Individual Variation in the Size of the Human Red and Green Visual Pigment Gene Array

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Purpose. To determine the size variation of the X-chromosomal human red and green visual pigment gene array in the general population using pulsed field gel electrophoresis and Southern blotting.

Methods. Peripheral blood lymphocytes were prepared from 67 anonymous males. The cells were embedded in agarose and the genomic DNA digested with restriction enzyme Not I. The resulting DNA fragments were resolved on a contour-clamped homogeneous electric field gel, and the Not I fragment containing the red and green pigment genes was visualized by Southern blot hybridization with a human green pigment cDNA probe.

Results. In DNA from each male, a single hybridizing fragment was observed in Not I-digested DNA. The lengths of the fragments from different males were observed to vary in steps of approximately 39 kilobases (kb), consistent with earlier studies showing a visual pigment gene repeat unit of 39 kb and a head-to-tail tandem arrangement of the red and green pigment genes. In the population studied, the number of repeat units per X-chromosome had a mean of 2.9 and a standard deviation of 0.94.

Conclusions. The sizes of visual pigment gene arrays observed in this study resemble those determined in earlier studies based on ratios of restriction fragments resolved by conventional gel electrophoresis and visualized by whole genome Southern blotting, but differ significantly from those determined using ratios of fragments obtained by the polymerase chain reaction. Invest Ophthalmol Vis Sci. 1997;38:1040–1043.

The human red and green pigment genes lie in a head-to-tail tandem array on the X-chromosome. Variation in the number and structure of these genes arises from unequal homologous recombination between nearly identical 39-kilobase (kb) repeat units, each of which contains a single gene. The common red–green dichromacies and anomalous trichromacies found in approximately 10% of human males arise from homologous recombination events that result in the loss of genes or the production of hybrid genes. Differences in the number of X-linked visual pigment genes also are found among normal trichromats and constitute one of the most common genetic variations in the human population.

The number of red and green pigment genes per X-chromosome in the general population has been measured by several laboratories with differing results. By conventional gel electrophoresis and quantitative Southern blotting, the average number of genes in the array was determined to be three, with the majority of arrays having a single red pigment gene and either one, two, or three green pigment genes. In these studies, arrays having six or more genes were found in, at most, a few percent of X-chromosomes. By contrast, a recent study using the polymerase chain reaction (PCR) concluded that the average number of genes in the array was greater than four, that visual pigment gene arrays with six or more genes are found on 30% of X-chromosomes, and that more than 40% of human X-chromosomes carry two or more red pigment genes. In these experiments, corresponding segments from each of the highly homologous genes in the X-linked visual pigment gene array were amplified by PCR, and the products derived from each gene were distinguished by restriction enzyme cleavage at sites that were known from gene sequencing to be present in only one or the other type of X-linked visual pigment gene. The PCR-based determination, if correct, would result in a substantial revision in current models of the evolution and physiology of human color vision.

In this report, we describe a determination of the size of the red and green visual pigment gene array in a group of 67 human X-chromosomes using pulsed field gel electrophoresis (PFGE) and Southern blotting. This method is distinguished from those used previously in that it does not rely on quantitating ratios of DNA fragments. In both the conventional gel electrophoresis–Southern blot method and the PCR method, the intensities of red and green pigment gene fragments are compared to one another and to an internal standard to obtain both the total number of genes and the ratio of gene types (Figs. 1A and 1B). In the PFGE–Southern blot method, the total number of visual pigment genes is determined by sizing a Not I fragment that carries the entire visual pigment gene array (Fig. 1C). This method relies on the observation that each 39-kb tandem repeat unit within the visual pigment gene array contains a single visual pigment...
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FIGURE 1. Methods for quantitating red and green pigment gene number. The example shown is for an array with one red and two green pigment genes. (A) Conventional gel electrophoresis and Southern blotting; R and G represent distinguishable restriction fragments derived from the red and green pigment genes, respectively. (B) Polymerase chain reaction (PCR), distinguishable PCR products are derived from the red (R) or green (G) pigment genes and from the most proximal (P) or from all of the distal (D) pigment genes. (C) Pulse field gel electrophoresis and Southern blotting.

MATERIALS AND METHODS. Blood samples from 67 anonymous males of unknown color vision phenotype were obtained from the Johns Hopkins Hospital. The blood samples were collected in accordance with the regulations of the Johns Hopkins Joint Committee on Clinical Investigation. This study adhered to the Declaration of Helsinki. An effort was made to match the ethnic composition of the populations studied previously by Nathans et al. and Drummond-Borg et al. by selecting subjects with names indicative of European ancestry. Peripheral blood lymphocytes were prepared by Ficoll–Paque purification (Pharmacia, Piscataway, NJ), embedded in agarose, and digested with Not I as described. Fragments were resolved on a contour-clamped homogeneous electric field gel (Bio-Rad, Melville, NY) for 14 hours at 200 V with a switch time ramping linearly from 0.2 to 22 seconds. The Not I fragment containing the red and green pigment genes was visualized by Southern blot hybridization using a human green pigment cDNA clone, hs2, which is 98% identical in sequence to the corresponding red pigment gene exons. In each of the 67 DNA samples examined, a single hybridizing Not I fragment was observed.

RESULTS. Figure 2 shows a typical Southern blot in which genomic DNA from 12 males selected randomly was digested with Not I, resolved by PFGE, and hybridized with a green pigment cDNA probe to visualize the Not I fragment containing the red and green pigment gene array. As expected, a single hybridizing fragment is seen in the DNA of each male, and the array lengths vary in steps of approximately 39 kb. The number of repeat units is determined readily by measuring the fragment lengths and is apparent by visual inspection (Fig. 2). Figure 3A shows a histogram of red and green pigment gene array lengths for 67 random males as determined by PFGE. The observed distribution of visual pigment gene number is: 1 gene, 3 subjects; 2 genes, 22 subjects; 3 genes, 26 subjects; 4 genes, 13 subjects; and 5 genes, 3 subjects.

DISCUSSION. In this report, we apply PFGE to determine the number of red and green pigment gene repeat units per X-chromosome in the human population. The size distribution of DNA fragments containing the entire red and green pigment gene locus observed by PFGE resembles closely the distributions determined previously by conventional gel electro-
an excess of even over odd numbers of visual pigment genes and a complete absence of subjects with a single green pigment gene (Figs. 3F, 3G). One explanation for these data is that in some or all of these 12 subjects, the ratio of PCR products used to determine the total number of genes is in error by a factor of 2, thus leading to a 2-fold overestimate of the total number of visual pigment genes. In contrast, the number of visual pigment genes in the 15 subjects who were reported to have a single red pigment gene (Fig. 3E) is consistent with the distributions obtained by the other methods (Figs. 3A, 3B, and 3C).

One source of variability that might account for the discrepancy in pigment gene distributions could arise from DNA sequence variations that alter the efficiency of PCR priming or amplification. For example, if 2 DNA segments exhibited a 3% difference in amplification efficiency per cycle, after 25 cycles of PCR, there would be a 2-fold difference in final product yield. The general possibility of a systematic error in the PCR method is suggested by the data presented in Table 1 of that article, which shows an unexpected distribution of visual pigment gene number in those 12 subjects who were reported to carry more than 1 red pigment gene. Among these 12 subjects, there is an excess of even over odd numbers of visual pigment genes and a complete absence of subjects with a single green pigment gene (Figs. 3F, 3G). One explanation for these data is that in some or all of these 12 subjects, the ratio of PCR products used to determine the total number of genes is in error by a factor of 2, thus leading to a 2-fold overestimate of the total number of visual pigment genes. In contrast, the number of visual pigment genes in the 15 subjects who were reported to have a single red pigment gene (Fig. 3E) is consistent with the distributions obtained by the 2 other methods (Figs. 3A, 3B, and 3C).
The distribution of red and green pigment gene number determined in this report supports the conclusion that the majority of human red and green pigment gene arrays consists of a single red pigment gene and between one and three green pigment genes. It now should be possible to determine how this basic arrangement can be modified by amino acid substitution and homologous recombination to generate the variety of red–green color vision types present in the human population.

Key Words
pulsed field gel electrophoresis, red–green color vision, visual pigments

Acknowledgment
The authors thank Ms. Ingeborg Hobbs (Johns Hopkins Hospital) for assistance in obtaining blood samples.

References

Intercellular Adhesion Molecule-1 in Proliferative Vitreoretinopathy


Purpose. To measure vitreous levels of the soluble intercellular adhesion molecule (sICAM-1) in eyes with rhegmatogenous retinal detachment (RRD) complicated or uncomplicated by proliferative vitreoretinopathy (PVR) to investigate whether levels of this molecule related to history of previous retinal surgery or to the duration and severity of PVR.

Methods. The authors measured vitreous sICAM-1 by enzyme-linked immunosorbent assay in 28 eyes with PVR and 35 eyes with uncomplicated RRD. Vitreous from 10 eyes with macular holes and from 12 cadaveric eye donors were used as control specimens.

Results. Vitreous sICAM-1 levels were higher in the group with RRD complicated by PVR as a whole than in the group with RRD alone or in the control groups. In patients with no previous retinal surgery, there was no difference in vitreous sICAM-1 levels between the groups with RRD alone and RRD complicated by PVR. However, in patients who had undergone previous external surgery, those with PVR showed higher levels of vitreous sICAM-1 than those with RRD alone. In PVR, raised levels of sICAM-1 were associated preferentially with a history of previous vitrectomy as well as with a longer duration of the condition, although these levels were not related to the grade of PVR. In eyes with RRD alone, the levels of sICAM-1 were not enhanced with the duration of the detachment. Despite showing high vitreous levels of sICAM-1, patients with PVR did not exhibit increased serum levels of this adhesion molecule.

Conclusions. The current observations suggest that those persons in whom PVR develops may have an impairment of the mechanisms that control the inflammatory response to retinal trauma. Persistently raised vitreous levels of sICAM-1 point to the continued operation of cytokine-mediated vascular reactions at the blood–retinal barrier. Invest Ophthalmol Vis Sci. 1997;38:1043–1048.

Proliferative vitreoretinopathy (PVR) is a severe complication of retinal detachment (RRD) characterized...