene pattern.

Protanopia—III-2 and III-4.—No A_r, C_r, or D_r bands were seen. III-2 had a B_g:B_r density ratio of 1.04. A_g, C_g, and D_g bands were present. These findings were interpreted as implying the presence of an intact green pigment gene and a 5′red-3′green fusion gene which had only a very small 5′ red pigment gene fragment (B_g). Individual III-4 had a similar restriction-fragment pattern. The B_g:B_r density ratio of 2.32 was consistent with a 1:1 fragment ratio (normal, 1.64 ± 0.81).

Mild deuteranomaly—III-5.—The A_g:A_r density ratio was 4.4 and represented the presence of four or five copies of the A_g fragment and one copy of the A_r fragment. The D_g:D_r density ratio of 1.9 represented the presence of three or four copies of the D_g fragment and two copies of the D_r fragment (see Interpretation section under Methods). The B_g:B_r and C_g:C_r density ratios were consistent with the presence of four or five copies of green fragments and one copy of a red fragment. These data indicated the presence of five or six genes (one red gene, one 5′green-3′red fusion gene, and three or four green genes); however, the results of his mother's (II-2) DNA studies (see below) favored the interpretation of the presence of six genes (one red gene, one 5′green-3′red fusion gene, and four green genes).

Females

Simple Heterozygotes

Obligatory heterozygote for protanopia—IV-2.—IV-2 had an A_g:A_r density ratio of 4.27, a D_g:D_r density ratio of 4.5, and a C_g:C_r density ratio of 3.47. These values

Table 3

Identifying Family Data and Color-Vision Phenotypes

Individual (Year of Birth)	Anomaloscopic Midpoint Match (Nagel Units)	Color-Vision Phenotypes[a]
I-1 (1892)	Accepts all matches	Protanopia
I-2 (1894)	45	Normal
II-1 (1918)	55.2	Severe protanomaly
II-2 (1919)	45.3	Normal
II-3 (1924)	26.0	Mild deuteranomaly
III-1 (1943)	46.1	Normal but decreased luminosity for right eye
III-2 (1944)	Accepts all matches	Protanopia
III-3 (1946)	45	Normal
III-4 (1948)	Accepts all matches	Protanopia
III-5 (1950)	22.1	Mild deuteranomaly
III-6 (1952)	30.0	Mild deuteranomaly
III-7 (1957)	Not done	Mild deuteranomaly
III-8 (1959)	Not done	Mild deuteranomaly
IV-1 (1977)	Not done	Mild deuteranomaly
IV-2 (1977)	Not done	Normal
IV-3 (1971)	Not done	Severe protanomaly
IV-4 (1975)	Not done	Normal

[a] See Thuline et al. (1969) for full color-vision test results.

were consistent with a green-pigment-gene-to-red pigment-gene ratio of 4:1. However, her B_g:B_r density ratio of 2.42 was not consistent with a 4:1 ratio and best fitted a 3:2 ratio of B_g and B_r gene fragments, since the total number of B_g and B_r fragments must equal five (see Interpretation section under Methods). As she must have inherited her father's (III-2) gene array of a small 5′red–large 3′green fusion gene and an intact green gene, these results indicated the inheritance of an intact red pigment gene and two intact green pigment genes from her mother.

Heterozygote for mild deuteranomaly—IV-4.—IV-4 must have inherited either the mild deuteranomalous gene array or the severe protanomalous gene array from her compound heterozygote mother (III-3). She had an A_g:A_r ratio of 2.8, which was interpreted as a 3:1 or 6:2 fragment ratio. Her B_g:B_r and C_g:C_r ratios were consistent with 3:1 or 6:2 fragment ratios, but her D_g:D_r ratio of 2.8 fitted best a 2:2, 4:4, or 5:3 fragment ratio. These density ratios were consistent with both the transmission of the mild deuteranomalous gene array (one intact red gene, a 5′green-3′red fusion gene, and four intact green genes) from her mother (III-4) and the paternal inheritance of a normal color-vision set consisting of one intact red gene and one intact green gene. This girl therefore was a heterozygote for mild deuteranomaly.

Compound Heterozygotes

Heterozygote for protanopia and mild deuteranomly—II-2.—II-2 had an A_g:A_r density ratio of 8.3, which was consistent with a ratio of seven to nine green genes to one red gene. The D_g:D_r density ratio of 2.85, however, fitted a ratio of five to seven green genes to two red genes. Her two protanopic sons (III-2 and III-4) had a gene arrangement of one fusion 5′red-3′green gene and one intact green gene. Thus, her deuteranomalous X chromosome must have comprised one red gene, one fusion 5′green-3′red gene, and four intact green genes to obtain the above density ratios. This interpretation was consistent with the results found in her mildly deuteranomalous son (III-5).

Heterozygotes for severe protanomaly and mild deuteranomaly—III-1 and III-3.—III-1 had an A_g:A_r density ratio of 2.9 and a D_g:D_r density ratio of 2.9. She inherited her father's X-chromosome color pigment gene array consisting of one 5′red-3′green fusion gene and one green gene. To obtain the above ratios she must have received a maternal X chromosome with one red gene, one 5′green-3′red fusion gene, and four intact green genes (fig. 1). The B_g:B_r and C_g:C_r ratios were consistent with the proposed arrays. The density ratios for III-3 (table 2) were consistent with the same gene arrangement as described for III-1 (fig. 1).

Discussion

Nathans et al. (1986*a*, 1986*b*) have carried out the pioneering study on the molecular basis of color vision. They demonstrated that most X-linked color-vision aberrations could be accounted for by various deletions of the color-vision pigment genes and by the existence of hybrid or fusion genes consisting of portions of the red and green pigment genes. Their findings on 25 color-defective males are summarized in figure 4. Some genetic implications of these and other findings have been discussed elsewhere (Motulsky 1988). Here we discuss the genetic implications of our findings.

Genotype-Phenotype Correlations

The three different color-vision phenotypes in the color-vision-defective males of this family were associated with three different gene restriction-fragment patterns (table 2, fig. 3). These patterns were consistent with the three different gene arrays illustrated for individuals II-1, III-2, and III-5 (fig. 1). These array assignments confirm Nathans et al.'s (1986*a*) studies suggesting that deuteranomaly, protanomaly, and protanopia are associated with fusion genes.

The anomaloscopic findings of affected family members, together with those for some of the individuals studied by Nathans et al. (1986*a*, 1986*b*) are shown in figure 5. Note that Nathans et al.'s (1986*a*) individuals 28 and 42, who apparently had the same genotypic array (one red-green fusion gene and two intact green genes) were protanopic and mildly protanomalous, respectively. Similarly, individual 31 of Nathans et al. (1986*a*) and individual II-1 from our study had the same genotype (one red-green fusion gene and one intact green gene). Yet, individual 31 was protanopic and II-1 was severely protanomalous. These phenotypic discrepancies could be due to slight differences in the crossover points between the red and green pigment genes, resulting in protein pigments with different in vivo light sensitivity, causing different color perception. The molecular methods used in such studies were unable to distinguish between small differences in the position of crossover points.

Heterozygotes and Compound Heterozygotes

The patterns found in female heterozygotes and compound heterozygotes fit those deduced from the pat-

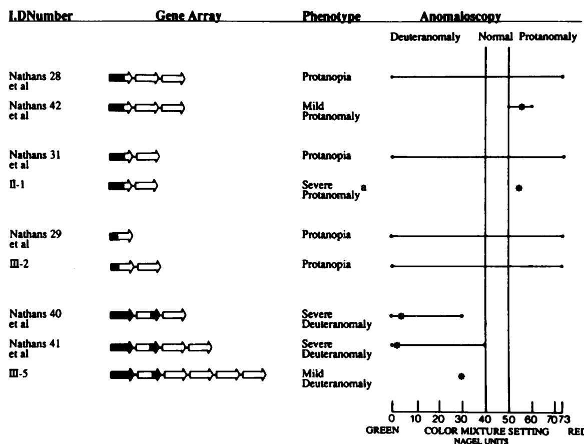

Figure 5 Genotype-phenotype correlations in color-vision defects. See legend to fig. 1 for explanation of gene symbols. Anomaloscopy results are expressed as the midpoints of the free-matching range in Nagel units. The range of accepted matches of mixtures of green and red light to a standard is provided, when available. The free-matching range is the range of acceptable matches when the subject is free to adjust the intensity of the red, green, and yellow lights to obtain a match. Dichromats such as protanopes and deuteranopes accept all mixtures as matches. Normals accept matches in a narrow range of 40–50 Nagel units. Deuteranomalous trichromats have free-ranging midpoint matches in the range <40 Nagel units. Protanomalous trichromats have free-ranging midpoint matches in the range >50 Nagel units. The wider the range of acceptable matches the poorer the chromatic discrimination.

[a]II-1 was classified as a severe protanope according to the Ishihara charts. On anomaloscopy this subject had a free-matching range midpoint in the protanomalous range. Thus, he was categorized as having severe protanomaly.

terns seen in their parents. It is of interest that even without a family study individuals II-2 and IV-4 were shown to be carriers of a gene array associated with a color-vision anomaly. Their A_g:A_r and D_g:D_r ratios (table 2) were not the same as would be expected in females who carried two normal sets of red and green color pigment genes. Thus, in II-2 and IV-4 one would infer the presence of a fusion gene producing the difference in their A_g:A_r and D_g:D_r ratios. Such females are almost certain to be heterozygotes for a color-vision anomaly. However, the findings in III-1, III-3, and IV-2 would have been interpreted as normal gene patterns, since A_g:A_r ratios were similar to D_g:D_r ratios in all these females. Thus, not all female heterozygotes and compound heterozygotes can be detected by molecular methods alone, but their genetic constitution for color pigment genes can often be deduced by appropriate family studies.

The distribution of red or green color-vision pigments in the retinal cones poses some interesting problems. Normal males have approximately equal numbers of cones expressing either the red or the green pigment genes but not both. The regulatory mechanism that limits gene expression to either the red or the green pigment gene complex is unknown. This process is quite different from that of Lyonization or random X inactivation, which inactivates both red and green pigment genes alongside most other X-chromosome genes on the same X chromosome. Among female heterozygotes for a deutan color-vision defect, the process of random X inactivation implies that about 50% of green cones will carry the abnormal gene array and that 50% will carry the normal green gene(s). Analogously, among protan heterozygotes, 50% of the red cones will carry the abnormal red pigment gene and 50% will have the normal red pigment gene. In both deutan or protan heterozygotes, the presence of green and red cones with normal pigment genes makes for normal color vision.

Compound heterozygotes with a protan defect on one X chromosome and a deutan defect on the other usually have grossly normal color vision (Jaeger and Lauer 1976). The three compound (protan/deutan) heterozygotes with normal color vision in our study inherited both an abnormal maternal and paternal pigment gene array (fig. 1). However, a normal green pigment gene was included in one gene array and a normal red pigment gene in the other. To understand the distribution of normal and abnormal color-vision gene products, the two different processes of (*a*) random X intactivation and (*b*) limitation of color-vision pigment gene expression to either the red or green pigment gene(s) in a given retinal cone cell must be considered. In a compound heterozygote for both deutan and protan abnormalities, Lyonization will on the average lead to suppression of 50% of X chromosomes with the deutan defect and of 50% of the X chromosomes with the protan defect. Among the expressing X chromosomes that are not inactivated, about 50% will only express the green pigment gene(s) and 50% will express the red pigment gene. Since the deutan X chromosome includes a normal red pigment gene and the protan X chromosomes include normal green pigment gene(s), 25% of the cone cells will express the normal green pigment gene(s), 25% will express the normal red pigment gene, 25% will express the mutant green-red fusion genes, and 25% will express the mutant red-green fusion gene. Thus, even though only 50% of the cone cells express normal color-vision pigment genes, and despite the expression of the fusion pigment gene in the other 50%, color vision is normal. However, one of the compound heterozygotes (III-1) had decreased

luminosity for yellow light in one eye only (Thuline et al. 1969). The significance of this finding is unknown.

The actual proportion of various color-sensitive cones resulting from X inactivation may vary from the 50:50 ratio. As an example, in simple heterozygotes most of the normal X chromosomes occasionally may be inactivated, leading to detectable color-vision defects in such carriers. The same process might occur in compound heterozygotes (deutan/protan), but the frequency of color-vision defects in such instances would be expected to be twice that among simple heterozygotes.

In conclusion, we have shown that the probes currently available for studying the X-linked color-vision pigment genes may not predict the exact color-vision phenotype within the deutan or protan class. More work needs to be done to determine the exact crossover points of the fusion genes and their effect. The influence of one or more intact green genes on color-vision phenotypes also needs further investigation. Not unexpectedly, carrier status cannot always be detected by the molecular methods currently used.

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