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ORIGINAL INVESTIGATION

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Spectrum of color gene deletions and phenotype in patients with blue cone monochromacy

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Abstract Blue cone monochromacy (BCM) is an X-linked ocular disease characterized by poor visual acuity, nystagmus, and photodysphoria in males with severely reduced color discrimination. Deletions, rearrangements and point mutations in the red and green pigment genes have been implicated in causing BCM. We assessed the spectrum of genetic alterations in ten families with BCM by Southern blot, polymerase chain reaction, and sequencing analysis, and the phenotype was characterized by ophthalmoscopy, fluorescein angiography, and a battery of tests to assess color vision in addition to routine ophthalmological examination. All families showed clinical features associated with BCM. Acuities were reduced in all affected males, and photopic b-wave was reduced by more than 90% in seven families. In three families, however, the photopic b-wave response showed uncharacteristic relative preservation of 30–80% (of the clinical low-normal value). The color vision was unusually preserved in two affected males, but this was not correlated with photopic electroretinography retention. Progressive macular atrophy was observed in affected members of two BCM families while the rest of the families presented with normal fundus. In nine families deletions were identified in the gene encoding the red-sensitive photopigment and/or in the region up to 17.8 kb upstream of the red gene which contains the locus control region and other regulatory sequences. In the same nine families the red pigment gene showed a range of deletions from the loss of a single exon to loss of the complete red gene. In one family no mutation was found in the exons of the red gene or the locus control region but showed loss of the complete green gene. No association was observed between the phenotypes and genotypes in these families.

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Introduction

Blue cone monochromacy (BCM; MIM303700) is a rare X chromosome linked ocular disorder in which affected males have reduced color vision caused by the absence of functional long-wavelength (L or "red") and medium-wavelength (M or "green") sensitive cone photoreceptors in the retina but with preservation of short-wavelength (S or "blue") sensitive "blue" cone photoreceptor function (Blackwell and Blackwell 1957). Affected individuals also show reduced visual acuity, photodysphoria, and congenital nystagmus (Berson et al. 1983). BCM has been shown to be associated with alterations in the genes that encode the long-wavelength and middle-wavelength sensitive photopigments in the cones (Nathans et al. 1989). These genes encoding the "red" and "green" sensitive pigments are present on the X chromosome, whereas the "blue" pigment is located on chromosome 7 (Nathans et al. 1997). We refer to the genes encoding the red- and green-sensitive pigments as the "red" gene and "green" gene, respectively.

The red and green pigment genes are arranged in a tandem array with a locus control region (LCR) located between 3.5 and 4.2 kb upstream from the red gene transcription initiation site (Nathans et al. 1986; Vollrath et al. 1988; Wang et al. 1992). The intervening sequence between the red and green genes has been shown to contain exons 2–5 of the *Tex 28* gene, while the complete *Tex 28* gene (exons 1–5) is located at the 3′ end of the green gene (Fig. 6). The *Tex 28* gene is transcribed in the opposite direction from the color genes and its function is not known (Hanna et al. 1997).

BCM is an infrequent condition, and as yet there have been only a limited number of reports describing the alterations in the red and green genes in these patients (Ayyagari et al. 1999a; Nathans et al. 1989, 1993; Reyniers et al. 1995). The genotype reported in BCM patients include deletions in the upstream red gene region encompassing the LCR, loss of part or complete red and/or green genes and deletions in combination with point mutations in red and green pigment genes.

The goal of the present study was to elucidate the spectrum of red and green color gene alterations involved in ten families with BCM. We report a comprehensive analysis of the red, green, and *Tex 28* genes as well as the upstream red gene region in these families.

Patients and methods

Ten unrelated families with BCM were studied. Affected individuals from these families were seen at the University of Michigan for clinical examination. Informed consent was obtained from all participating individuals.

Clinical examination

A standard ophthalmological examination including measurement of visual acuity, Goldmann visual field testing, and ophthalmoscopy was followed by extensive color vision testing, dark-adapted thresholds, and electroretinography (ERG). Color vision testing included the Ishihara plates, Berson's BCM plates (Berson et al. 1983), and the Farnsworth Dichotomous D-15 test, performed under CIE Standard Illuminant C from a MacBeth Easel lamp. Additionally, two-degree Rayleigh matches were determined on a Nagel anomaloscope to check for residual L-cone or M-cone function in affected members of five families (families A, B, F, H, and I). Dark-adapted thresholds were obtained using a Goldmann-Weekers Adaptometer after 45 min of dark adaptation. Standard clinical ERGs were recorded from fully dilated pupils (Sieving 1995) using 10-μs full-field xenon flashes and Burian-Allan bipolar corneal electrodes (Hansen Ophthalmic Instruments, Iowa City, Iowa, USA).

Molecular analysis

DNA from at least two affected males in generations separated by one meiosis, one female carrier, and one unaffected male were studied in each family. In some families we have also analyzed females that are not obligate carriers. DNA from venous blood was isolated using standard protocols. The structure of the red, green, and *Tex 28* genes, as well as the 5′ upstream red gene region up to 18 kb from the red gene transcription initiation site were analyzed. Southern blot analysis was performed using probes Br, Cr, Dr, Bg, Cg, Dg, and Zr as described by Nathans et al. (1993) to detect gross structural changes in the red and green pigment genes and the upstream red gene region.

We assembled a contig of the sequences spanning a 120-kb region encompassing the red, green, *Tex 28* genes, transketolase pseudogene (*TKL*), and a 30-kb sequence upstream to the red pigment gene, by scanning the National Center for Biotechnology Information database and identifying overlapping sequences (http://ncbi.nlm.nih.gov). The upstream red gene sequence was analyzed for the presence of known genes or coding sequences (expressed sequence tags, ESTs) by BLAST analysis (http://ncbi.nlm.nih.gov).

The 5′ upstream red gene region up to 18 kb from the red gene transcription start site, and all six exons of the red and green genes, and exons 1–5 of the *Tex 28* gene were amplified from genomic DNA using primers listed in Table 1 and described elsewhere (Ayyagari et al. 1999a). The PCR products were analyzed on agarose or polyacrylamide gels to check for deletions. The sequence between the red gene transcription initiation site and 6 kb upstream was amplified by PCR as overlapping segments of 500–800 bp. The region between 6 and 18 kb from the red gene transcription start site was analyzed by amplifying as 0.2–1.5 kb segments.

Mutation analyses of the exons of the red and green pigment genes and the 600-bp LCR region, were carried out by sequencing the amplified PCR products using a cycle sequencing kit (Amersham, Arlington Heights, Ill., USA) as described earlier (Caldwell et al. 1999).

Table 1 Primers used for the analysis of upstream red gene region

	Location[a]	Accession no.	Sequence 5′-3′
1	842–861	Z47066	tct agc ggg act cat cac ct
2	1176–1157	Z47066	ctc tct ctt cag ggc agt gg
3	2661–2680	Z47066	aaa aca ata tga ggc aca ag
4	4076–4057	Z47066	tgt gct tct aag ata tca gc
5	4151–4170	Z47066	tgg tgt tct gtg cac agt gg
6	4603–4584	Z47066	aat cac ggt agt tag tgt ac
7	6131–6150	Z47066	act acc tga aca tct gtg tg
8	6570–6551	Z47066	ctc tgc act cca tca tac ag
9	7573–7592	Z47066	cga tac atg ctg aaa ttc ag
10	8264–8245	Z47066	ttc atc cca aga agt tat gc

[a] Position of the primers 5′ to 3′ internal to the sequence of the accession number

Results

Clinical phenotype

We analyzed ten families with BCM for alterations in the red and green genes and the upstream red gene region. All affected individuals who were examined showed typical signs of BCM. The pattern of inheritance of disease in all the ten families is X-linked recessive. Although severe loss of color discrimination was observed on the Ishihara and D-15 tests, all were able to pass the BCM plate test, consistent with retention of the short-wavelength sensitive pigment in the blue cones. The two-degree Rayleigh matches obtained from individuals of five families (Fig. 1) extended across the entire intensity range of the test light, indicating an absence of red-green color discrimination in

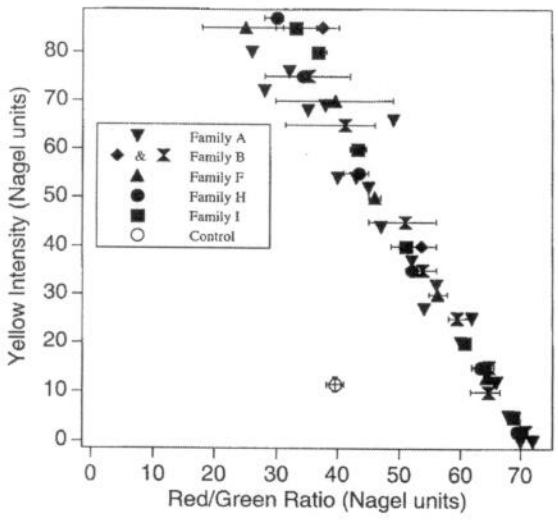

Fig. 1 Two-degree Rayleigh matches for seven affected members of families A (*inverted triangles*), B (*diamond and bow tie symbols*), F (*triangles*), H (*filled circles*), and I (*squares*) measured on the Nagel anomaloscope. *Symbols* in the graph distinguish individuals; *error bars* range of settings that produced a match; *gray shaded area* range of normal settings

the macula. All affected males had poor visual acuity of 20/80 or worse. ERGs of affected males generally showed preservation of rod function under dark-adapted conditions but a marked attenuation of cone function to photopic single stimuli and the 30-Hz flicker (Fig. 2). The dark-adapted dim blue flash condition elicits responses nearly exclusively from the rod system, with little or no contribution from cones. These rod responses had the appropriate wave form, but the amplitudes were on the marginally small side of the normal control population, as has previously been noted for BCM (Sieving 1995). The functional integrity of the rod system is noted by the normal psychophysical rod absolute threshold sensitivity in the dark-adapted condition for all these affected males.

The photopic cone-driven responses generally were reduced to levels near noise, indicating a retinal-wide absence of red and green cones which normally dominate the light-adapted response (Gouras and MacKay 1990). However, three families (A, B, and F) had individuals with anomalous relative preservation of the photopic b-wave. The lowest normal photopic b-wave on our system is approximately 55 μV. Figure 2 shows photopic response of 25–30 μV in a 25-year-old affected male (individual KB) and his 22-year-old brother (individual SB) in family B.

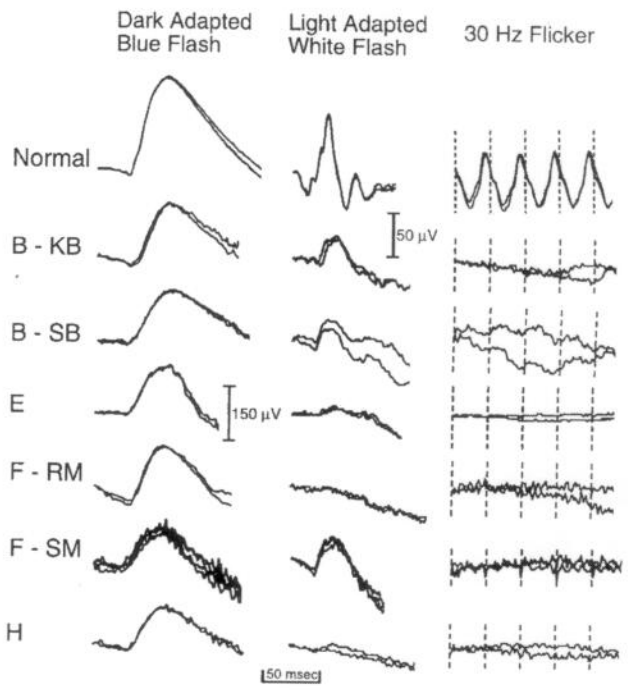

Fig. 2 ERG of affected males from four BCM families using standard conditions. Scotopic rod ERG was elicited with −1.86 log cd s^{-1} m^{-2} flashes after 45 min of dark-adaptation. Cone responses were recorded with 30-Hz flicker (4 cd s^{-1} m^{-2}/flash) and by photopic adaptation of 3.3 log scotopic trolands and "white" flashes of 1.0 log cd s^{-1} m^{-2}. The photopic response in family B (individuals KB and SB) is anomalously large. In family F individual SM had 40- to 45-μV photopic b-waves, whereas his cousin (individual RM) showed no photopic responses

For both brothers the responses were of equal amplitude for both eyes and were the same size when the ERG was recorded again several months later. These responses are anomalous in lacking an a-wave. Individual SB showed a residual response of 30-Hz flickers, whereas KB did not. We also recorded photopic responses to long-duration stimuli (methodology as in Sieving 1993) and again saw the absence of an a-wave. The predominant response for this long stimulus was a positive-going wave at the termination of the stimulus termed the d-wave (not shown). Both brothers were considerably photodysphoric, however, and the waveforms were quite noisy; consequently we could not definitively establish that the predominant component was the d-wave.

Both brothers in family "B" had reduced acuities: the 22-year-old had 20/100 OU, and the 25-year-old had 20/200 OU, best corrected. Both were about 9 diopters myopic, but their fundi showed no changes beyond myopic retinal pigment epithelium (RPE) thinning. Color discrimination was similar for both of them. They correctly identified all of the Berson BCM plates, but they identified only two plates correctly out of 14 AO plates (beyond the test plate). D-15 testing showed errors for both of them considerably less than random, as illustrated in Fig. 3A for individual SB: each eye showed one arrangement with only minor errors, a second with primarily one major error, and a third result with three or four major errors. While the D15 testing indicated some degree of color discrimination at better than chance levels, Nagel anomaloscope two-degree Rayleigh match testing (shown in Fig. 1 for both brothers in family "B") gave matches across the entire range and thus failed to indicate residual red or green cone function. Both had reduced acuity in keeping with BCM and considerably worse than the normal acuity associated with protanopia or deuteranopia.

In family B one affected male at age 56 years and his carrier sister at age 70 years showed RPE changes just outside the macular arcade vessels, with RPE thinning and course granularity. The sister had acuities of 20/30 best corrected for both eyes, not explained by lens changes. She was examined in the field, and only limited testing could be performed. Her affected brother had 20/200 acuity bilaterally and was a 6 diopter myope which accounted for the mild peripapillary RPE changes but did not explain the perimacular RPE granularity and patch of mild atrophy. Only two other affected males in the family were the brothers KB and SB, and neither showed macular abnormalities. No obvious macular abnormalities were noted in BCM patients of the remaining nine families.

In family A one young affected male also retained a 15-μV photopic b-wave (not shown). He was only 2.5 years old and was too young for formal color testing. Nagel anomaloscope testing of his affected grandfather gave matches across the entire range and was not different from the other families tested; an ERG was not obtained on this grandfather.

In family F a 30-year-old affected male (SM) showed anomalous 40- to 45-μV photopic b-waves in both eyes

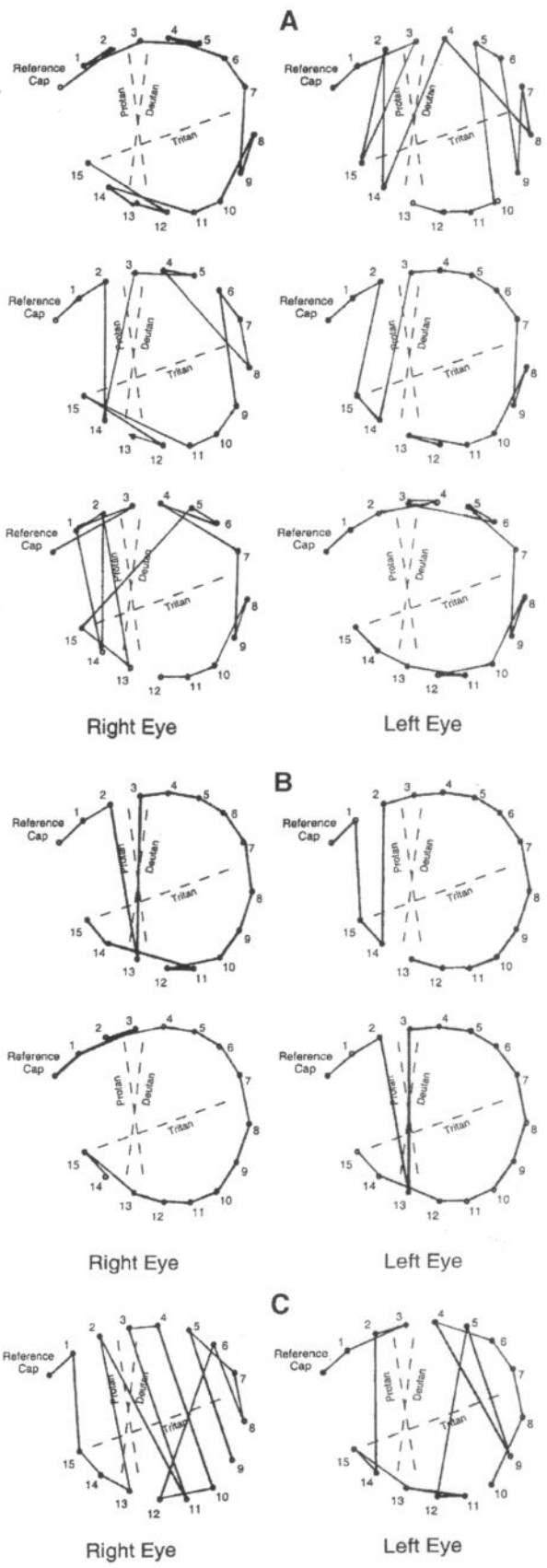

Fig. 3 Farnsworth D-15 color test results on subject SB from family B, performed three times for each eye separately on a single occasion (**A**), and subjects RM (**B**) and his affected cousin SM (**C**) from family F

(Fig. 2) but no 30-Hz flicker response. He had 20/300 acuities. On D-15 testing he made seven major crossings for one eye and four for the fellow eye (Fig. 3C). His affected cousin (individual RM) gave the best performance in arranging the D-15 test of all individuals we tested (Fig. 3B) but he showed essentially no photopic flash or flicker ERG responses (Fig. 2). On the Nagel anomaloscope he matched across the entire red-green range (des-

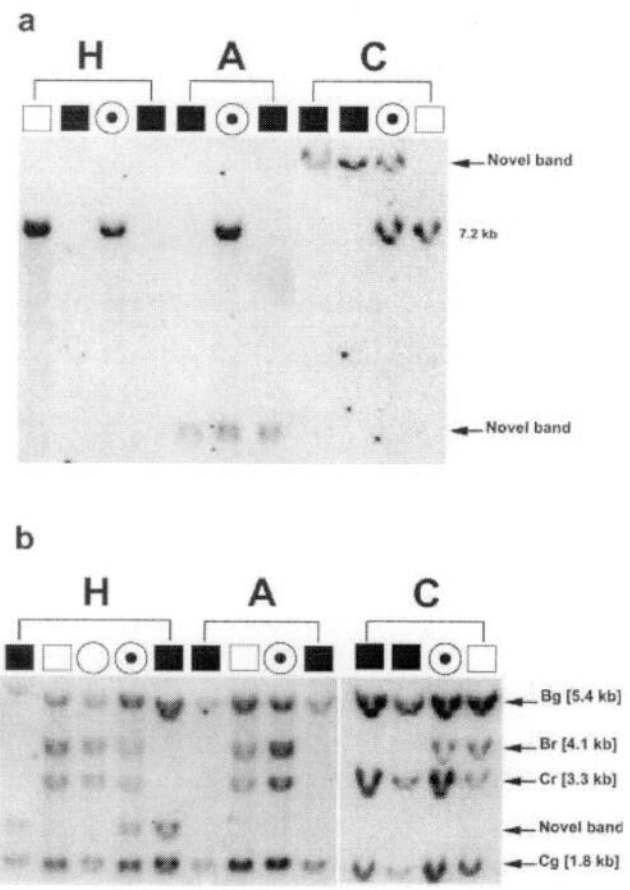

Fig. 4 Southern blot analysis on families A, C, and H. *Filled squares* Affected males; *circles with dot* carrier females; *open squares* unaffected males; *open circles* unaffected females. **a** Analysis of upstream red gene region. *Hin*dIII digests probed with an 800 bp *Bam*HI-*Hin*dIII partial digest fragment located between 8.2 and 9.0 kb upstream of the red gene. In all the three families absence of the expected 7.2-kb fragment can be seen in affected individuals with the presence of novel bands in families A and C. **b** Southern blot showing the analysis of red and green genes. *Bam*HI-*Eco*RI double digests probed with the segment encompassing exon 1 and the 5′ half of exon 2 of the red/green genes. As described earlier, Br and Cr correspond to red gene exons and Bg and Cg to exons of the green gene (Nathans et al. 1989)

ignated as family F in Fig. 1). However, on D-15 testing, he made only two minor errors on one attempt, and his performance on the three other trials gave only two major crossing errors with no or only one minor errors (Fig. 3B). This can be interpreted as a minimal color discrimination deficiency according to Pokorny et al. (1979): "Subjects with normal color vision will sometimes make one or two minor errors or a single major error, as, for example, when the observer reverses part of the series... such as placement of cap 15 next to cap 7. Occasionally an observer will make a few minor errors and a few major errors. In this case, a retest is required." By these criteria, he performed nearly normally on the D-15 test.

Analysis of the LCR

Southern blot of a *Hin*dIII digest of genomic DNA was probed with the Zr fragment to detect the alterations in the upstream red gene region. The Zr probe is an 800-bp segment between 8.2 kb and 9.0 kb upstream from the red gene transcription initiation site (Nathans et al. 1993). Nine of the ten families tested showed deletions in the upstream red gene region (Fig. 5). The *Hin*dIII digests of human genomic DNA hybridized with the Zr probe did not identify either the expected 7.2-kb fragment or any novel fragments in families B, E, F, G, H, and I (Fig. 4A, shows family H). This suggests the presence of a deletion in the upstream red gene sequence including the region of the probe. Systematic analysis of the 18-kb region upstream to the red gene, employing multiple PCRs, indicated that the deletion in families G, H, and I extends up to 9.7-kb upstream from the red gene transcription start site (Fig. 5), whereas the deletion in families B and F extends up to 17.8 and 10.5 kb, respectively.

The Zr probe detected a fragment smaller than 7.2 kb in family A (Fig. 4A), whereas bands larger than 7.2 kb were identified in families C, D, and E (Fig. 4A shows family C) consistent with deletions in the upstream red gene region. Hybridization of the Zr probe to novel *Hin*dIII fragments suggests that the 5′ boundary of deletions in these families does not extend beyond the region corresponding to the Zr probe.

Results of the PCR analysis with 46 primers derived from the upstream red gene region (Ayyagari et al. 1999a; Table 1) showed the loss of the upstream red gene region up to 8.2 kb, and the presence of the region between 8.2 kb and 18 kb upstream (Fig. 5) in families A and E. Families C and D showed that the 5′ boundary of the deletion is close to 6.3 kb upstream from the red pigment gene (Fig. 5). In aggregate, the size of the deletions in the upstream red gene ranged from 6.3 to 17.8 kb (Fig. 5). All of these deletions included the LCR, positive and negative regulatory elements involved in controlling the expression of red and green pigment genes (Shaaban and Deeb 1998; Wang et al. 1992).

Analysis of the red gene

All affected individuals with deletions upstream of the red gene also showed a loss of at least part or the entire red gene. Both Southern blot (Fig. 4B) and PCR analysis revealed the loss of complete red gene in families A and B

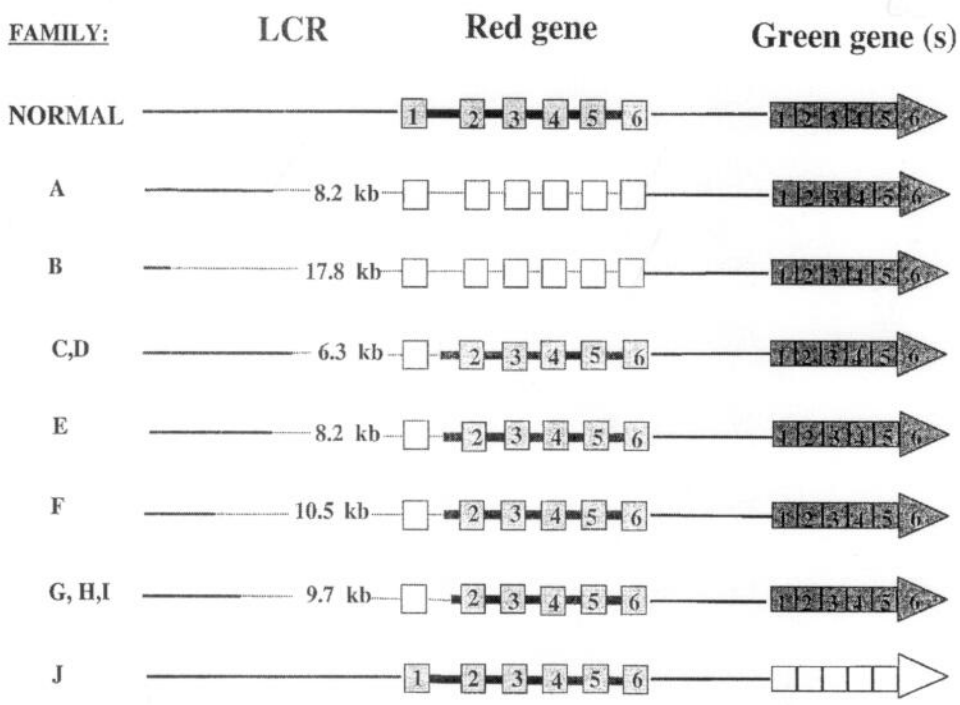

Fig. 5 Deletions in color genes in ten families with BCM. Organization of red and green genes in unaffected individuals shown at the top. *Solid line upstream to the red gene* 18-kb region upstream from the red gene transcription initiation start site; *each box in red and green genes* an exon; *solid line in the red gene* introns; *filled boxes* presence of the corresponding exons; *open boxes* missing exons; *dotted lines* deleted regions. Size of the deletion in the upstream red gene region is indicated. The 3′ boundary of the deletion is similar in families C, D, E, and F, and in families G, H, and I. The 3′ end of the deletion in families G, H and I extends 800 bp further 3′ into intron 1 of the red gene than the 3′ end of the deletion in families C, D, E, and F

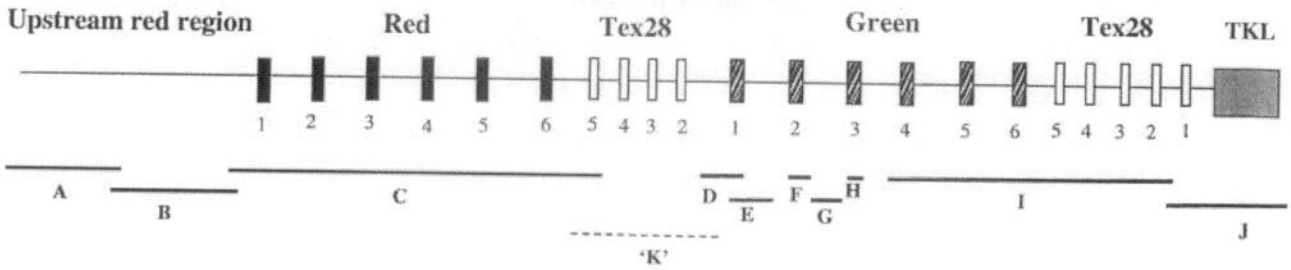

Fig. 6 Contig of sequences encompassing the red, green, and *Tex 28* genes, and Transketolase pseudogene (*TKL*). *I* Organization of red, green, *Tex 28* and transketolase genes are shown. Exons of each gene were shown as boxes with numbers below representing the corresponding exons. *Tex 28* gene is transcribed in the opposite direction. The genes and sequences are not shown to the scale. *II* Contig of sequences. *Horizontal bars* position of each segment of sequence; *letters below each* the corresponding sequence. Gene bank numbers of sequences are: *A* Z47046 (15.25 kb), *B* Z47066 (13.540 kb), *C* Z68193 (21.480 kb), *D* U93721 (2.467 kb), E. M13306 (0.609 kb), F. K03490 (0.290 kb), G. X76097 (1.987 kb), H. K03491 (0.182 kb), *I* Z46936 (28.230 kb), *J* Z49258 (36.429 kb). Sequence K is shown as *dotted line* is the sequence of Z46936 (*I*) between 4.931 and 28.230 kb. Overlapping regions *between horizontal bars* indicate the presence of overlapping sequences

(Fig. 5). Southern blot analysis with probes corresponding to red and green genes (Nathans et al. 1989) detected the absence of the 4.1 kb fragment corresponding to exon 1 and the 3.3 kb 5′ end sequence of intron 1 of the red gene in families C, D, E, and F (Fig. 4B). The total size of intron 1 is 6.4 kb. Presence of the 3.3 kb Cr band, which corresponds to the 3′ half of intron 1 (3.1 kb) and part of exon 2, suggests that the 3′ boundary of the deletion in these families lies in intron 1. Analysis by PCR further confirmed the loss of exon 1 and the presence of remaining exons 2–6 of the red gene.

Families G, H, and I showed the loss of both the 4.1 (Br) and the 3.3 kb (Cr) bands and the presence of an additional 2.5 kb novel band (Fig. 4B). Analysis of the red gene by PCR indicated the loss of exon 1 and the presence of exons 2–6 in these families. These results suggest that most of intron 1 (4.1 kb) is deleted in these individuals. The novel 2.5 kb *Eco*RI-*Bam*HI fragment observed on the Southern blot analysis (Fig. 4B) corresponds to exon 2 (200 bp) and the 3′ end of the intron 1 (2.3 kb).

Analysis of the green gene

Analysis of the green gene by Southern blot and PCR showed the presence of at least one copy of the green gene in nine of the ten families tested (Fig. 5). Family J showed the loss of all the exons of the green gene.

Analysis of the other genes in the region

The *Tex 28* gene with all 5 exons was present in all ten families whom we analyzed. A contig of the sequences spanning a 120-kb interval was constructed using the sequence data available from the GenBank (Fig. 6). Presence of neither known genes nor coding sequences including ESTs was detected in the 18-kb upstream region.

All the deletions observed in patients with BCM cosegregated with the disease in available members of the respective families (Fig. 4). These deletions were found to be stable in sequential generations.

Discussion

We studied ten BCM families in which affected members all showed deletions in the red and green pigment genes and in the upstream red gene region (Fig. 5) that included the LCR and other regulatory elements involved in controlling the expression of the red and green genes (Shaaban and Deeb 1998; Wang et al. 1992). Loss of the LCR can result in the loss of functional red and green pigment genes (Nathans et al. 1989).

The upstream red gene deletions extended from the red gene transcription initiation site to as far as 17.8 kb upstream (Fig. 5). The deleted region encompasses the 600-bp LCR and the positive and negative regulatory elements (Shaaban and Deeb 1998; Wang et al. 1992). BLAST analysis of the 18-kb upstream sequence did not identify any sequence homologous to coding sequences, indicating that the large deletions observed in our BCM families (Fig. 5) comprise no other known genes.

Families A, B, and F had affected males with anomalous relative preservation of the photopic ERG b-wave up to 45 μV. The low but normal value for this clinical laboratory is 55 μV. This extent of preservation is not ordinarily associated with the BCM (Berson et al. 1986). The deleted region in all three families includes exon 1 of the red gene and extends upstream to 8.2 kb from the transcription start site in family E, to 17.8 kb in family B, and to 10.5 kb in family F. This should result in the loss of functional red and green gene expression (Shaaben and Deeb 1998; Wang et al. 1992) and thereby cause a severe reduction in the photopic b-wave response (Berson et al. 1986; Gouras and MacKay 1990; van Norren and Padmos 1973). Loss of functional red and green cones leaves only the rods and blue cones. Blue cones alone cannot account for the 30–40% relative retention of ERG photopic b-wave responses (referenced to the lowest normal values for our system). The 43 cd/m^2 photopic background light is equivalent to about 3 log troland intensity for dilated pupils and is sufficient to desensitize rods (Aguilar and Stiles

1954) and eliminate any rod ERG contribution (Marmor et al. 1989).

In family B one affected male (aged 56 years) and his carrier sister (aged 70 years) showed macular changes. The deletion in family B extends about 10 kb further upstream than that in family A which had a loss of 8.2 upstream red gene region. No known genes or ESTs were found to be homologous to the 10-kb sequence that lies between the 5′ boundaries of the deletions observed in both these families to account for the maculopathy in family B.

Progressive macular atrophy was reported earlier in three other BCM families (Ayyagari et al. 1999a; Fleischman and O'Donnell 1981; Nathans et al. 1989). These occurred by young middle age in our BCM families and hence seemed different from age-related macular degeneration (Ayyagari et al. 1999b). We are not certain what molecular features of color gene mutations may lead to macular atrophy, since families C–I and family A with similar deletions did not show macular atrophy. Additionally, a family with X-linked cone degeneration and loss of part of the red gene has been reported to have macular degeneration in some older males (Reichel et al. 1989).

Large deletions in the red gene in addition to the upstream red gene sequence were observed in all of these patients. Two families had loss of the complete red gene. The 3′ boundary of the deletion in seven families (C–I) lies in intron 1 of the red gene. Although the exact boundaries of the deletions are not known, the Southern blot analysis clearly indicates that the 3′ boundary of the deletion in families G, H, and I is located at about 800 bp downstream to the 3′ boundary of the deletion in families C–F (Figs. 4, 5). These three unrelated families G, H, and I showed the loss of exon 1 and most of intron 1 of the red gene and a 9.7-kb deletion in the upstream red gene region. Similarly, families C and D had a deletion of 6.3 kb in the upstream red gene region in addition to the loss of exon 1 of the red gene. No genealogical relationship is known between the families with similar deletions, and therefore no comment is possible regarding a common origin of the deletions observed in these two groups, but not all have the same ethnic background. Furthermore, the exact boundaries of the deletions in these families are not known. The identification of five different types of deletions in eight families (Fig. 5) suggests an independent origin of each type of deletion observed in spite of the broad range of genotype. The phenotype observed in the families presented here did not show much deviation from the typical symptoms of BCM, except for three families A, B, and F which had partially preserved cone ERG response.

It has long been recognized that blue-cone monochromats display residual color discrimination (Daw 1973). Performance of these individuals on the Farnsworth D-15 test is described as "... somewhat between... the random array of π_0 monochromacy... and the unequivocal dichromatous axis of confusions exhibited by the ordinary protan, deutan and tritan colour defectives" (Alpern et al. 1971). Other studies find that individuals with typical total π_0 monochromacy arrange the D-15 caps according to the scotopic luminosity function, which is the following cap order: 1, 2, 3, 4, 15, 14, 5, 6, 13, 7, 12, 11, 10, 8, 9 (Sloan 1954). None of the BCM individuals whom we tested arranged the D-15 hues according to these scotopic luminosity values, and thus they display evidence of color discrimination beyond that of π_0 rod function and involving discrimination afforded by blue cone function in the least. Several of our subjects did far better than chance and in some cases performed close to normal (Fig. 3). However, no particular association was noted between those with even fairly normal D-15 arrangement and the extent of the residual photopic b-wave response. This is demonstrated by individual RM of family F who had the best D-15 performance (Fig. 3A) but had a photopic ERG reduced essentially to noise level. Intrafamilial variation was seen both in the degree of performance on the D-15 color test and on the photopic b-wave, as illustrated by the two males in family F. Four cases were found with a considerable residual b-wave (one each in families "A" and "F," and two in family "B"). We cannot explain this phenomenon in these BCM families.

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