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Human Tritanopia Associated with a Third Amino Acid Substitution in the Blue-sensitive Visual Pigment

To the Editor:

Tritanopia is an autosomal dominant color-vision disorder characterized by insensitivity to the blue region of the spectrum (Boynton 1979). Recently, we used PCR and denaturing gradient gel electrophoresis (DGGE) to screen for mutations in the coding region and intron-exon boundaries of the gene encoding the blue-sensitive visual pigment in nine unrelated tritanopes and available members of their families (Weitz et al. 1992). In four of the nine probands we found two examples each of two point mutations leading, respectively, to the amino acid substitutions G79R and S214P. The mutations segregated with tritanopia in the relevant pedigrees, and no example of either of these mutations were detected in 43 and 84 control subjects, respectively. In five of the nine probands we failed to detect any sequence variants that were candidates for causal mutations, although in one proband (C2; for numbering of subjects, see Weitz et al. [1992]) we detected, within the splice-donor consensus sequence of intron 3, a single-base-pair deletion that did not segregate with tritanopia.

We now report three independent examples of a third point mutation, leading to the amino acid substitution P264S. This mutation was not detected in our original experiments in which exon 4 (including its flanking sequences and "GC-clamp") was amplified as a single product of 423 bp, but it is clearly revealed by DGGE when the exon 4 sequence is analyzed as two smaller overlapping products of 253 bp (exon 4A) and 257 bp (exon 4B). Figure 1 shows the DGGE analysis of exon 4A PCR products from relevant subjects in our original study (Weitz et al. 1992). Tritanopes from family C (lanes 3 and 7), proband D1 (lane 8), and a tritanope studied by Wooten and Wald (1973) (lane 9) are shown to be heterozygous for exon 4A sequences, with one allele having a mobility indistinguishable from the control (lane 1) and with the other having a lower mobility (apparently the same in all four tritanopes); an unresolved pair of heteroduplexes is evident as a band of yet lower mobility. Unaffected subjects from family C (lanes 2 and 4–6) show a single allele indistinguishable from that of the control.

The exon 4 PCR products corresponding to the wild-type and variant alleles from proband C2 were subcloned and sequenced as described by Weitz et al. (1992; see fig. 2 legend). The variant allele differed from the wild-type allele only in the substitution of C^{1199} by T (fig. 2) (for numbering, see Nathans et al. 1986), leading to the amino acid substitution P264S in the predicted sixth membrane-spanning segment of the blue-sensitive visual pigment (fig. 3).

Exon 4 PCR products from the subjects described in figure 1 and from 64 control subjects of northern European ancestry were "slot blotted" and probed, as
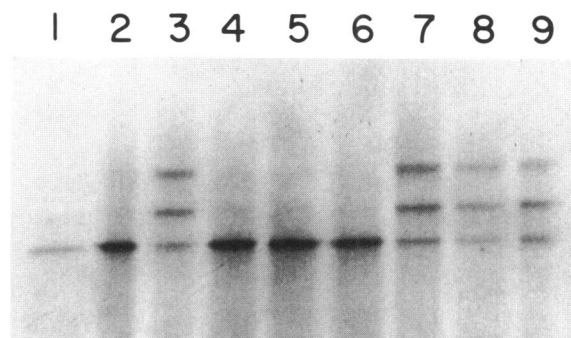

Figure 1   DGGE profile of exon 4A PCR products from subjects described by Weitz et al. (1992). Lane 1, Wild-type control. Lanes 2, 4, 5, and 6, Unaffected subjects C1, C3, C4, and C5, respectively. Lanes 3 and 7, Affected subjects C2 and C6, respectively. Lane 8, Affected subject D1. Lane 9, Tritanope studied by Wooten and Wald (1973). PCR and DGGE were performed as described for exon 4 (Weitz et al. 1992), except that the following primer was substituted for the second member of the primer pair in PCR amplification of genomic DNA: 5′-ACGGTTGTTGACCATGTACAT-3′.
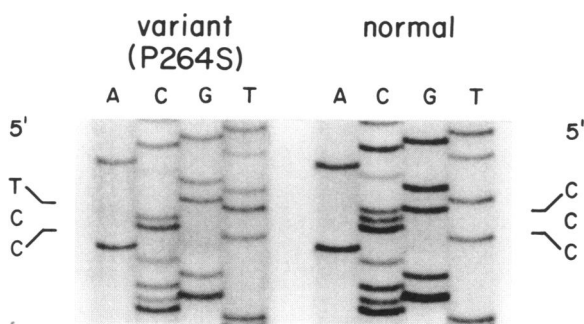
Figure 2 Sequences of exon 4 alleles amplified from the genomic DNA of proband C2. *Left,* Variant allele. *Right,* Wild-type allele. Amplification, subcloning, and allele assignment was performed as described by Weitz et al. (1992). PCR primers containing restriction sites for subcloning exon 4 were 5'-CAACGAATTCAG-CATCCAGAGGGCCAGGAA-3' and 5'-TTTGAAGCTTTAAA-AGTCAATGGTGAGAAA-3'.

described elsewhere (Weitz et al. 1992), with a 13-mer oligonucleotide complementary to the P264S sequence centered at position 1199. As suggested by the DGGE analysis shown in figure 1, all four tritanopes and none of the unaffected subjects were found to have the P264S allele; no examples of the mutation were found in the control population (data not shown). If the data from our previous study are included here, (Weitz et al. 1992), P264S is present in three of the nine tritanope probands, as compared with zero of 64 controls ($\chi^2 = 40.1$; $P < 10^{-5}$).

Taken together, these data strongly argue that P264S is the mutation causing autosomal dominant tritanopia in these subjects. This mutation has also been found independently in two large pedigrees of European ancestry segregating tritanopia (Li et al.,
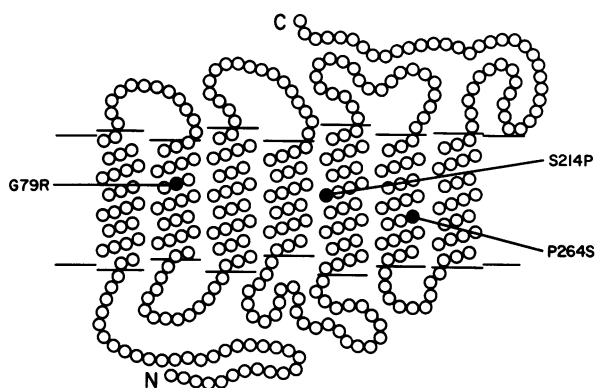
Figure 3 Model of the transmembrane topology of the blue-sensitive opsin, showing the locations of the three amino acid substitutions associated with tritanopia.

submitted). Like the G79R and S214P substitutions we found previously, it is not yet known how the P264S substitution leads to a loss of blue-sensitive cone function or viability. The fact the proline-264 is highly conserved in both visual pigments (Nathans et al. 1986) and in other members of the superfamily of receptors that couple to G-proteins (McFarland et al. 1989) suggests that this substitution might perturb the folding, stability, or transport of the blue-sensitive opsin protein.

In addition to the P264S mutation, the proband of family C (subject C2) has, in his other blue-sensitive opsin gene, a coincidental deletion of a single G in the splice donor sequence of intron 3 (Weitz et al. 1992), whereas his affected son (subject C6) has the P264S and wild-type alleles. The similarity in the psychophysical test results for these two subjects (Higgins et al. 1983) suggests that the presence of the splice-donor G deletion in the other chromosomal copy of the blue-sensitive opsin gene has little or no effect on the severity of the dominant P264S phenotype.

Acknowledgments

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Mitochondrial tRNA^{Thr} Mutations and Lethal Infantile Mitochondrial Myopathy

To the Editor:

Recently, Yoon et al. (1991) attributed certain cases
of lethal infantile mitochondrial myopathy (LIMM)
to mutations in the mtDNA threonine transfer RNA
(tRNA^{Thr}) gene. The two patients in their study had
severe respiratory chain enzyme deficiency and associ-
ated lactic acidosis and died within days after birth.
Separate mtDNA tRNA^{Thr} mutations were detected by
DNA sequence analysis at nucleotide pair (np) 15924
in one patient and at np 15923 in the other patient.
Both mutations occur within the tRNA anticodon
stem-loop structure. No unaffected controls were
screened for the presence of these mutations.

We have screened three LIMM and multiple control
subjects for these mutations and report here that the
np 15924 mutation found in one of the above patients
is not a primary cause of LIMM. mtDNA was isolated
from transformed lymphocytes of three LIMM pa-
tients, all of whom had mitochondrial respiratory en-
zyme deficiency and lactic acidosis. The tRNA^{Thr} genes
from all three were sequenced, but only one was found
to differ from the normal sequence. It contained the
np 15924 A-to-G mutation. This Caucasian male died
at 4 mo postpartum, of severe lactic acidosis and heart
and muscle mitochondrial defects (Zheng et al. 1989).
His mother had the same mutation, and both individu-
als were homoplasmic. However, the mother showed
no evidence of disease even after extensive biochemi-
cal, histological, and clinical analyses (Zheng et al.
1989).

In order to determine the frequencies of the np
15924 and 15923 mutations in the general popula-
tion, mutation-specific restriction-endonuclease di-
gestion assays were developed. PCR primers were pre-
pared in which the sequence near the mutation was
altered to create a diagnostic restriction enzyme–rec-
ognition site after PCR amplification (Seibel et al.
1990). To test for the np 15924 mutation, a Fnu4HI
site was created using PCR primers at np 15409–
15428 and np 15925–15944 (mismatched G at np
15928). To test for the np 15923 mutation, a MaeI
site was generated using PCR primers at np 15903–
15922 (mismatched C at np 15920) and at np 16527–
16557. With this procedure, a number of unaffected
controls were screened for the mutations.

The np 15924 mutation was confirmed in our Cau-
casian LIMM patient and his mother, but it was also
detected in approximately 11% (11/103) of Cauca-
sian controls. Thus, while deleterious interactions
with other genes cannot be completely excluded, the
presence of the np 15924 mutation in the unaffected
mother and its polymorphic frequency in the general
population strongly suggest that this mutation is a
common mtDNA polymorphism and not the primary
cause of LIMM.

The np 15923 mutation was not detected in 91 Cau-
casians, 35 Africans, and 57 Asians. Consequently,
the role of this mutation in causing LIMM remains to
be clarified.

In conclusion, recent studies have demonstrated
both the genetic heterogeneity of LIMM (Zheng et al.
1989; Tanaka et al. 1990; Moraes et al. 1991) and
the extensive sequence variation of human mtDNAs
(Wallace et al. 1991). Therefore, it is extremely im-
portant to ascertain the frequency of a sequence vari-
ant in the general population before attributing a
pathological role to it.

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