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Genetics

Molecular genetics of X chromosome-linked color vision among populations of African and Japanese ancestry: High frequency of a shortened red pigment gene among Afro-Americans

(visual pigment genes/color vision defects/molecular evolution/Afro-American and Japanese populations)

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ABSTRACT **Red–green color vision in humans is mediated by the X chromosome-linked highly homologous red and green pigment genes. Color vision defects are caused by deletions and fusions involving these genes. However, we found the frequency of molecular abnormalities among Caucasians to be twice as high as that of phenotypic color vision defects. Among Japanese the frequency of phenotypic and molecular color vision defects was similar (≈5%). Among Afro-Americans, molecular defects (largely green–red fusion genes) were at least five times more frequent (21%) than phenotypic color vision defects (≈4%). In addition, 35% of Afro-Americans, 2% of Japanese, and <1% of Caucasians had a shortened red pigment gene not associated with phenotypic color vision defects. This gene lacked 1.9 kilobases in its first intron and had the identical size as the green pigment gene from which it presumably originated by gene conversion in an ancestral African population. This gene and the closely linked glucose-6-phosphate dehydrogenase A^+ variant were in linkage equilibrium. A model for the evolutionary origin of the color vision pigment genes in higher primates is portrayed.**

Normal human red–green color vision is mediated by a single red pigment gene and one or more green pigment gene(s) that are located on the long arm of the X chromosome (q28). The X-linked pigment genes are arranged in a head-to-tail array tandem configuration with the red pigment gene 5′ to the green pigment gene(s) (1–4). The red and green pigment genes are highly homologous and their deduced protein products differ by at most 15 amino acids. The green pigment gene further differs from the red pigment gene by a first intron that is ≈1.9 kilobases (kb) shorter (1). Color vision defects of red and green perception are caused by deletions or fusions between the red and green pigment genes that are caused by illegitimate recombination between the highly homologous red and green pigment genes (2, 5). However, in a study of 134 men of European ancestry we found a higher frequency of molecular abnormalities affecting X-linked color vision pigment genes than we expected considering the frequency of phenotypic color vision defects in such a population (16% observed vs. 8–10% expected) (6). We suggested that some fusion genes comprising both green and red pigment gene sequences may *not* be associated with color vision defects.

A lower frequency of *phenotypic* color vision anomalies is found among Japanese (5%) (7) and Afro-American males (3.9%) (8) as compared with Caucasians. We hypothesized that this lower frequency might be due to the presence of fewer green pigment genes among individuals with normal color vision in these populations. With fewer green pigment genes the opportunities for illegitimate recombination would be lessened causing a lower frequency of deletions or fusion genes. To delineate molecular variants of color pigment genes, we used Southern blot hybridization to analyze the molecular patterns of the X-linked color pigment genes in 102 unselected males of Afro-American descent and 101 unselected males of Japanese descent.

METHODS

Sample Populations and Southern Blot Analysis. A population of 101 unselected adult Japanese (Nisei) males from Seattle with no known admixture from other ethnic groups was used for this study. The blood specimens had been obtained for studies on the frequency of non-insulin-dependent diabetes and were used anonymously. No phenotypic color vision testing was done. Afro-American blood specimens were collected from 102 unselected newborn males during a newborn screening program for sickle cell anemia in Georgia. Male sex in this population was confirmed by dot blot hybridization of the DNA to a Y chromosome-specific probe (9). DNA extraction, gel electrophoresis, Southern blotting, hybridization and washing blots, autoradiography, and densitometry have been described (5, 6). The probes for the color vision pigment genes were supplied by J. Nathans (Johns Hopkins University, Baltimore).

Interpretation of Southern Blot Patterns. *Eco*RI restriction fragments (Figs. 1 and 2) denoted A encompass the 5′ and middle part of the pigment genes, whereas B and C are subfragments of A generated by digesting genomic DNA with *Eco*RI and *Bam*HI. A, B, and C fragments were detected on Southern blots by a 350-base-pair (bp) cDNA probe encompassing exon 1 and the 5′ part of exon 2 of the standard red pigment gene. The *Rsa* I fragments denoted D are derived from the 3′ region of the pigment genes and were detected on Southern blots by a 400-bp genomic DNA probe from the 3′ end of the fourth intron of the green pigment gene (1). A_r, B_r, C_r, and D_r are standard red pigment gene fragments; $A_{r\text{-}s}$ (s for short) and $C_{r\text{-}s}$ are shortened red pigment gene fragments. A_g, B_g, C_g, and D_g are green pigment gene fragments. The interpretation of various molecular patterns previously encountered has been described (5, 6).

Fig. 2 shows autoradiogram patterns characteristic for genotypes with the shortened red pigment gene (individual 10380) and for genotypes with the standard red pigment gene (individual 9743). Each lane contains 10 μg of genomic DNA. It can be inferred from the green/red fragment ratios that both individuals carry two green pigment genes along with one red pigment gene. The structure of the shortened red pigment gene (Fig. 1*B*) is deduced from these results. Individual 10380 lacks an A_r fragment (Fig. 2*A*). It is replaced by a new fragment $A_{r\text{-}s}$, which comigrates with the A_g fragment forming an $A_{r\text{-}s}$ + A_g band that is more intense than the corresponding A_g band in individual 9743. Fragment $A_{r\text{-}s}$ therefore has the same size as fragment A_g. Similarly, frag-

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Abbreviation: G6PD, glucose-6-phosphate dehydrogenase.

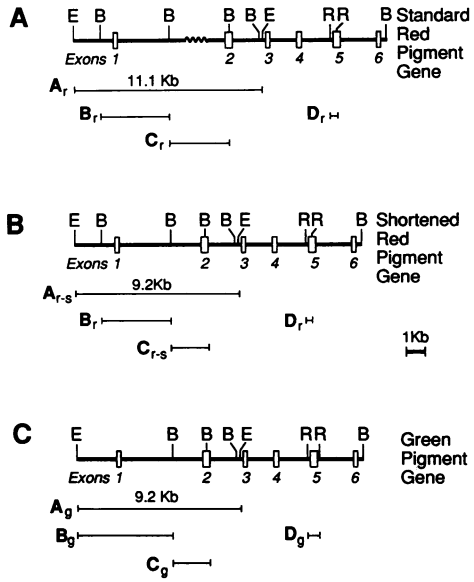

FIG. 1. Restriction maps of X-linked color vision pigment genes. *A*, standard red pigment gene; *B*, shortened red pigment gene; *C*, green pigment gene; E, *Eco*RI; B, *Bam*HI; R, *Rsa* I. The restriction fragments correspond to bands on the Southern blots in Fig. 2. A_r, B_r, C_r, and D_r are standard red pigment gene fragments; A_{r-s} (s for short) and C_{r-s} are shortened red pigment gene fragments; A_g, B_g, C_g, and D_g are green pigment gene fragments. Fragments A_{r-s} and A_g are of equal length, and fragments C_{r-s} and C_g are of equal length. The corresponding standard red pigment gene fragments A_r and C_r are larger due to an ≈1.9-kb longer first intron (wavy line). A, B, and C fragments were detected by a 350-bp cDNA probe encompassing exon 1 and part of exon 2 of the standard red pigment gene. D fragments were detected by a 400-bp genomic DNA probe from the 3′ end of the fourth intron of the green pigment gene (obtained from J. Nathans).

ment C_r (Fig. 2*C*) is replaced by a new fragment C_{r-s}. It comigrates with the C_g fragment forming a C_{r-s} + C_g band more intense than the corresponding C_g band in individual 9743. The new C_{r-s} fragment thus has the same size as the C_g fragment. No other fragments are detected in Fig. 2*A* and *C*.

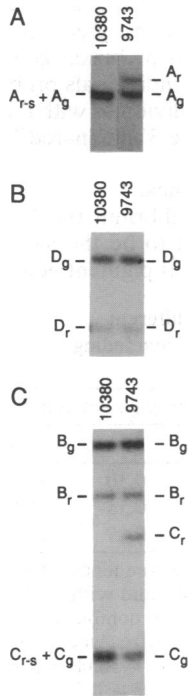

FIG. 2. Autoradiographs of restriction fragments of X-linked color vision genes (Southern blots). Individual 10380 has a shortened red pigment gene, whereas individual 9743 has a standard red pigment gene. Both persons have two green pigment genes. The restriction fragments correspond to those given in Fig. 1. Note that 10380 is lacking an A_r fragment (*A*), which is replaced by a new fragment A_{r-s} that has the same size as the A_g fragment and comigrates with it (*A*). Similarly, the C_r fragment (*C*) has the same size as the C_g fragment and is denoted as C_{r-s} (*C*). No other bands were detected. Since the A_{r-s} and C_{r-s} fragments were 1.9 kb shorter than the corresponding A_r and C_r fragments, we conclude that the new red pigment gene is 1.9 kb shorter in the 3′ segment of its first intron (see Fig. 1).

No changes are observed in the B_r fragment and D_r fragment (Fig. 2*B*).

The green pigment gene is shorter than the standard red pigment gene due to a 1.9-kb shorter first intron (1). Since fragment A_g and fragment C_g (subfragment of A_g) are correspondingly reduced in size (1.9 kb), intronic sequences of fragment C_r (wavy line, Fig. 1*A*) are absent, making for a shorter first intron of the green pigment gene. Similarly, the shortened red pigment gene is 1.9 kb shorter than the standard red pigment gene due to absence from fragment C_{r-s} of 1.9-kb intronic sequences of fragment C_r of the standard red pigment gene. It is highly likely that these missing sequences are identical to those absent from fragment C_g of the green pigment gene. The alternative explanation that the shortened red pigment gene is in fact a standard red pigment gene with an extra *Bam*HI site 1.9 kb downstream of the *Bam*HI site in the first intron and an extra *Eco*RI site 1.9 kb from one of the *Eco*RI sites is incompatible with the Southern blots.

Nathans *et al.* (2) found that males with normal color vision have equivalent A_g/A_r, B_g/B_r, C_g/C_r, and D_g/D_r density ratios, as measured by densitometry on autoradiograms. These ratios can be 1:1, 2:1, 3:1, and possibly up to 5:1 depending on the presence of one or more green pigment genes along with a single red pigment gene. In genotypes with the shortened red pigment gene (individual 10380 in Fig. 2) the A_g/A_{r-s} and C_g/C_{r-s} ratios (shown as ∞ in Figs. 3 and 4) are impossible to establish since fragments A_g and A_{r-s} and fragments C_g and C_{r-s} comigrate. In these genotypes, however, equivalent B_g/B_r and D_g/D_r ratios indicate the presence of gene arrays consistent with normal color vision. Inequality in the green/red fragment ratios in any individual indicates the presence of an abnormal gene array—i.e., complete gene deletion or fusion genes comprising pieces of both red and green pigment genes.

Glucose-6-Phosphate Dehydrogenase (G6PD) Genotyping. The relevant nucleotide base substitutions were detected by polymerase chain reaction amplification followed by digestion with *Fok* I and polyacrylamide gel (10%) electrophoresis for G6PD A^+ and with *Nla* III and electrophoresis on 12% acrylamide gels for G6PD A^- (10). Neither G6PD enzyme assays nor electrophoretic *phenotyping* could be done for technical reasons. Note that although all G6PD A^+ carriers have the characteristic polymorphisms detectable by *Fok* I, all G6PD A^- alleles carry this A^+ substitution in addition to a second mutation detectable by *Nla* III. However, a few G6PD A^- carriers have other substitutions (11) that would not have been detected in this study.

RESULTS

A "New" Shortened Red Pigment Gene. We discovered "new" red color vision pigment gene, distinctively different from the standard red color vision pigment gene (Fig. 1*B* and Fig. 2). Based on our analysis of restriction fragment sizes (see *Methods* and Fig. 2), we conclude that the first intron of this new red pigment gene is shorter by ≈1.9 kb as compared with the standard red pigment gene (Fig. 1). This new shortened red pigment gene has the same size as the green pigment gene, which also lacks this 1.9-kb sequence (Fig. 1). The new red pigment gene was present in 35% (36/102) of unrelated Afro-Americans but only in 2 of 101 of the Japanese (Fig. 3) and in 1 of the 134 Caucasians previously studied. Reinterpretation of the restriction pattern of individual 1238 in our previous study (ref. 6; see Fig. 5) appears most compatible with the presence of the new shortened red pigment gene. Since the frequency of detectable phenotypic color vision defects among Afro-American males is only 4% (8) while the new gene was observed in about a third of this population, we conclude that this genotype is not associated with phenotypically abnormal color vision. Ongoing anomaloscopy studies confirmed that the new gene has no phe-

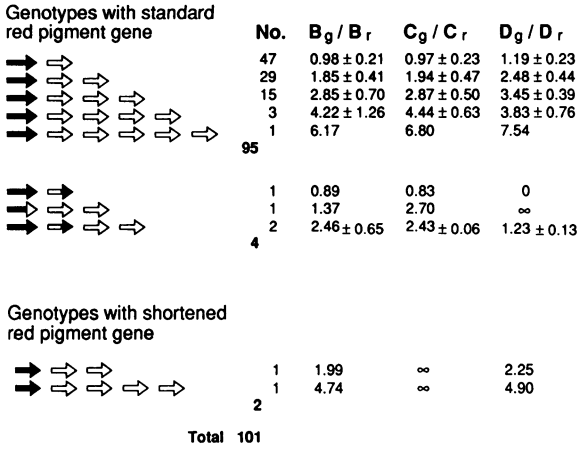

FIG. 3. X-linked color vision pigment gene pattern in 101 *unselected* adult Japanese (Nisei) males from Seattle. No., number of individuals with a given genotype; B_g/B_r, C_g/C_r, and D_g/D_r are densitometry ratios obtained from autoradiograms (see Fig. 2). Values are means ± standard deviation. Arrows are oriented in 5′ → 3′ direction. Solid and open arrows represent red and green pigment genes, respectively. Fusion genes consist of both green and red pigment gene segments, as illustrated. The position of fusion genes in the array is arbitrarily shown next to the red pigment gene. The shortened red pigment gene and the green pigment gene are drawn to equal size, whereas the standard red pigment gene is drawn longer reflecting the ≈1.9-kb longer first intron (Fig. 1).

notypic effects on color vision. In any case, phenotypic effects would be unlikely since the molecular alteration affects an intron and not an exon or intronic splice junctions.

No Allelic Association Between the Shortened Red Pigment Gene and G6PD A. The color vision locus and the G6PD locus are about 500 kb (or 0.5 recombination unit) apart on the X chromosome (q28) (3, 12, 13). The G6PD variants A^+ and A^- are common among Black populations (14). Since the high frequency of G6PD variants among populations of African origin is most likely caused by malarial selection (see ref. 15 for references), we tested for allelic association of the common G6PD variants and the common shortened red pigment gene on the hypothesis that the high frequency of the shortened red pigment gene might be caused by cosegregation with the closely linked G6PD A gene. The frequencies of G6PD variants among carriers of the standard and the shortened red pigment genes are given in Table 1. The genotype in 28% (28/99) of this Afro-American sample was G6PD A^+, whereas 4% (4/99) carried the G6PD A^- variant detectable by *Nla* III. Wild-type G6PD B was found in 68% (67/99) of the cases. The shortened red pigment gene was present in 10 (35.7%) of the 28 G6PD A^+ carriers and in 2 (50%) of the 4 G6PD A^- carriers. Among 67 G6PD B carriers the shortened red pigment gene was found in 24 (35.8%). Thus, the frequency of the new shortened red pigment gene was identical in G6PD A^+ and wild-type G6PD B carriers—i.e., there was no allelic association between G6PD A^+ and the shortened color vision locus. The data for G6PD A^- (detectable by *Nla* III) were similar but the numbers were small. The gene frequencies of the new shortened red pigment gene and G6PD A^+ were sufficiently high to be certain that the conclusion of no allelic association (or linkage equilibrium) between these loci in this sample of 99 individuals was real and not spurious as is possible with low allele frequency of one of these genes under study (16).

Table 1. Frequencies of G6PD variants among carriers of standard and shortened red pigment gene

G6PD genotype	Red color vision pigment gene genotype: Standard	Shortened (%)	Total
B (wild-type)	43	24 (35.8)	67
A^+	18	10 (35.7)	28
A^-	2	2 (50)	4
Total	63	36 (36.3)	99

Includes all cases, with and without color vision molecular abnormalities (3 of the 102 specimens could not be analyzed for G6PD genotype). Note that there is no difference in shortened red pigment gene frequency between carriers of G6PD A variants (A^+ and A^-) and normals (B).

Molecular Pigment Gene Variants Among Japanese. Among 101 Japanese males tested, 97 had normal molecular color vision genotypes. The number of green pigment genes varied from one to five, similar to the findings among Caucasoids (6). However, about one-half of these men (48.5%) had only one green pigment gene as opposed to about one-fifth (22%) among Caucasoids (Table 2). This difference may explain the low frequency of color vision defects among Japanese (see *Discussion*) (Fig. 3). Four percent (4/101) had molecular color vision abnormalities as might be expected from the 5% frequency of phenotypic color vision defects. One individual had a 5′ red–green 3′ fusion gene along with two normal green genes and may be classified as protan [R^- or R′: protanopic (R^-) and protanomalous (R′) patterns are not distinguishable by current molecular methodology]. Three individuals had a 5′ green–red 3′ fusion gene. One had no normal green gene and most likely was deuteranopic (G^-); two had two normal green genes and most likely would be classified as deuteranomalous (G′).

Molecular Pigment Gene Variants Among Afro-Americans. The molecular patterns of color vision pigment genes among males of African ancestry were highly heterogeneous. We found 21 different genotypes among the 102 males tested, and aside from the high frequency (35%) of the new shortened red pigment gene, another 21% (21/102) of this population had molecular color vision gene variants (Fig. 4). Three individuals with the standard red pigment gene lacked any green genes and may be classified as deuteranopes (G^-). Four individuals had a 5′ green–red 3′ fusion gene along with one or more green genes and based on studies in Caucasians would be classified as deuteranomalous (G′). The color vision gene arrays of two individuals were interpreted as the product of a double intragenic crossover event between standard red and green pigment genes producing a 5′ green–red-green 3′ fusion gene alongside three green pigment genes. These individuals probably have normal color vision (6). Twelve individuals with the shortened red pigment gene had one or more 5′ green–red 3′ fusion genes. One of the 12 individuals had no green pigment gene, whereas 11 had one or more green genes.

In a population in Hardy–Weinberg equilibrium the frequency of fusion genes would be expected to be the same among individuals with either type of the red pigment gene.

Table 2. Distribution of green pigment gene in different populations with "normal" molecular genotypes (excluding individuals with deletions or fusion genes)

Male population	*n*	Number of green pigment genes: 1*	2	3	≥4
Afro-American	81	0.42	0.42	0.10	0.06
Japanese	97	0.48	0.31	0.15	0.05
Caucasian	113	0.22	0.51	0.19	0.08

Since there was no difference ($P > 0.5$) in the frequency of the number of green pigment genes among those with and without the shortened red pigment gene, these two groups were combined.
*Proportion of single green genes: Caucasians vs. Afro-Americans, $\chi^2 = 8.79 = P < 0.01$; Caucasians vs. Japanese, $\chi^2 = 16.08 = P < 0.001$; Afro-Americans vs. Japanese, $\chi^2 = 0.74 = P > 0.3$.

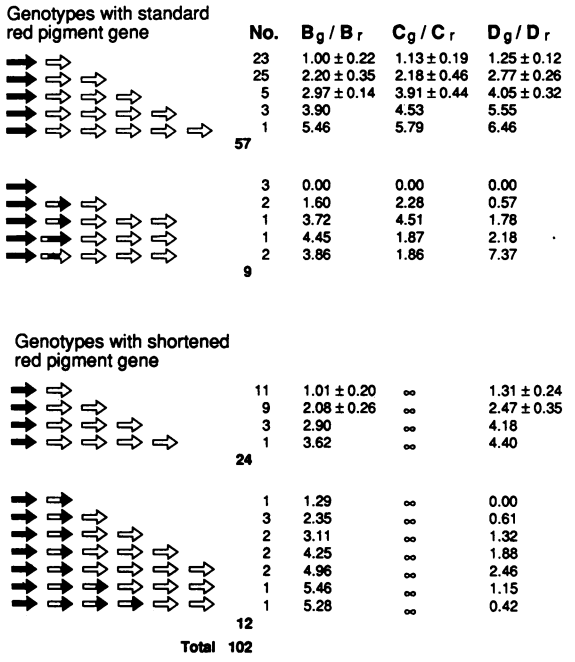

FIG. 4. X-linked color vision pigment gene pattern in 102 *unselected* newborn Afro-American males. Note the absence of 5′ red–green 3′ fusion genes. Symbols and abbreviations are as described in the legend to Fig. 3.

However, the frequency (12/36, 33%) of fusion genes among individuals with the shortened red pigment gene was 3.7 times higher than the frequency (6/66, 9%) among those with the standard red pigment gene ($P < 0.01$). Furthermore, three of the six fusion genes found among individuals with the standard red pigment gene contain fragment C_r, indicating that the illegitimate recombinations involved standard red pigment genes. In contrast, out of a total of 15 fusion genes among individuals with the shortened red pigment gene, none contained a C_r fragment. Thus, all fusion genes among these individuals can be accounted for by participation of green pigment genes and only shortened red pigment genes. In view of the low frequency of *phenotypic* color vision defects among Afro-Americans (4%) (8), most variants detected in this study of unselected males are not likely to be associated with phenotypic color vision defects. All fusion genes have previously been observed among individuals of European origin (6). The reciprocal product of an unequal crossover event resulting in a 5′ green–red 3′ fusion gene is a 5′ red–green 3′ fusion gene. However, no such 5′ red–green 3′ fusion genes suggestive of a protan defect were identified among Afro-Americans.

Among the 81 Afro-American males with normal molecular color vision genotypes, the number of green pigment genes varied from one to five, similar to the findings among Europeans and Japanese. The number of individuals with a single green gene was higher (42%) than among Europeans (22%) and was similar to the Japanese (48%) (Table 2). The frequency of a single green pigment gene was similar (40% vs. 46%) among individuals with the standard and the shortened red pigment gene.

DISCUSSION

We observed a high frequency of a shortened red pigment gene among Afro-Americans. Over one-third of this population carried a red pigment gene that was 1.9 kb shorter in its first intron as compared with the standard or wild-type red pigment gene. The high frequency of this variant (35%) in the face of a low frequency of color vision defects in the Afro-American population (4%) as well as the fact that the deletion is located in an intron indicated that the variant short red pigment gene had no phenotypic consequences. Anomaloscopic assessment of color vision in an individual with this variant who is not part of this study confirmed this inference. The shortened red pigment gene is identical in length to the green pigment gene, which also lacks 1.9 kb from its first intron.

The red and green pigment genes presumably arose from duplication of an ancestral color pigment gene during the last ≈30 million years (17). Our data from ongoing studies in gorillas and chimpanzees (*Gorilla gorilla* and *Pan troglodytes*) suggest that the standard human red pigment gene represents the ancestral gene since only this gene and not the new shortened red pigment gene were found in these primate species. In addition, our findings show that the red and green pigment genes in gorillas, unlike in most humans, are of equal length (data not shown). An evolutionary scheme (Fig. 5) of X-linked color vision pigment genes in higher primates can be suggested from these results. After duplication of the ancestral color vision gene, divergence created the *equal*-sized red and green pigment genes, as found in gorillas. Later in primate evolution, a deletion in intron 1 of the green pigment gene resulted in a gene that is 1.9 kb shorter than the standard red pigment gene. Gene conversion between this shortened green pigment gene and the "standard" red pigment gene presumably took place in an ancestral human African population and created the newly discovered shortened red pigment gene now observed in about one-third of Afro-Americans and among a few individuals of Japanese and European ancestry. A less likely mechanism for the generation of the shortened red pigment gene is the occurrence of a double crossover (between the green and standard red genes) that flanks the ≈1.9-kb fragment.

Our failure to find allelic association between the shortened red pigment gene and the closely linked G6PD A^+ locus indicates that the high frequency of this color vision pigment variant among Africans cannot be explained by selection for both of these genes. Our findings were consistent with an ancient origin of the shortened color vision red pigment gene. Using the data in Table 1, calculations of linkage disequilibrium ($D = h - pq$) (16) showed a value of $D = -0.00367$,

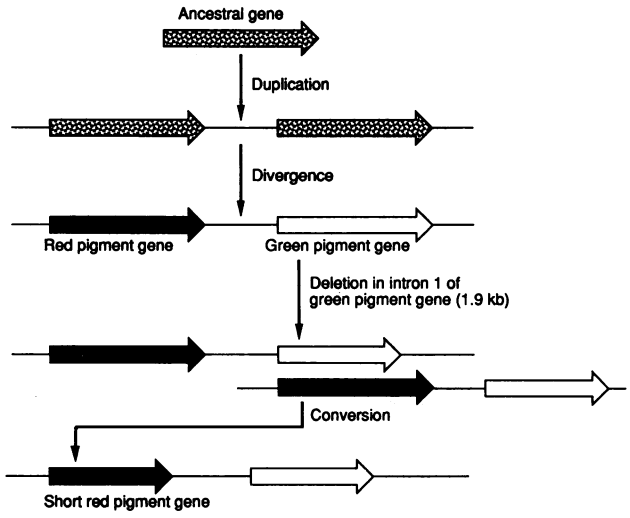

FIG. 5. Evolution of X-linked color vision pigment genes and formation of the shortened red pigment gene. The ancestral pigment gene and its duplication products are represented by dotted arrows. Divergence into the red (solid) and green (open) pigment gene is followed by an ≈1.9-kb deletion in the green pigment gene. The shortened red pigment gene is formed by gene conversion from this green pigment gene. This event is postulated to have occurred in an ancestral human African population.

which was not significantly different from 0—i.e., consistent with linkage equilibrium; p = frequency of G6PD A (A^+ 0.28 and A^- 0.04) = (0.32); q = frequency of shortened red pigment gene (0.36) (36/99). Thus, the shortened red pigment gene and G6PD A variants, common among Black populations, were in linkage equilibrium indicating that a sufficiently large number of generations had occurred to separate these loci from each other following the initial mutation. Despite their proximity (500 kb) and even if the disequilibrium between these two loci were as high as its possible maximum values ($D = -1.15$) (16), it would take 687 generations (or about 17,000 years) from the initial mutational event to attain the currently observed disequilibrium value of $D = -0.00367$ (see *Results*). This time period fits the hypothesis that the shortened red pigment gene originated in an ancestral human African population.

Further studies on the distribution of the shortened red pigment gene among various African and other populations will be of interest since study of the frequencies of this gene may aid in tracing affinities among various African and other populations.

We hypothesized (6) that the lower frequency of phenotypic X-linked color vision anomalies among Japanese (5%) and Afro-American males (3.9%) as compared with Caucasoids (8–10%) was due to the presence of fewer green pigment genes among individuals with normal color vision in these populations. We reasoned that whenever there are fewer green pigment genes in an individual, the opportunities for homologous unequal crossovers between the red pigment and green pigment genes lessen and there will be fewer deletions and fusions involving these genes. As expected from the low frequency of phenotypic color vision defects, a larger number (48%) of Japanese males with normal molecular color vision had only one green pigment gene as compared with 22% Caucasians with one green pigment gene (Table 2). Consistent with the hypothesis, a corresponding lower frequency of deletions and fusion genes was detected (Fig. 3): 4% among Japanese vs. 15.7% among Caucasians (6). In contrast to the findings in the Caucasian (6) and Afro-American (this study) populations, the frequencies of molecular (4%) and phenotypic (5%) color vision defects in the Japanese population are almost the same, suggesting that all molecular defects observed have phenotypic effect. However, since the total number of molecular color vision defects among the 101 Japanese was 4, the 95% confidence limits for this value range up to 8 or 8%. It is therefore possible that a higher frequency of molecular color vision defects observed in Caucasians and Afro-Americans also applies to the Japanese.

In the Afro-American population (Fig. 4) we observed a frequency (21%) of molecular color vision abnormalities at least five times the frequency of phenotypic color vision defects (4%). This finding is even more remarkable as the distribution of green pigment genes among those individuals *with no molecular abnormalities* proved to be similar to the Japanese with fewer multiple green gene carriers (Table 2). The apparent contradiction of the hypothesized relationship between the frequencies of multiple green genes and fusion genes in Afro-Americans probably is explained by the high frequency (35%) of the shortened red pigment gene in this population. Since the shortened red pigment gene resembles the green pigment gene because of the 1.9-kb deletion in the first intron, illegitimate meiotic pairing between these genes and intragenic recombination are facilitated, making for a high frequency of fusion genes.

The unexpected finding that the frequency of fusion genes was 3.7 times higher in individuals with the shortened red pigment gene would suggest that the population was not in Hardy–Weinberg equilibrium and indicates long-term separation of populations with different frequencies of the two variants of the red pigment gene. This is further supported by the observation that none of the fusion genes among individuals with the shortened red pigment gene contained the C_r fragment of the standard red pigment gene. These observations suggest that the frequency of the shortened red pigment gene varied among the populations from which Afro-Americans originate.

The discrepancy between fewer phenotypic color vision defects and more molecular abnormalities at the X-linked color vision loci among Africans is in keeping with our data from U.S. Caucasians (6) and other more limited data from France (18). The much higher frequency of molecular abnormalities in the U.S. Black population presumably relates to the high frequency of the shortened red pigment gene that allows more frequent illegitimate recombination between the red and green genes. However, this explanation does not account for the absence of color vision deficiencies in many individuals with 5′ green–red 3′ fusion genes. Studies on the exact site of the fusion genes may aid in resolving this dilemma. Furthermore, we lack information regarding the expression of the color vision genes in the retina. What are the regulatory signals that specify expression of normal genes and various fusion genes? Are multiple green genes expressed? Does the exact position of a given color vision gene in the color vision gene array affect expression of color vision phenotypes? More work regarding these issues needs to be done to elucidate phenotype–genotype relationships at this complex genetic locus.

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