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Correlation of Gene Structure and Psychophysical Measurement in Red-Green Color Vision Deficiency in Chinese

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Purpose: To study the correlation of genotype for X-linked red-green gene array with color vision phenotype in 58 subjects with red-green color vision deficiency.

Methods: The molecular structure of red and green pigment genes on 58 X chromosomes was studied exon-by-exon by using heteroduplex-SSCP analysis and sequencing. The color vision of these subjects was determined by a Neitz anomaloscope.

Results: Variations in the red and green pigment genes were detected in 43 subjects and a hybrid gene was found in 27 subjects. About 50% of the fusion sites occurred at intron 2-3. All 3 anomalous trichromats with intron 4 fusion were mild type but another 3 with intron 2-3 fusion were severe type. No subjects with mild type of color vision defects had a fusion site at intron 2-3 or its upstream. Three subjects with complete deletion of the green pigment gene manifested deuteranomaly.

Conclusions: Protans can be differentiated from deutans on the basis of genotype. It is still difficult to establish a clear correlation of different anomalous trichromats with genotype. The fusion site of a hybrid gene affects the phenotype to some degree. Intron 2-3 is the common place for gene crossover. **Jpn J Ophthalmol 2000;44:596–600** © 2000 Japanese Ophthalmological Society

Key Words: Deutan, gene variation, genotype, phenotype, protan.

Introduction

Color vision in the human eye is mediated by three opsins encoded by red, green, and blue visual pigment genes.[1] The red and green visual pigment genes are 98% identical in DNA sequences and lie at Xq28 in a head-to-tail tandem array. On the X chromosome of a male subject with normal color vision there is one red pigment gene and one or more green pigment genes with the red in the upstream.[1,2] Each of the red or green pigment genes has six exons with the same exons 1 and 6 between these two genes.[1] The difference in absorption spectrum between the red and green opsins has been shown to be determined by 7 of their 15 different amino acid residues.[3] This difference only presents in exons 2, 3, 4, and 5 of the red and green pigment genes.[1]

Congenital red and green color vision deficiency is the most common hereditary variation in human beings. It is X-linked and occurs in about 5.4% of the Chinese Han population. Previous studies showed that gene deletion and/or hybrid gene formation in the red and green pigment genes, resulting from homologous unequal recombination, are the common molecular basis for this deficiency.[4–7] Protan subjects had a deficiency in the red visual pigment gene that is usually replaced by a red-green hybrid gene. Deutan subjects had a deficiency in the green pigment genes that may be deleted in all or may be partly re-

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placed by a green-red hybrid gene. Persons with the complete loss of red or green pigment sensitivity are dichromats, such as protanopes or deuteranopes. Persons with abnormal red or green pigment sensitivity are anomalous trichromats, such as persons having protanomaly or deuteranomaly.

We have studied the red and green gene variations by using Southern blotting, and the exon 5 of these two genes by using heteroduplex-SSCP analysis in Chinese subjects with congenital red-green color vision deficiency.[6,8] In order to determine the fusion sites of a hybrid gene and the correlation of genotype with its corresponding phenotype, the exons 2, 3, 4, 5 and the promoter of the red and green visual pigment genes in 58 red-green color deficient subjects and in 5 normal subjects were analyzed by using heteroduplex-SSCP analysis and sequencing. Previously we have found that a few subjects with complete deletion of green pigment genes could manifest deuteranomaly but not deuteranopia.[6] This has also been described by other researchers.[5,9] The gene structure in these subjects was further studied.

Materials and Methods

Subjects

Fifty-eight subjects with congenital red and green color vision deficiency were male Han nationality Chinese recruited from our outpatient clinic and high-school screening. Of the 58, the red and green pigment genes in 27 subjects have been analyzed by Southern blot hybridization previously, and exon 5 of these genes in 28 subjects was studied by PCR-heteroduplex-SSCP analysis.[6,8] The 5 normal subjects were male Han nationality Chinese from our staff and university students. Informed consent was given prior to psychophysical test and collection of peripheral venous blood.

Psychophysical Test

Color vision for all subjects was tested by pseudoisochromatic plates and a Neitz anomaloscope (Tokyo). Classification criteria related to the dichromat and mild or severe type of anomalous trichromat were the same as described previously.[8] Subjects who made color matches at both the green end and the red end were dichromats. Those who made color matches at either the green end (0) or the red end (73) were a severe type of anomalous trichromats; those who made color matches beyond the normal range, but not at the extremes, were a mild type of trichromats. Protan and deutan subjects were differentiated by how much yellow light was needed to make the color matches at different points.

DNA Preparation

Genomic DNA from each subject was prepared from leukocytes of peripheral venous blood by whole blood lysis, followed by phenol-chloroform extraction and ethanol precipitation. Then the DNA pellet was dissolved in TE buffer (pH 8.0).

Polymerase Chain Reaction Amplification

Exons 2, 3, 4, 5, and the promoter region of the red and green pigment genes in each subject were amplified by polymerase chain reaction (PCR). The primer sequences and the amplification conditions are listed in Table 1.

Exon 1 and exon 6 regions of these two genes were not amplified, as these regions are the same for the red and the green genes. In general, 30 cycles of

Table 1. Primer Sequence, Length, of Polymerase Chain Reaction Products and Amplification Condition

Primers	Nt Position*	Sequence (5–3)	Products (bp)	Annealing Temperature (°C)
Promoter	264–284	CCAGCAAATCCCTCTGAGCCG	229	60
	472–492	CTATGGAAAGCCCTGTCCCCG		
Exon 2	666–686	GTCTGGATGATCTTTGTGGTC	198	60
	844–863	GTGGCCCAGCACGAAGTAGC		
Exon 3	906–926	ATCACAGGTCTCTGGTCTCTG	167	60
	1052–1072	CTGCTCCAACCAAAGATGGGC		
Exon 4	1073–1094	GTACTGGCCCCACGGCCTGAAG	166	60
	1218–1238	CGCTCGGATGGCCAGCCACAC		
Exon 5	intr-1247	TCTCCCTTAGGTGGCAAAG	260	58
	1470-intr	TGTTGCTTACCTGCCGGTT		

intr: Primer sequence with 10 nt intron sequence.
* Number system refers to Nathans et al., p. 193.

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amplification were carried out at 94°C for 30 seconds, at 58°C or 60°C for 30 seconds (Table 1), and at 72°C for 1 minute by using a Thermolyne thermal cycler, with an initial denaturation at 96°C for 4 minutes and a final extension at 72°C for 5 minutes. All primer pairs were located in regions where both the red and green pigment genes share the same sequence. Both red and green gene fragments in the promoter and in exons 2, 3, 4, and 5 can be amplified at the same time by using these universal primers.

Heteroduplex-SSCP Analysis

Heteroduplex-SSCP analysis was used to examine the origin and composition of each exon and the promoter in the red and green pigment gene. An aliquot of PCR products was mixed with an equal volume of formamide dye (97% formamide, 20 mM EDTA, 0.05% bromophenol blue and xylene cyanol), denatured at 96°C for 5 minutes and immediately placed on ice; then electrophoresed separately in at least two polyacrylamide nondenaturing gels (1 mm × 40 cm × 30 cm) containing 10% glycerol : (1) on 6% gel (acrylamide:bisacrylamide = 99:1) in 1 × TBE buffer for 7–9 hours with fan; (2) on 8% gel (49:1) in 0.5 × TBE buffer for 9 hours at room temperature. The gels were stained by silver staining to visualize the DNA bands.

Determination of Red-Green Gene Arrays

The promoter and exons 2, 3, 4, and 5 of the red and green pigment genes in 3 subjects with a single gene in the red-green pigment gene array were sequenced and served as standards. The nucleotide sequence for fragments of interest was determined from both directions by the dideoxy chain termination method using ABI310 and ABI377 sequencer.

Through comparison of band patterns on heteroduplex-SSCP analysis with known standard sequence, individual red and green pigment exons and their respective variants could be identified in each subject.

Results

Color Vision Test

Based on anomaloscopic examination, the 63 subjects were divided into 58 color deficient subjects and 5 normal subjects. Of the 58 subjects with color vision deficiency, 24 were protan and 34 were deutan. The 24 protan included 19 with protanopia and 5 with protanomaly. The 34 deutan subjects included 24 with deuteranopia and 10 with deuteranomaly. Figure 1 shows the anomaloscopic results.

Protan Subjects

Twenty-four of the 58 subjects with color vision deficiency were protan. Figure 2 and Table 2 show the variation in the red and green visual pigment genes which can be divided into two patterns: (1) a single red-green hybrid gene; (2) a red-green hybrid gene plus green pigment genes. The normal red pigment gene in all protan subjects was replaced by a red-green hybrid gene so that no protan had an intact red pigment gene. The fusion sites of the hybrid gene in the 24 protan subjects were located in intron 1 (7 cases), intron 2-3 (11 cases), and intron 4 (7 cases). Six subjects had only a single red-green hybrid gene, two of whom had a specific hybrid gene. One of the 2 had a red-green-red hybrid gene and the other had a red-green-red-green hybrid gene where 2 or more fusion sites were present.

The fusion sites were upstream of exon 5 in all protan but one (Figure 2). Fusion sites in exon 5 were found only in our study. The normal exon 5 of the red pigment gene was absent in all 24 protan.

Protan subjects with a single hybrid gene (6 cases) or with a fusion point in intron 1 were protanopes. Protan subjects with a fusion in intron 2-4 and additional normal green pigment genes may have protanopia or protanomaly.

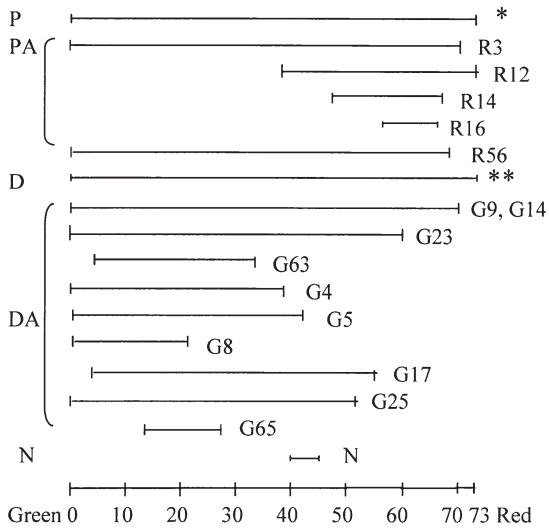

Figure 1. Anomaloscopic match ranges of subjects with red-green color vision deficiency and normals. Horizontal lines represent match range of red-green mixture with yellow light. Yellow scale setting which differentiates protan from deutan is not shown here. P: protanomaly; PA: protanomaly D: deuteranopia; DA: deuteranomaly; N: normals. *R2,R4–11, R13, R15, R17–19, R52, R55, R61, R69, R70. **G1–3, G6, G7, G10, G12, G13, G15, G16, G18, G19, G24, G26–29, G51, G53, G57–60.

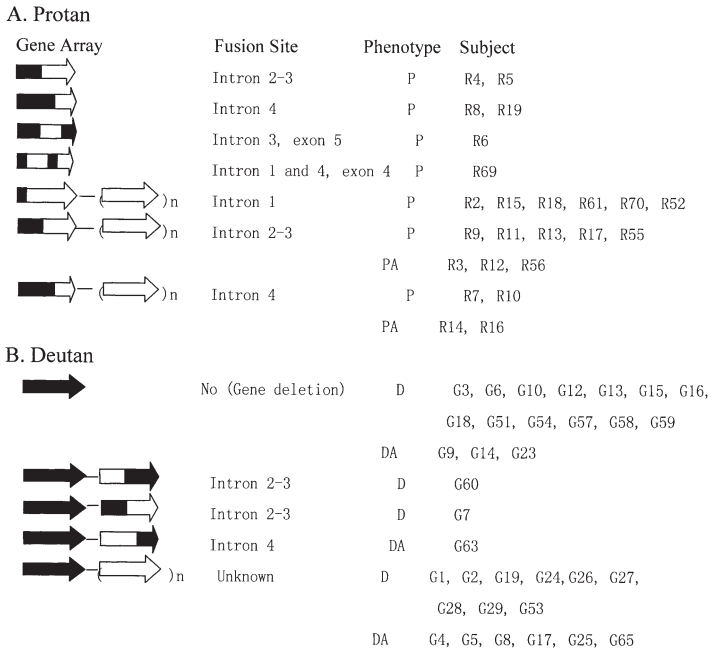

Figure 2. Red and green arrays in protan and deutan subjects. ■: Red pigment gene; □: green pigment gene. P: protanopia; PA: protanomaly; D: deuteranopia; DA: deuteranomaly; n: varied number of gene copies.

Deutan Subjects

Thirty-four of the 58 subjects with color vision deficiency were deutan. Figure 2 shows the variation in their red and green pigment genes that can be divided into three patterns: (1) red pigment gene only, (2) red pigment gene plus green-red hybrid gene, and (3) red pigment gene plus an entirely green pigment gene. All deutan subjects had a normal red pigment gene. Complete absence of a green pigment gene was found in 16 of the 34 deutan. Of the 16, 13 had deuteranopia and 3 had deuteranomaly. It is very interesting that 3 deuteranomalous subjects had only a red pigment gene, the same as subjects with deuteranopia. This is in accordance with previous studies.[5,6]

Three of the 34 deutan were found to have a hybrid gene besides the normal red pigment gene (Table 2, Figure 2). Of the 3, one having deuteranopia (G60) and one having deuteranomaly (G63) had a green-red hybrid gene. The third deuteranope (G7) had a red-green hybrid gene that has not been described before in deutan. This hybrid gene may not express in this subject as there is a preceding normal red pigment gene.[11–12]

Fusion Sites

A hybrid gene in the red-green gene array was detected in 27 color-deficient subjects (Figure 2 and Table 2). Fusions occurred at intron 1 in 7 cases, at intron 2-3 in 13 cases, and at intron 4 in 8 cases (two fusion sites were counted in one case). The SSCP band patterns, which indicate the frequency of a sequence variation, occur much more frequently in exon 3 than in other exons.

Discussion

Congenital red-green color vision deficiencies can be classified into protan and deutan through gene analysis. All protans have no intact red pigment but

Table 2. Gene Variation Detected in Red-Green Color Vision Deficiencies

Test of Color Vision	No. of Cases	Hybrid Gene	Single Gene	Green Gene Deletion	Fusion Site of Intron*		
					1	2,3	4
Protanopia	19	19	6	0	7	8	5
Protanomaly	5	5	0	0	0	3	2
Deuteranopia	24	2	13	13	0	2	0
Deuteranomaly	10	1	3	3	0	0	1

*Two cases had more than one fusion site as shown in Figure 2.

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all deutans have a normal red pigment gene. On this point, phenotype is closely associated with genotype.

In our 24 protan subjects, the normal red pigment gene is replaced by a red-green hybrid gene in all protan but two with specific hybrid genes: red-green-red hybrid gene in one and red-green-red-green in the other. Absence of the normal exon 5 of the red pigment gene in all 24 protan indicates that exon 5 is important for the characteristic spectrum of a red pigment. This also can be a diagnostic marker for protan. Six subjects with a single hybrid gene but without the normal red and green visual pigment genes manifested protanopia.

Six protan subjects with a hybrid gene of intron 1 fusion and one or more green pigment genes manifested protanopia where the hybrid gene encodes a pigment the same as the green pigment. Subjects with a hybrid gene of intron 2-4 fusion and additional green pigment genes may manifest protanopia or protanomaly. In fact, all these subjects should have been protanopes, because the absorption spectrum of these hybrid gene products should be similar to a green pigment gene as exon 5 plays a major role in spectrum absorption. This phenotype difference implies that other factors may affect color recognition.

In our 34 deutan subjects, only 16 had the red pigment gene with complete deletion of the green pigment gene. Of the 16, 13 had deuteranopia and three had deuteranomaly (Figure 2), which confirms our previous finding.[6] The red pigment gene in the 3 deuteranomaly subjects is the same as that in the deuteranopia subjects. There is no evidence in our study or in others[5] that there may be two red pigment genes with a different absorption spectrum in one X chromosome. Therefore, the 3 deuteranomaly subjects with red pigment genes but without green pigment genes indicate that the psychophysical test may be less than perfect. In addition, we cannot exclude other factors that may influence the color recognition phenotype.[9]

Three deutan subjects had a hybrid gene in addition to a normal red pigment gene. One deuteranope (G7) had a red-green hybrid gene with intron 2-3 fusion in addition to the red pigment gene. This is interesting since the red-green hybrid gene has been found before only in protan but not in deutan. This subject (G7) should not have been a dichromat as the red-green hybrid gene should encode a product similar to that of a green pigment gene. Selective gene expression in the red-green array may explain this phenomenon.[10–12]

A hybrid gene in the red-green gene array was detected in 27 color-deficient subjects. Intron 2-3 is the common site for gene crossover since about 50% of the fusion sites occurred in this region. Our analysis of exon 3 PCR products has confirmed that the electrophoretic SSCP band patterns occur much more frequently in exon 3 than in other exons. The fusion sites of a hybrid gene have never been found in exon 5 and its downstream except in subject R6. All 3 anomalous trichromats with intron 4 fusion (R14, R16, and G63) were the mild type but another 3 anomalous trichromats with intron 2-3 fusion (R3, R12, and R56) were the severe type. No subject with a mild form of red-green color vision deficiency had a fusion site at intron 2-3 or its upstream. Therefore, the fusion site of a hybrid gene affects the color-deficient phenotype to some degree.

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