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Genetics

A frequent tyrosinase gene mutation in classic, tyrosinase-negative (type IA) oculocutaneous albinism

(albino/pigment disorders/melanin)

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ABSTRACT **We have identified a tyrosinase gene mutation in several patients with classic, tyrosinase-negative (type IA) oculocutaneous albinism. This mutation, which results in a proline → leucine substitution at codon 81 of the tyrosinase polypeptide (EC 1.14.18.1), was observed in 20% (6 of 30) of oculocutaneous albinism alleles from independent probands, but it was not observed in any normal individuals. This mutation thus appears to be a frequent cause of tyrosinase-negative oculocutaneous albinism.**

Oculocutaneous albinism (OCA) is a heterogeneous group of autosomal-recessive inherited disorders of pigmentation. Affected individuals are characterized by reduced or absent biosynthesis of melanin pigment in melanocytes of the skin, hair follicle, and eye (1, 2). Type I OCA is associated with deficient activity of melanocyte tyrosinase, the first enzyme in the melanin biosynthetic pathway. In type IA OCA, complete lack of melanin synthesis results from completely absent tyrosinase activity. In type IB OCA, various less severe hypopigmentation phenotypes are associated with greatly reduced tyrosinase activity, possibly resulting from compound heterozygosity with a variety of type I OCA alleles.

Tyrosinase (monophenol, L-dopa:oxygen oxidoreductase, EC 1.14.18.1) is a bifunctional copper-containing glycoprotein that catalyzes the conversion first of tyrosine to dopa and then of dopa and dopaquinone in melanocytes (3, 4). Recently, cDNAs encoding human (5, 6) and mouse (7, 8) tyrosinase have been cloned, and the 528-amino acid sequence of the 58-kDA tyrosinase polypeptide (9) has been deduced. Here, we report a missense mutation in the tyrosinase gene coding region in several individuals with classic tyrosinase-negative (type IA) OCA. This mutation appears to account for a substantial fraction of type IA OCA mutant alleles, at least among individuals of northern European Caucasian ethnic origin.

MATERIALS AND METHODS

Genetic Linkage Analysis. All OCA affected individuals in the two families studied were evaluated by clinical examination, quantitative hairbulb tyrosinase (tyrosine hydroxylase) assay (10), and electron microscopy of hairbulb melanocytes. OCA carrier status of family members was also determined by quantitative hairbulb tyrosinase assay (10).

For tyrosinase gene restriction fragment length polymorphism (RFLP) analyses, DNA was isolated from peripheral blood leukocytes, digested with *Taq* I and *Bgl* II, and analyzed by Southern blot hybridization (11) using cloned human tyrosinase cDNA (5) as probe. Autoradiograms were scored for polymorphic 2.8-kilobase (kb) versus 2.4-kb *Taq* I tyrosinase gene fragments (12) and 5.8-kb versus 5.6-kb *Bgl* II tyrosinase gene fragments (13), and *Taq* I/*Bgl* II haplotypes were assigned based on phase relationships determined from the pedigrees. Haplotype 1: *Taq* I, 2.8 kb; *Bgl* II, 5.8 kb. Haplotype 2: *Taq* I, 2.4 kb; *Bgl* II, 5.6 kb. Haplotype 3: *Taq* I, 2.4 kb; *Bgl* II, 5.8 kb. Haplotype 4: *Taq* I, 2.8 kb; *Bgl* II, 5.6 kb. Linkage between the tyrosinase RFLP haplotypes and type I OCA was assessed by determination of logarithm of odds (lod) scores using version 3 (1986) of the LIPED program (14).

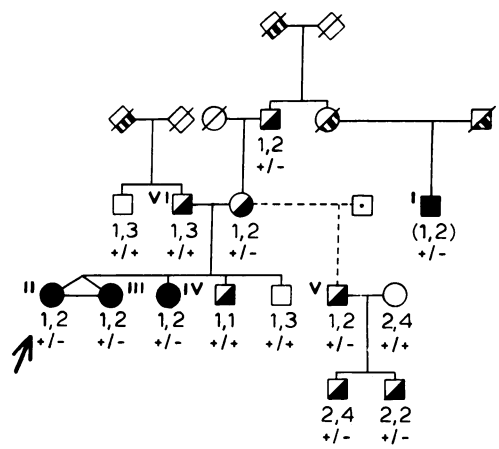

FIG. 1. Pedigree of family 1. *Taq* I/*Bgl* II RFLP haplotypes are indicated (see text). Parentheses indicate an individual in whom the linkage phase of the *Taq* I and *Bgl* II RFLPs could not be inferred; the most probable haplotypes are shown. Arrow denotes the proband. Paternity testing (18) of individual V, performed because of his seemingly discrepant data, excluded individual VI as his father. Solid symbols, individuals with OCA; half-solid symbols, OCA heterozygotes (obligate and/or ascertained by hairbulb tyrosinase assay); hatched symbols, presumed OCA heterozygotes; open symbol with dot, heterozygote status indeterminate. *Hae* III codon 81 mutation analysis: +, normal allele; −, codon 81 mutant allele. Roman numerals denote individuals described in the text.

Polymerase Chain Reaction (PCR) Amplification and Sequencing of Genomic DNA. Genomic mapping (not shown) demonstrated that the human tyrosinase gene contains four intervening sequences, with exon–intron boundaries identical to the mouse gene (15). Each of the five exons of the human tyrosinase gene was amplified independently from genomic DNA (0.1–1 μg) from the probands of families 1 and

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Abbreviations: OCA, oculocutaneous albinism; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; lod, logarithm of odds.

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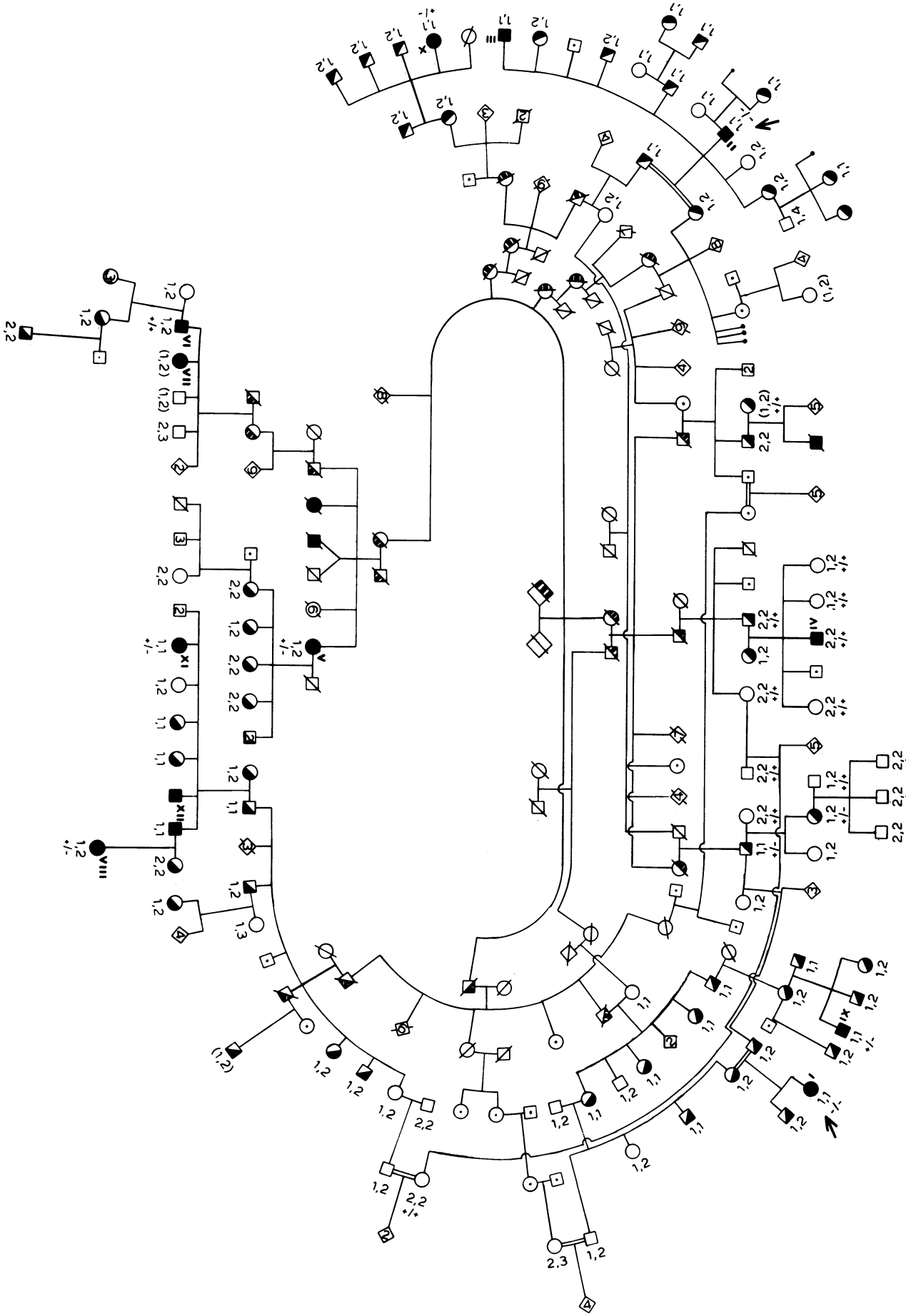

FIG. 2. Pedigree of family 2. Details are as given in Fig. 1.

2 by 40 cycles of PCR (16) by using 19- or 20-mer oligonucleotide primers (see Fig. 3), *Thermus aquaticus* (*Taq*) DNA polymerase (Perkin–Elmer/Cetus), and an automated thermal cycler (Coy Laboratory Products, Ann Arbor, MI). Each cycle consisted of 1.5 min each at 94, 50, and 72°C. For family 1, the amplified PCR products were purified by electrophoresis in 1.5% agarose gels, isolated using GeneClean (Bio 101, La Jolla, CA), and the DNA sequences of the double-stranded PCR products were determined completely on both strands by using the products of at least two independent PCR reactions, essentially by the method of Sanger *et al.* (17). PCR fragments containing exons 1, 2, 3, and 5 were also gel purified on 4% polyacrylamide gels and subcloned into bacteriophage vector M13mp18 or M13mp19. The nucleotide sequences of at least six independent subclones per PCR fragment were then determined (17). For family 2, PCR fragments containing all five exons were purified, subcloned into M13, and sequenced as for family 1.

RESULTS

Genetic Linkage Analysis. Tyrosinase haplotypes were assigned by using *Taq* I and *Bgl* II tyrosinase gene RFLPs (12, 13). The frequency of the four *Taq* I/*Bgl* II tyrosinase gene haplotypes (see *Materials and Methods*) among 40 unrelated normal individuals was 42.5% for haplotype 1, 45% for haplotype 2, 7.5% for haplotype 3, and 5% for haplotype 4. The polymorphic *Taq* I site is located within intervening sequence 1 of the tyrosinase gene, within 7.5 kb of the polymorphic *Bgl* II site. We have never observed recombination between the polymorphic *Taq* I and *Bgl* II sites.

Family 1 (Fig. 1) is of English descent and has been described previously (19). One affected individual (I) has typical tyrosinase-negative (type IA) OCA. In a different sibship within the family, three sisters (individuals II, III, and IV) are affected with a milder (type IB) form of OCA and are apparently compound heterozygotes for type IA and type IB OCA alleles.

Genetic linkage analysis of family 1 using the *Taq* I and *Bgl* II tyrosinase gene RFLPs demonstrated complete linkage between OCA and the tyrosinase gene (lod score = 1.50; θ = 0). The common familial type IA OCA allele segregates with a tyrosinase *Taq* I/*Bgl* II haplotype 2 allele, and the type IB OCA mutation segregates with a haplotype 1 allele.

Family 2 (Fig. 2) is unrelated to family 1 and consists of 10 sibships with various forms of OCA. Most family members are descended from two individuals who migrated from England in 1841; their descendants now number ≈10,000–25,000 individuals. Individuals I and II were independently ascertained as probands and were subsequently determined to be related. Individuals I–VIII are affected with typical tyrosinase-negative (type IA) OCA. Individuals I, II, and III are all apparently homozygous by descent for the common familial type IA OCA allele, and all three have identical OCA phenotypes. In addition, several other tyrosinase-deficient OCA alleles appear to be segregating in this kindred. Individuals IX and X are affected with two quite different type IB phenotypes, severe and mild, respectively, and presumably are compound heterozygotes for the common familial type IA allele and two different type IB OCA alleles. Individuals XI and XII exhibit a novel type I OCA phenotype, in which the distribution of pigment is related to local body temperature. They appear to be compound heterozygotes for the common familial type IA OCA allele and an OCA allele that produces a temperature-sensitive form of tyrosinase (R.A.K. and R.A.S., unpublished data), similar to Himalayan mice and Siamese cats.

Genetic linkage analysis of family 2 demonstrated complete linkage between the common familial type IA OCA allele and the tyrosinase gene RFLPs (lod score = 6.17; θ = 0). The common familial type IA OCA allele segregates with a tyrosinase *Taq* I/*Bgl* II haplotype 1 allele in this kindred.

Nucleotide Sequence Analysis of the Type IA OCA Tyrosinase Alleles. Southern blot hybridization analysis of the tyrosinase genes of all of the probands described here showed no detectable deletions or rearrangements (data not shown). Therefore, tyrosinase gene segments were amplified by PCR and sequenced from probands from both families. As shown in Fig. 3, exon 1, 2, and 5 primers were derived from the published cDNA sequences (6) and thus amplify only the respective exon sequences. Exon 3 and 4 primers were derived both from the exons and also from the sequences of the adjacent introns (L.B.G., K.M.S., and R.A.S., unpublished data) and thus amplified the exons plus portions of the adjacent introns.

Individual II of family 1 is an apparent type IA/IB OCA compound heterozygote (19). In our sequence analysis of her tyrosinase genes, we found only a single base difference from that of normal human tyrosinase cDNA (6). This change, within codon 81 (CCT → CTT) results in a proline → leucine substitution at this site (Fig. 4). As expected, the proband was heterozygous for this substitution; her other tyrosinase allele contained no abnormalities within the coding region, suggesting that the defect in that allele is either in a noncoding region or is in the first or last 20 bases of exon 1, 2, or 5, which comprised the primers used in our PCR analyses and thus could not be analyzed.

The codon 81 substitution abolishes an *Hae* III restriction site within exon 1. Therefore, we were able to rapidly screen individuals for this substitution by PCR amplification of exon 1 followed by *Hae* III cleavage. Analysis of all individuals of family 1 confirmed the association of the codon 81 mutation with *Taq* I/*Bgl* II haplotype 2 in this family (see Fig. 1). The codon 81 substitution in family 1 is associated with TCT (serine) at codon 192, which exhibits a common nonpathologic polymorphism (serine vs. tyrosine) among normal tyrosinase alleles in Caucasians (20).

Sequence analysis of the tyrosinase genes of individual I of family 2, who is homozygous for the common familial type IA OCA allele, also demonstrated only a single base difference from normal human tyrosinase cDNA (6), the same codon 81 CCT → CTT substitution, although in family 2 it is associated with TAT (tyrosine) at the polymorphic codon 192. *Hae* III analysis of exon 1 PCR products from members of family 2 showed that individuals I, II, and III are all homozygous for this allele; that individuals V, VIII, IX, X, XI, and XII are

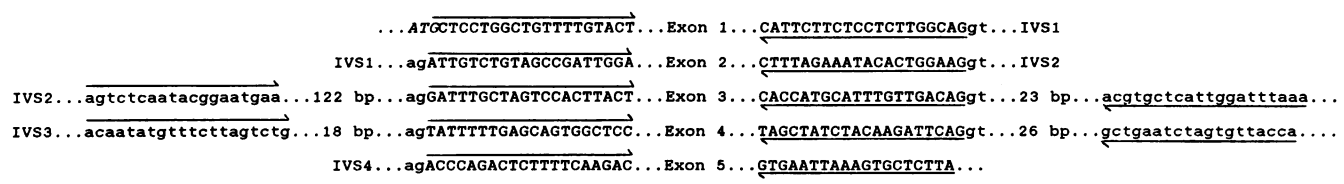

FIG. 3. PCR oligonucleotide primers. The rightward arrows indicate the 5′ PCR primers, and the leftward arrows indicate the sequences from which the 3′ PCR primers were derived; the reverse complements of these sequences were actually used as 3′ primers. Uppercase letters indicate exon sequences, and lowercase letters indicate intron sequences determined by analysis of genomic human tyrosinase clones (L.B.G., K.M.S., and R.A.S., unpublished data). The translational initiation codon in exon 1 is italicized. IVS, intervening sequence; bp, base pairs.

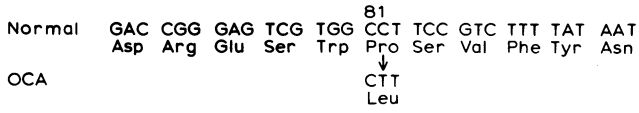

FIG. 4. Sequences in the region of the codon 81 mutation.

compound heterozygotes for this and different mutant tyrosinase alleles; and that individuals IV, VI, and VII, although they have type IA OCA, do not carry the codon 81 mutant allele at all (see Fig. 2).

At least 10 unrelated heterozygotes for type I OCA apparently introduced at least five, but possibly more, different type I OCA alleles into family 2. This number of type I OCA heterozygotes is consistent with their estimated frequency of 1% (1, 2), given the very large number of individuals (10,000–25,000) in this kindred, only a fraction of which is illustrated in Fig. 2.

Frequency of the Codon 81 Mutation Among OCA Alleles. To determine whether the codon 81 mutation is simply a common nonpathologic polymorphism, we screened 25 unrelated normal individuals of northern European Caucasian ethnic origin by *Hae* III cleavage analysis of tyrosinase exon 1 PCR fragments (data not shown). None of their 50 tyrosinase alleles contained the codon 81 mutant allele. To determine the frequency of the codon 81 substitution among type I OCA tyrosinase alleles, we also performed *Hae* III screening (data not shown) on DNAs from a total of 15 independently ascertained type I OCA probands of northern European ethnic origin: individual II from family 1, individuals I and II from family 2, and 12 unrelated individuals with type IA OCA. We detected the codon 81 mutant allele in one additional family; the proband with type IA OCA is heterozygous for this allele. Altogether, we detected the codon 81 mutant allele in 4 of the 15 independently ascertained type I OCA probands; of their 30 total OCA tyrosinase alleles, 6 contained the codon 81 mutation, yielding an overall frequency of this allele among these type I OCA probands of 0.2.

DISCUSSION

Patients with classic type IA OCA have no detectable activity of tyrosinase, the first enzyme in the melanin biosynthetic pathway, and thus produce no melanin pigment. We first demonstrated complete genetic linkage between type IA OCA and the tyrosinase gene in two families (lod score = 7.67; $\theta = 0$). We then identified a tyrosinase gene mutation in these families, at codon 81 (CCT → CTT), that results in a proline → leucine substitution at this site. Individuals homozygous for this substitution have typical type IA OCA, with no pigment and no detectable tyrosinase activity in anagen hairbulbs. We detected the codon 81 mutation in 6 of 30 total OCA alleles (20%) among the 15 independently ascertained type I OCA probands that we tested but not in any of 50 normal tyrosinase alleles from 25 unrelated normal individuals ($P = 0.002$ by Fisher's exact test). Thus, the codon 81 proline → leucine substitution is not simply a common nonpathologic polymorphism but instead is apparently causative for type IA OCA. The codon 81 proline → leucine substitution does not occur at any of the putative signal peptide, transmembrane, copper-binding, or glycosylation sequences within the tyrosinase polypeptide (5–8), and so the reason for its deleterious effect is not apparent. However, proline-81 is conserved between human (5, 6) and mouse (7, 8, 15) tyrosinases, suggesting that it may be important for function.

In the two families studied here, the codon 81 substitution was associated with two different *Taq* I/*Bgl* II RFLP haplotypes, haplotypes 1 and 2. This is surprising because the polymorphic *Taq* I site is located within intervening sequence 1 only 1.8 kb 3′ to codon 81. Furthermore, the codon 81 mutant alleles of the two families also differ from each other at codon 192 (serine vs. tyrosine), a common nonpathologic polymorphism in Caucasians (20) located only 333 bases 3′ to the codon 81 substitution. Thus, either there has been genetic recombination or conversion within this 333-base region or else the codon 81 substitutions on the tyrosinase alleles in the two families originated independently. At average rates, the probabilities of either recombination in such a small region or of recurrent mutation are both exceedingly small. This suggests that either the codon 81 mutation is very ancient or the rate of recombination or mutation at this site is relatively high.

The recent isolation of human tyrosinase cDNAs has made possible molecular analysis of abnormal tyrosinase genes from OCA patients. During the preparation of this report, Tomita *et al.* (21) described a tyrosinase gene frameshift mutation in two unrelated Japanese patients with type IA OCA. Whereas that allele does not appear to be frequent among OCA patients of European Caucasian ethnic origin, the codon 81 mutation described here is a frequent OCA allele in this ethnic group. Both should prove useful for improved carrier detection and possible prenatal diagnosis of this important genetic disorder.

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