Visual Pigment Gene Changes in Adrenoleukodystrophy

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Purpose. The gene for X-linked adrenoleukodystrophy, a neurodegenerative disorder, is closely linked to the red/green color pigment genes on the distal X-chromosome Xq28 and one kindred is known to have a genetic change affecting both loci. The purpose of this article is to perform a systematic assessment of the frequency of this situation in many affected kindreds.

Methods. Recombinant DNA probes were used in blot hybridization studies to determine the structure of the color pigment genes in affected males from 59 different adrenoleukodystrophy kindreds. Whenever possible, color vision was measured using the Farnsworth 100-Hue test.

Results. Eleven of the 59 kindreds had abnormal color pigment gene clusters; these included fusion genes and changes in gene number. Only one kindred had a deletion of sequences immediately 5' to the color pigment genes.

Conclusions. The incidence of color pigment gene changes in our 59 adrenoleukodystrophy kindreds is approximately twice the frequency of defective color vision reported in historic studies but is about the same as that found in studies of the actual genes in large populations. However, the range of changes in the color pigment genes in adrenoleukodystrophy is broader than encountered in most populations. Changes in the highly conserved color pigment genes reflect reorganizations in the Xq28 chromosomal region, some of which involve the contiguous gene for adrenoleukodystrophy. Invest Ophthalmol Vis Sci. 1993;34:2634–2637

X-linked Adrenoleukodystrophy (ALD) is a devastating neurodegenerative disease affecting boys 6 to 12 years old. Although the underlying metabolic abnormality is unknown, ALD is associated with diagnostic accumulations of very long chain fatty acids.[1]

To localize the ALD gene on the human X-chromosome we showed that ALD and the anonymous Xq28 DNA fragment DXS52 cosegregate with a lod score >13 at $\theta = 0.0$ in seven kindreds.[2] Subsequently,

we found frequent color vision abnormalities in ALD kindreds as well as changes in red/green color pigment (R/GCP) genes in a study of eight kindreds.[3]

We then studied men with adrenomyeloneuropathy (AMN), another manifestation of the same X-linked metabolic defect, characterized by intact cognition and various neurologic impairments.[1] (For convenience in this report we will refer to both as ALD unless specific differentiation is needed.) We used the Farnsworth-Munsell 100-Hue test to screen color vision in these patients and found abnormal scores in 12 of 27 males with AMN.[4] This incidence (44%) was significantly higher than the frequency of 6–8% reported in earlier physiologic studies.[5,6] The axes of bipolarity for color perception in the 12 AMN males testing abnormally varied widely, compatible with the notion that diverse gene changes were responsible. Our kindred "O"[3,4] showed cosegregation of DNA changes (including a chromosomal deletion) with ALD in the

kindred[7] and a unique change in color perception in a male with AMN.

We now have studied and report here our studies of the visual pigment genes in 59 ALD kindreds (emphasizing those identified earlier as having abnormal color vision[4] as well as those ascertained subsequently). We compare these changes with those we found earlier in ALD kindreds,[3] in males with blue-cone monochromacy,[8] in males whose color vision status was unknown,[9] and with a group of French ALD patients not studied for color vision defects.[10]

METHODS

Patients

We studied an affected male from each of 59 different kindreds. Forty test subjects had AMN and 19 had ALD; all were ascertained by very long chain fatty acid screening and clinical analysis.[1] We followed the tenets of the Declaration of Helsinki and performed this work with the approval of the Joint Committee on Clinical Investigation. After obtaining informed consent, lymphoblast lines were established; these were the sources of DNA for these studies. When possible, color vision screening was performed for males with AMN using the Farnsworth-Munsell 100-Hue (F-M) test.[4]

DNA Probes and Hybridizations (Figure 1)

For the repeated units of the R/GCP gene cluster we used probes hs7 (a 1.2 kb fragment of the red pigment gene cDNA) and 9Δ6 (a 400 bp genomic fragment originating from the 3' end of intron 4) representing 5' and 3' regions of the human visual pigment genes, respectively.[3] Note that these probes recognize sequences in both red and green pigment genes that are distinguished by the sizes of the hybridizing fragments. We also used probe JHN60, a fragment of the region immediately 5' to the R/GCP gene cluster, which is essential for R/GCP gene expression.[8]
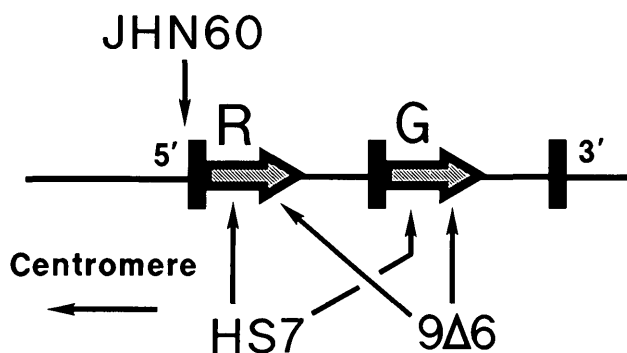

FIGURE 1. Map of probe locations with respect to the R/GCP gene cluster in Xq28. Heavy vertical lines indicate the 40kb repeat units for each pigment gene.

TABLE 1. Ratios of Green/Red Color Pigment Genes in Otherwise Normal Clusters

(Green/Red)	1	2	3	4	5	Σ
AMN	8	9	6	3	1	27
ALD	—	7	1	1	—	9
Σ	8	16	7	4	1	36

10 μg of DNA was digested to completion using enzymes purchased from Boehringer-Mannheim (Indianapolis, IN). Electrophoresis, transfer to Gene-Screen Plus® membranes, hybridization to ^{32}P-labeled probes ($>10^9$ cpm/μg), washing, and autoradiography were performed as reported.[3,8] Band densities in individual lanes were compared by densitometric scanning; the ratios were used to estimate R/GCP gene structures.[3,9]

RESULTS

The R/GCP gene clusters in 48 of 59 kindreds differed only in the numbers of green pigment genes; 89% showed a ratio of 3:1 or lower as shown in Table 1.

Eleven kindreds had abnormal hybridization patterns as shown in Panels A and B of Figure 2; Panel C presents models for these 11 R/GCP gene regions. The single red pigment gene pattern in patients "M" and "S" is consistent with patterns reported for deuteranopia.[9] Patients N-16, D-1, and D-12 have no intact red pigment gene, as reported for patients with protanopic or protanomalous color perception. By contrast, although patient O had a single apparently green pigment gene (compatible with simple protanopia) his color perception abnormality is complex and likely influenced by his DNA rearrangement.[7] All other patients have fusion genes with occasionally large numbers of gene copies as shown.

Probe JHN60 showed a normal band in all patients except for patient O in whom there was no hybridizing material as discussed earlier in the context of his complex deletion/rearrangement.[7]

Color vision was normal in 16 of 35 (46%) males with AMN (27 from our earlier report,[4] of whom 12 were known to have abnormal scores, and 8 ascertained subsequently). Seven of these 16 (6 of the 12 from our earlier study and 1 from the newly ascertained group of 8) showed R/GCP gene changes (44%). Five of the 47 newly ascertained patients (11%), whether tested for color vision or not, showed changes in the R/GCP genes, a moderate elevation (1.5–2X) over the frequency of color vision defects in unselected populations.[5,6] Thus, not surprisingly, selecting AMN patients with abnormal F-M test scores produced a group intentionally enriched for R/GCP gene changes.
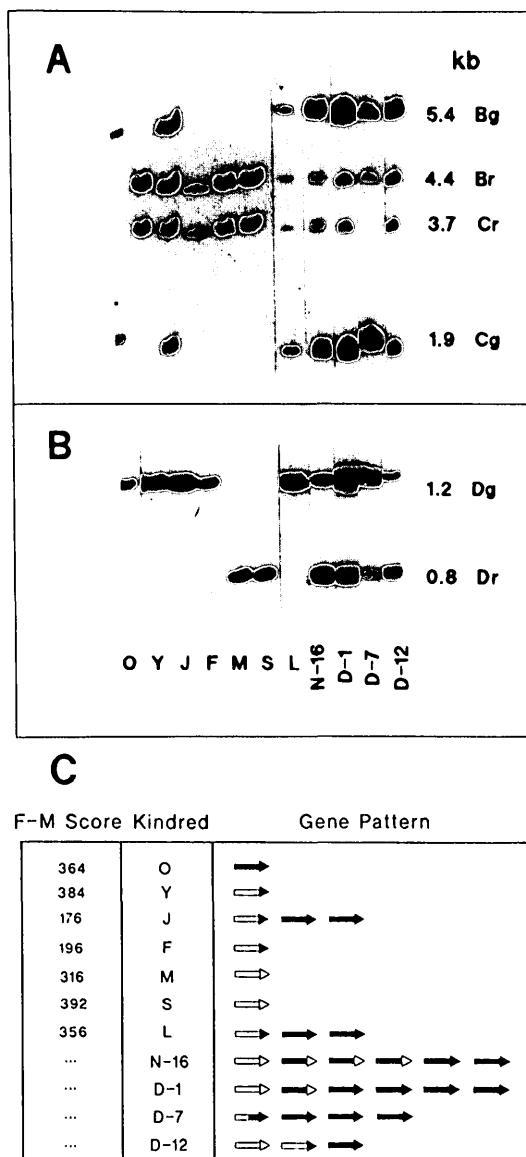
FIGURE 2. Abnormal hybridization patterns of R/GCP gene probes to genomic DNA from 11 patients identified by letter corresponding (where appropriate) to the studies of color vision reported earlier.[4] The identifying letters for the bands are based on previous studies.[8] (A) Probe hs7 hybridized to DNA digested with EcoRI/BamHI and separated on 1% agarose gels. Sizes and positions of Bg, Br, Cg, and Cr are indicated. (B) Probe 9Δ6 hybridized to the same DNA digested with RsaI and separated on 1.4% agarose. Sizes and positions of bands Dg and Dr are indicated.(C) Models for organization of the R/GCP gene clusters for the 11 patients described above. The F-M Score was obtained by color vision testing;[4] average values are given when the person was tested more than once. Deletions, reorganizations, and gene number(s) are based on densitometric studies of the autoradiograms shown in A and B and on earlier studies.[3,8,9] Open boxes indicate red gene sequences; closed boxes represent green genes.

DISCUSSION

These observations extend our earlier report[3] of R/GCP gene alterations in ALD. They add an important perspective to our tests of color vision in these patients[4] and are compatible with our earlier proposal that no single genetic alteration was likely to explain all of the visual changes.

Although F-M test scores are included in Figure 2C, we cannot relate them directly to the R/GCP gene changes. Functional hybrid transcription units may have been formed between red and green genes in patients J, F, L, Y, N-16, D-1, D-7, and D-12. Although potentially important in terms of the contributions of specific domains to the spectral sensitivities of these pigments, our findings will require further study, including DNA sequencing, to establish any direct relationships. Also, it is not currently possible to know if these hybrid genes are transcribed in our patients' retinal cones. Our recent studies of color vision in patient O have shown that his remaining pigment gene is transcribed despite the replacement of the transcriptional control element(s) known to reside in the JHN60 region with a novel sequence brought in by the recombination.[7]

Most AMN patients studied here were identified following our earlier color vision testing.[4] As shown in Figure 2C, patient M had an elevated F-M test score result of 316; N-16 could not be tested because of foreign language problems. The gene pattern in M resembles that reported in deuteranopia, which certainly would be consistent with its frequency.[5,6] By contrast, patient S, who also lacked identifiable green gene sequences, had test scores of 392 and 400 and a profound disturbance in color vision (more severe than dichromats; B. Drum, personal communication). These patients are candidates for detailed vision analyses that are beyond the scope of this report. However, subtle demyelination changes can occur in AMN, potentially affecting color perception and frustrating attempts to correlate visual and DNA changes. We documented such a psychophysical change during a 5-year period in an AMN patient from the O kindred.[3]

We intentionally biased our patient selection to emphasize defective color vision because we sought DNA changes likely to assist in localizing the ALD gene; our findings of unusual R/GCP gene changes likely reflect this bias. Although several patients (M, S, F, Y, and N-16) have changes grossly similar to reported changes, others are novel. Taken together, our findings indicate that a broader spectrum of R/GCP gene changes is found in ALD than is encountered in most color blind males. The frequency of these changes (10.6%) is 1.5–2 times the reported frequency of deficient color vision[5,6] but is very similar to the frequency of gene changes found in 134 men not

tested for color vision (11.2%).[9] We emphasize, however, that the clinical tests were performed 60 years before the genes were isolated and cannot be related directly to the R/GCP genes. Resolving this discrepancy and determining accurate frequencies will require extensive physiologic and genetic testing to eliminate ascertainment bias.

In 34 French ALD patients, Aubourg et al[10] found that 79% had 1–3 green pigment genes. No hybridization data were presented but they stated that seven patients had hybrid genes—an incidence of 21% (7 of 34), similar to that which we have found (about 2–3 times that in the normal population) but also constrained by ascertainment bias. In studying the DNA flanking the R/GCP genes they found intact DNA up to 210 kb from the green (3') end of the cluster in all patients. Although O had a deletion extending 5' from the red end of the cluster, the immediate 5' region appeared grossly intact in all others. The latter region includes probe JHN60 containing transcriptional controls for the R/GCP genes; changes within it affect color vision by altering pigment gene transcription.[8]

Because our study patients were chosen to emphasize those with abnormal color vision whenever possible, it is difficult to assess the frequency of this problem in ALD. However, we note: (1) F-M scores were abnormal in 16 of 35 (46%) AMN patients tested (12 of 27 from our earlier study,[4] and 4 of 8 additional patients included here); (2) only 7 of these 16 AMN patients with abnormal F-M tests had abnormal R/GCP genes (44%); (3) One of the 20 untested AMN patients (N-16) had abnormal R/GCP genes; (4) 3 of 19 ALD patients (16%), who could not be studied with the F-M test, had important R/GCP gene changes; (5) there were 4 R/GCP gene changes in all 39 patients who were not tested for color vision; and (6) considering all of our 59 kindreds, there were 11 with abnormal color pigment genes (18%). These observations imply more frequent R/GCP gene changes in ALD than the 6–8% frequency of male color blindness reported in the earlier surveys but not strikingly different from the 11.2% frequency of gene changes in anonymous population studies.[9]

Nine of our 16 AMN males with abnormal F-M test scores had no gross R/GCP gene changes. Because the hybridizations used are rather crude measures of gene integrity we cannot exclude small mutations or changes in expression. Concomitant imaging and clinical studies did not provide evidence for demyelination or cognitive dysfunction in these men but such changes may be subtle and we thus have no unequivocal explanation for this situation.

The ALD gene region is subtelomeric on Xq28

and may be near regions where changes develop because of repetitive sequences. Further analysis of this region will require long-range mapping. Fortunately, a number of clones are becoming available and a regional map is emerging. It is now especially important to examine the regional organization of the DNA in ALD patients to identify more deletions, inversions, or other changes. A systematic approach based on associated, possibly contiguous, genetic lesions is likely to lead to the isolation of the ALD gene as well as help clarify the structure and the potential for variation of this important chromosomal region.

Key Words

Acknowledgments

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