Tyrosinase Gene Mutations Associated with Type IB ("Yellow") Oculocutaneous Albinism

Lutz B. Giebel,* Ram K. Tripathi,* Kathleen M. Strunk,* Jon M. Hanifin,† Charles E. Jackson,‡ Richard A. King,§ and Richard A. Spritz*

*Departments of Medical Genetics and Pediatrics, University of Wisconsin, Madison; †Department of Dermatology, Oregon Health Sciences University, Portland; ‡Division of Clinical and Molecular Genetics, Department of Medicine, Henry Ford Hospital, Detroit; and §Department of Medicine and Institute of Human Genetics, University of Minnesota, Minneapolis

Summary

We have identified three different tyrosinase gene mutant alleles in four unrelated patients with type IB ("yellow") oculocutaneous albinism (OCA) and thus have demonstrated that type IB OCA is allelic to type IA (tyrosinase negative) OCA. In an inbred Amish kindred, type IB OCA results from homozygosity for a Pro→Leu substitution at codon 406. In the second family, type IB OCA results from compound heterozygosity for a type IA OCA allele (codon 81 Pro→Leu) and a novel type IB allele (codon 275 Val→Phe). In the third patient, type IB OCA results from compound heterozygosity for the same type IB allele (codon 275 Val→Phe) and a novel type IB OCA allele. In a fourth patient, type IB OCA results from compound heterozygosity for the codon 81 type IA OCA allele and a type IB allele that contains no identifiable abnormalities; dysfunction of this type IB allele apparently results from a mutation either well within one of the large introns or at some distance from the tyrosinase gene. In vitro expression of the Amish type IB allele in nonpigmented HeLa cells demonstrates that the Pro→Leu substitution at codon 406 greatly reduces but does not abolish tyrosinase enzymatic activity, a finding consistent with the clinical phenotype.

Introduction

Human oculocutaneous albinism (OCA) is a heterogeneous group of autosomal recessive genetic disorders of pigmentation. Affected individuals are characterized by reduced or absent melanin synthesis in pigment cells of the skin, hair follicles, and eyes. Hypopigmentation of the retina is associated with decreased visual acuity, photophobia, and varying degrees of nystagmus (reviewed by King and Summers 1988; Witkop et al. 1989). Type I OCA results from deficient activity of melanocyte tyrosinase (E.C.1.14.18.1), the enzyme that catalyzes the first two steps in the melanin biosynthetic pathway: (1) the hydroxylation of tyrosine to dihydroxyphenylalanine (dopa) and (2) the

subsequent oxidation of dopa to dopaquinone (Lerner and Fitzpatrick 1950). In classic, type IA (tyrosinase-negative) OCA, tyrosinase activity and melanin biosynthesis are entirely absent, whereas, in type IB OCA, tyrosinase activity and melanin production are greatly reduced.

Type IB ("yellow") OCA was first described in an inbred Amish kindred (Nance et al. 1970) and was later identified in other populations. Patients with type IB OCA completely lack detectable pigment at birth and initially are indistinguishable from patients with classic, type IA OCA. However, patients with type IB OCA accumulate some melanin pigment, principally the yellow-red pheomelanins, during childhood and adulthood. The activity of hairbulb tyrosinase is greatly reduced in type IB OCA, and the existence of apparent type IA/IB OCA compound heterozygotes suggests that type IA and type IB OCA are allelic (Hu et al. 1980; Giebel et al. 1990). Cloning and sequence analysis of the human tyrosinase gene, located in chromosome segment 11q14→q21 (Barton et al. 1988), has permitted the recent identification of several different

tyrosinase gene mutations in patients with type I OCA (Tomita et al. 1989; Giebel et al. 1990, 1991b, and in press; Kikuchi et al. 1990; Spritz et al. 1990, 1991; Takeda et al. 1990). In the present paper we directly demonstrate that types IA and IB OCA are allelic and describe two novel missense mutations of the tyrosinase gene in four patients with type IB OCA. Furthermore, we show that the "Amish" type IB OCA mutation results in a >90% reduction in tyrosinase enzymatic activity in HeLa cells transfected with the mutant gene, as compared with that in cells transfected with the normal gene. This correlates well with the accumulation of a small amount of melanin pigment by individuals homozygous for this allele.

Patients and Methods

Patients

Patient 1 (fig. 1A) was an adult Caucasian male, one of several members of an inbred Amish kindred with OCA type IB. This is the same kindred reported in the original description of type IB OCA (Nance et al. 1970); patient 1 is individual VII.9 in that report. Note that considerable dark eumelanin pigment has accumulated in his scalp and facial hair over time.

Patient 2 (fig. 1B) was an adult Caucasian female with typical features of OCA type IB. Detailed clinical descriptions and genetic analyses of her family have also been published previously (Hu et al. 1980; Giebel et al. 1990); patient 2 is patient 11 of family 1 in Giebel et al. (1990). Note that a moderate amount of yellow-red pheomelanin has accumulated in her scalp and facial hair over time.

Patient 3 (fig. 1C) was a 19-year-old Caucasian male with severe type IB OCA. There was very little detectable melanin pigment in his skin, hair, and eyes. His irides were pale blue and transilluminated completely, and his retinas had almost no detectable pigment. The visual acuity was greatly reduced, with foveal hypoplasia, constant nystagmus, and photophobia. A small amount of brown eumelanin pigment was evident in both his facial hair and the hair on his extremities.

Patient 4 (fig. 1D) was a 15-year-old Caucasian male with typical type IB OCA. An extensive study of his very large family has been published (Giebel et al. 1990); patient 4 is patient IX of family 2 in that same report. Small to moderate amounts of both yellow pheomelanin and dark eumelanin pigment have accumulated in his scalp and facial hair over time.

Quantitative assay of tyrosinase (tyrosine hydroxylase) in freshly epilated anagen hairbulbs (Kind and

Olds 1985) revealed no detectable activity, except for patient 3, in whom tyrosinase activity was 0.255 pmol tyrosine oxidized/120 min/hairbulb (normal range for blond individuals 1.50 ± 0.85; $n = 13$).

Analysis of Genetic Linkage

DNA was isolated from peripheral blood leukocytes of patient 1 and many members of his Amish kindred (fig. 2), was digested with TaqI, and was analyzed by Southern blot hybridization (Southern 1975) using cloned human tyrosinase cDNA as probe (Kwon et al. 1987). Autoradiograms were scored for polymorphic 2.8-kb versus 2.4-kb TaqI tyrosinase gene fragments (Spritz et al. 1988), and TaqI haplotypes were assigned to each individual. The OCA carrier status of all family members was defined by quantitative assay of tyrosinase (tyrosine hydroxylase) activity in anagen hairbulbs (King and Olds 1985). Linkage between the tyrosinase TaqI RFLP and type IB OCA was assessed by determination of logarithm-of-odds (lod) scores by using version 3 (1987) of the LIPED program (Ott 1974). Tyrosinase gene RFLP linkage analyses of the families of patients 2 and 4 have been published previously (Giebel et al. 1990).

PCR and DNA Sequencing

DNA fragments corresponding to the five exons plus adjacent flanking regions of the human tyrosinase gene (Giebel et al. 1991a) were PCR amplified from 0.1–1.0 µg DNA of the four patients and were gel purified, cloned into bacteriophage vectors M13mp18 or mp19, and sequenced, exactly as described elsewhere (Spritz et al. 1991).

Allele-specific Oligonucleotide Hybridization

Tyrosinase exon 2 gene fragments were PCR amplified from genomic DNA of OCA patients, their family members, and 30 unrelated normally pigmented individuals, exactly as described elsewhere (Spritz et al. 1991). Twenty microliters of each PCR reaction was transferred to MAGNA nylon membranes (Micron Separations) with a Bio-Dot SF microfiltration device (Bio-Rad). Allele-specific oligonucleotide hybridizations of replicate filters were performed (Kogan and Gitschier 1990) by using 5'-radiolabeled 19-mer oligonucleotides corresponding to the normal (5'-ATTGTC-TGTAGCCGATTGG-3') and mutant (5'-ATTTTC-TGTAGCCGATTGG-3')– forms of codon 275.

Restriction-Enzyme Cleavage Analysis

Tyrosinase exon 4 gene fragments of patient 1,

Figure 1   Photographs of four patients with type IB OCA. *A*, Patient 1. *B*, Patient 2. *C*, Patient 3. *D*, Patient 4.

many members of his kindred (fig. 2), and four unrelated patients with type I OCA were PCR amplified exactly as described elsewhere (Spritz et al. 1991). Amplification products were digested with *Mnl*I and were analyzed by PAGE for the presence of the codon 406 mutation.

Site-directed Mutagenesis and In Vitro Expression of Cloned Tyrosinase cDNAs

The codon 406 CCT (Pro)→CTT (Leu) IB OCA mutation was introduced into the human tyrosinase cDNA expression plasmid pcTYR (Bouchard et al.

A

B
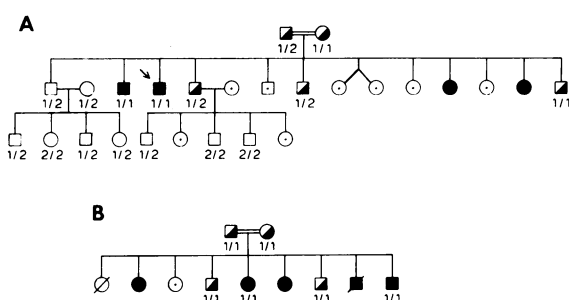
Figure 2   Pedigrees of two portions of Amish kindred with type IB OCA. *Taq*I RFLP haplotypes are indicated: haplotype 1 is a 2.8-kb polymorphic fragment, and haplotype 2 is a 2.4-kb fragment. The arrow denotes patient 1. A more complete pedigree of the extended kindred has been published by Nance et al. (1970).

1989) according to a method described elsewhere (Giebel and Spritz 1990*b*). Exon 4 of the tyrosinase gene was PCR amplified from DNA (30 ng) of an M13 exon 4 clone containing the codon 406 mutation, by using 20-mer oligonucleotide primers (5'-TATTTT-TGAGCAGTGGCTCC-3' and 5'-TAGCTATCT-ACAAGATTCAG-3'). PCR conditions were as described elsewhere (Giebel and Spritz 1990*b*), except that (*a*) only 25 cycles were performed and (*b*) the annealing step was two min. An exon 2-3-4 product was then amplified from 30 ng of tyrosinase cDNA by using as one primer 5 pmol gel-purified mutant exon 4 PCR product and as second primer a 20-mer oligonucleotide (5'-ATTGTCTGTAGCCGATTGGA-3') derived from the 5' end of exon 2. The resultant mutant exon 2-3-4 PCR product contained the codon 406 mutation and unique *Pvu*II and *Bgl*II sites; these sites were used to replace the *Pvu*II/*Bgl*II fragment of pcTYR by the corresponding codon 406 mutant fragment. The nucleotide sequence of the mutant expression plasmid pcTYR-T406 was verified by double-stranded DNA sequencing (Zhang et al. 1988).

Expression of tyrosinase and chloramphenicol acetyltransferase (CAT) was assayed by transient cotransfection of either pcTYR or pcTYR-T406 plus pSV2CAT into cultured HeLa cells by using the calcium phosphate precipitation procedure (Gorman et al. 1982). Cells were harvested 64 h after transfection, were lysed in 20 mM sodium phosphate buffer pH 6.8, 0.5% Triton X-100, and were sonicated. After centrifugation the supernatant was dialyzed twice against 5 mM sodium phosphate buffer, pH 6.8. Fluorometric assay of cell extracts for tyrosinase (tyrosine hydroxylase) activity was done according to a method described elsewhere (Husain et al. 1982), except that

4 mM ascorbic acid was included as reductant for tyrosine hydroxylation (Tripathi et al. 1988) and to reduce any dopaquinone formed by tyrosinase back to dopa; thus, measurement of amount of dopa formed provides a true estimate of the tyrosine hydroxylase activity of tyrosinase. The 55-μl reactions containing 0.1 mM L-tyrosine, 5 μM L-dopa, 4 mM ascorbic acid, 40 mM sodium phosphate buffer pH 6.8, and 15 μl cell extract were incubated for 4 h. Production of dopa was measured by specific fluorescence at 360-nm excitation and 490-nm emission wavelengths. Protein concentrations of cell extracts were determined according to the method of Peterson (1977) and CAT activities were determined according to the method of Gorman et al. (1982).

Results

Type IB OCA Is Linked to the Tyrosinase Gene

*Taq*I digests were used to assign tyrosinase RFLP haplotypes to patient 1 and other members of the original Amish kindred with type IB OCA (fig. 2). Genetic linkage analysis of this family demonstrated linkage between OCA and the tyrosinase gene (lod score = 1.07, θ = 0). Although part of this kindred was relatively uninformative (fig. 2*B*), the OCA IB trait cosegregated with the 2.8-kb polymorphic *Taq*I tyrosinase gene fragment; all individuals studied in the Amish kindred with homozygous type IB OCA were homozygous for the 2.8-kb *Taq*I fragment.

We have previously published genetic linkage analyses of the families of patients 2 and 4 (Giebel et al. 1990) and have shown that in both families the type I OCA mutations are linked to the tyrosinase gene. Lod scores in these two families were 1.50 (θ = 0) and 6.17 (θ = 0), respectively. In both families individuals with type IB OCA were apparent compound heterozygotes for a type IB OCA allele and a frequent type IA OCA allele, containing a Pro→Leu substitution at codon 81 (Giebel et al. 1990).

Patient 1 Is Homozygous for a Novel Missense Mutation at Codon 406
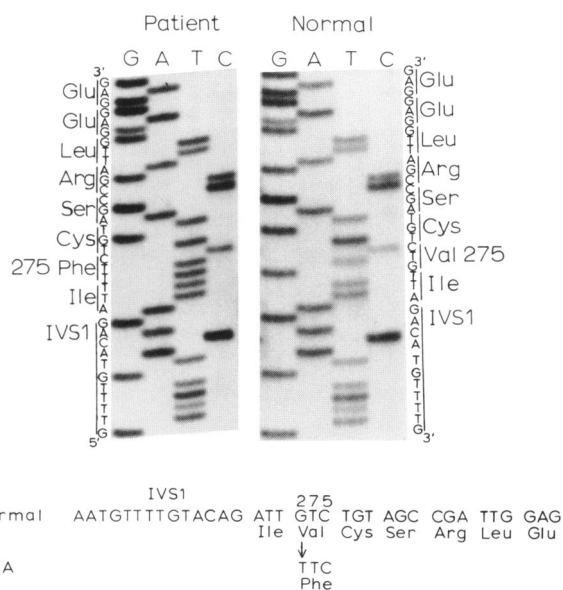
To identify mutations that cause type IB OCA, we PCR amplified each of the five exons of the tyrosinase gene plus adjacent noncoding sequences from genomic DNA of each of the four patients by using oligonucleotide primers derived from the normal tyrosinase gene sequence (Giebel et al. 1991*a*). Amplification products were cloned in M13 vectors, and their DNA sequences were determined.
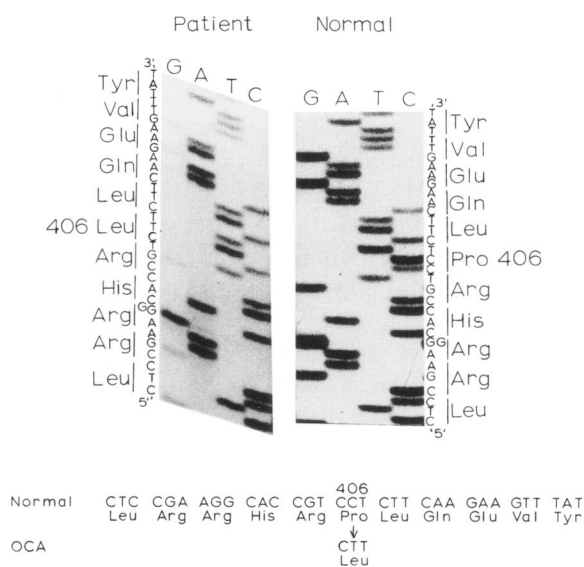
Figure 3 Tyrosinase gene sequences in region of codon 406 mutation of patient 1. The sequences shown are those of the coding strand.

Figure 4 Tyrosinase gene sequences in region of codon 275 mutation of patients 2 and 3.

Analysis of the tyrosinase gene sequence of patient 1 revealed that he is homozygous for a novel missense substitution, CCT (Pro)→CTT (Leu), at codon 406 (fig. 3). No other abnormalities were found. At two nonpathological polymorphic sites within the tyrosinase-coding sequence, codon 192 TCT (Ser) versus TAT (Tyr) (Giebel and Spritz 1990*a*) and codon 402 CGA (Arg) versus CAA (Gln) (Tripathi et al., in press), patient 1 was homozygous, having TAT (Tyr) and CGA (Arg), respectively. Both of these polymorphisms are commonly found among normal individuals.

The codon 406 CCT→CTT substitution abolishes an *Mn*lI restriction site, enabling us to easily test whether this substitution is a type IB OCA mutation or a nonpathologic polymorphism. Exon 4 tyrosinase gene fragments were amplified from genomic DNAs of all members of the Amish kindred shown in figure 2, were digested with *Mn*lI, and were analyzed by gel electrophoresis (data not shown). The distribution of the codon 406 substitution correlated perfectly with carrier status as determined by assay of tyrosinase enzymatic activity and RFLP data. All individuals with type IB OCA were homozygous for the codon 406 mutation; all OCA carriers were heterozygous for the codon 406 mutant allele; and all of the noncarriers had only the normal allele. We also analyzed the DNAs of 14 unrelated patients with type I OCA, including patients 2–4 of the present study; none had the codon

406 mutant allele. We have also not detected the codon 406 substitution by DNA sequence analyses of 16 additional unrelated type IA OCA patients and two normal individuals. Therefore, the codon 406 substitution is not a common polymorphism but instead appears to be a private type IB OCA mutation in the Amish.

Patients 2 and 3 are Compound Heterozygotes for a Novel Missense Substitution at Codon 275 and for Different Type IA OCA Mutations

The nucleotide sequence of tyrosinase gene fragments of patient 2 showed her to be a compound heterozygote for two different OCA alleles, each containing a different missense substitution. One allele, containing a CCT (Pro)→CTT (Leu) substitution at codon 81, is a relatively frequent type IA OCA allele (Giebel et al. 1990; authors' unpublished data). The patient's type IB OCA allele contains a novel substitution, GTC (Val)→TTC (Phe) at codon 275 (fig. 4). No other abnormalities were found. Patient 2 was homozygous for TCT (Ser) at polymorphic codon 192 and for CGA (Arg) at polymorphic codon 402.

Tyrosinase gene DNA sequence analysis of patient 3 showed that he is also a compound heterozygote, for the same codon 275 Pro→Leu type IB OCA mutant allele (fig. 4) and a novel allele, containing a frameshift (CGT→CCGT) at codon 501 (fig. 5). The frameshift
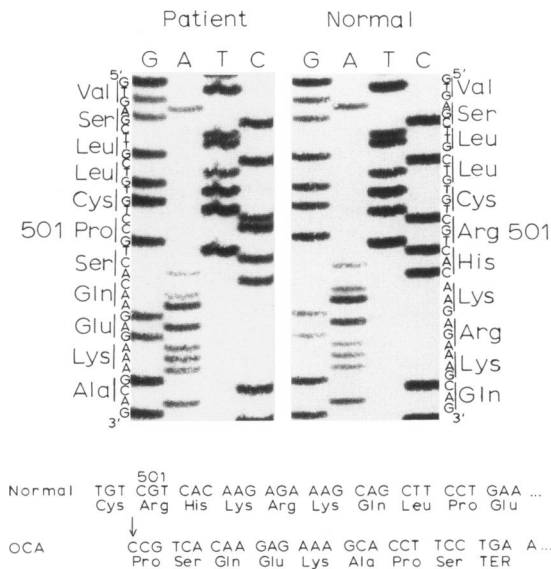
Figure 5   Tyrosinase gene sequences in region of codon 501 mutation of patient 3.

at codon 501 results in alteration or deletion of the 29 carboxyl-terminal residues of tyrosinase; therefore, this is most likely a type IA OCA allele. No other abnormalities were found. Patient 3 was homozygous for TCT (Ser) at polymorphic codon 192 and was heterozygous CGA (Arg)/CAA (Gln) at polymorphic codon 402.

To determine whether the codon 275 tyrosinase gene substitution is causative of type IB OCA or is simply a nonpathologic polymorphism, we PCR amplified exon 2 gene fragments from DNA of patient 2, her entire family (see fig. 1 in Giebel et al. 1990), and 30 unrelated normal individuals. The exon 2 amplification products were then analyzed by allele-specific oligonucleotide hybridization (data not shown). The distribution of the codon 275 substitution correlated perfectly with tyrosinase enzymatic activity and RFLP data. All three individuals with type IB OCA in this family were compound heterozygotes for the codon 275 and codon 81 mutant alleles; all type IB OCA carriers were heterozygous for only the codon 275 mutant allele; and all of the noncarriers and the 30 unrelated normal individuals had only normal alleles.

The Type IB OCA Mutant Allele of Patient 4 Contains No Identifiable Abnormality

Previous family studies involving patient 4 indicated that he is a compound heterozygote for the codon 81 Pro→Leu IB OCA mutant allele and an unknown type

IB OCA allele (Giebel et al. 1990). Nucleotide sequence analysis of the tyrosinase genes of patient 4 confirmed this, demonstrating the codon 81 substitution in one of his two alleles. However, no abnormality could be identified in the other allele, including in the five exons, 39–87 bases of the adjacent intervening sequences, 693 bases of the 5' promoter region, and 388 bases of the 3' flanking region. These data suggest that the type IB OCA mutation in patient 4 may be either deep within one of the four large intervening sequences or at some distance from the tyrosinase gene. This apparent type IB OCA allele is associated with TAT (Tyr) at polymorphic codon 192 and with CAA (Gln) at polymorphic codon 402.

The Amish Codon 406 Pro→Leu Substitution Drastically Reduces Tyrosinase Enzymatic Activity

To investigate the effect of the Amish codon 406 CCT (Pro)→CTT (Leu) type IB OCA mutation, we introduced this substitution into the human tyrosinase cDNA expression plasmid pcTYR (Bouchard et al. 1989) by site-directed in vitro mutagenesis. The wild-type (pcTYR) and the mutant tyrosinase (pcTYR-T406) expression plasmids were each transiently cotransfected along with pSV2CAT into cultured HeLa cells. The cells were harvested 64 h after transfection, and tyrosinase (tyrosine hydroxylase) activity, chloramphenicol acetyltransferase (CAT) activity, and protein concentrations were determined (table 1). The CAT activity served as an internal control for transfection efficiency. As shown in table 1, the tyrosinase activity in HeLa cells transfected with the codon 406 Pro→Leu mutant tyrosinase expression plasmid was only ~7% of the activity in cells transfected with the wild-type plasmid.

It is interesting that, in general, the amount of pigment apparent in the four type IB OCA patients in the present study correlates only modestly well with their genotypes. Patient 1, who has the most apparent melanin pigment (fig. 1A), is homoallelic for type IB OCA, whereas patients 2–4, who all have considerably less melanin pigment (fig. 1B–D), are all type IA/IB compound heterozygotes. The pigmentation phenotype of type IB OCA is quite homogeneous within sibships (family 1 and family 2). However, there can be considerable phenotypic differences among unrelated type IB OCA patients, even among those with quite similar genotypes, and significant age-related accumulation of predominantly yellow-red pheomelanin is not a constant feature. In addition, the clinical phenotype among patients with type IB OCA does not correlate

Table I

Activities of Normal and Codon 406 Pro→Leu
Mutant Tyrosinases in Transfected
HeLa Cells

Plasmid[a]	Tyrosinase Specific Activity[b]
pcTYR........................	5.6 ± .3 (100 ± 5.4%)
pcTYR-T406...............	.4 ± .1 (7.1 ± 1.8%)

[a] pcTYR is the expression plasmid containing the normal human tyrosinase cDNA; pcTYR-T406 is the corresponding plasmid containing the codon 406 Pro→Leu substitution.

[b] Each experiment was performed in triplicate; the value presented is the average value for the three plates. 1 unit of tyrosinase activity was defined as 1 pmol dopa formed/min. The tyrosinase specific activities shown were corrected for slight plate-to-plate variation in transfection efficiency, by dividing by the CAT activity (percentage of ^{14}C-chloramphenicol converted to the two forms of acetyl-chloramphenicol) in the extract of each plate of transfected HeLa cells.

well with measured levels of hairbulb tyrosinase activity, the assay for which is unreliable toward the low end of its range. Patients 2 and 3 are both type IA/IB compound heterozygotes and share the same codon 275 Val→Phe type IB OCA allele (fig. 4). However, patient 2 (fig. 1B) has much more apparent pigment than does patient 3 (fig. 1C), who appears almost as severely hypopigmented as do patients with type IA OCA, despite having the highest hairbulb tyrosinase activity among the patients studied here, at the low end of the normal range. These differences of pigmentation phenotypes, along with poor correlation between clinical phenotype and measured tyrosinase enzymatic activity in type IB OCA, most likely result from epistatic phenomena acting on a background of low residual tyrosinase activity. By way of illustration, we note that the normally pigmented parents and sibs of patient 2 are very darkly complected, whereas those of patient 3 are very lightly complected; therefore, in the absence of OCA, patient 2 might otherwise have been darkly complected, whereas patient 3 might otherwise have been lightly complected.

We have shown that the phenotypes of both type IA OCA and type IB OCA result from homozygosity or compound heterozygosity for a series of allelic mutations at the human tyrosinase locus. This will greatly enhance genetic counseling for the various forms of type I OCA. The occurrence of the codon 275 mutant allele in both proband 2 and proband 3, who are unrelated, suggests that this may be a frequent allele in patients with type IB OCA. Furthermore, the availability of carrier detection for the Amish codon 406

mutant allele offers obvious advantages for genetic counseling of this high-risk population.

Discussion

Type IB OCA was first described by Nance et al. (1970) in an inbred Amish kindred. Tyrosinase enzymatic activity is decreased or undetectable, respectively, in type IB OCA and type IA OCA, and the observation of families with apparent type IA/IB OCA compound heterozygotes strongly suggested that these two forms of OCA are allelic (Hu et al. 1980; Giebel et al. 1990). Recent advances in recombinant-DNA technology made it possible to investigate the molecular relationship between type IA OCA and type IB OCA. In the present study we have considered four unrelated patients with type IB OCA, including one of the original inbred Amish kindred described by Nance et al. (1970). Tyrosinase gene RFLP linkage analyses were consistent with allelism of types IA and IB OCA. Nucleotide sequence analysis of the tyrosinase genes of patient 1, the Amish patient, demonstrated that he is homozygous for a novel Pro→Leu substitution at codon 406 (fig. 3). Patient 2 was a compound heterozygote for a previously published (Giebel et al. 1990) type IA OCA allele, i.e., codon 81 Pro→Leu, and a novel allele containing a type IB OCA substitution, i.e., codon 275 Val→Phe (fig. 4). Patient 3 was a compound heterozygote for the same codon 275 Val→Phe type IB OCA allele and a novel type IA OCA allele, containing a frameshift at codon 501. Patient 4 was also apparently a compound heterozygote, for the codon 81 Pro→Leu type IA OCA allele and a tyrosinase allele in which we detected no abnormality. It is likely that this allele contains a mutation either well within one of the four large intervening sequences or at some distance from the tyrosinase structural gene. Thus, at least three different tyrosinase alleles can be associated with type IB OCA.

We tested the effect that the Amish codon 406 Pro→ Leu substitution had on tyrosinase enzymatic activity, by expressing a tyrosinase cDNA containing this mutation in HeLa cells. We found that the codon 406 substitution greatly decreases, but does not abolish, tyrosinase activity in vitro, a finding consistent with the "leaky" in vivo phenotype of type IB OCA in patients homozygous for this mutant allele.

It is interesting that, in general, the amount of pigment apparent in the four type IB OCA patients in the present study correlates only modestly well with their genotypes. Patient 1, who has the most apparent mela-

nin pigment (fig. 1A), is homoallelic for type IB OCA, whereas patients 2–4, who all have considerably less melanin pigment (fig. 1B–D), are all type IA/IB compound heterozygotes. The pigmentation phenotype of type IB OCA is quite homogeneous within sibships (family 1 and family 2). However, there can be considerable phenotypic differences among unrelated type IB OCA patients, even among those with quite similar genotypes, and significant age-related accumulation of predominantly yellow-red pheomelanin is not a constant feature. In addition, the clinical phenotype among patients with type IB OCA does not correlate well with measured levels of hairbulb tyrosinase activity, the assay for which is unreliable toward the low end of its range. Patients 2 and 3 are both type IA/IB compound heterozygotes and share the same codon 275 Val→Phe type IB OCA allele (fig. 4). However, patient 2 (fig. 1B) has much more apparent pigment than does patient 3 (fig. 1C), who appears almost as severely hypopigmented as do patients with type IA OCA, despite having the highest hairbulb tyrosinase activity among the patients studied here, at the low end of the normal range. These differences of pigmentation phenotypes, along with poor correlation between clinical phenotype and measured tyrosinase enzymatic activity in type IB OCA, most likely result from epistatic phenomena acting on a background of low residual tyrosinase activity. By way of illustration, we note that the normally pigmented parents and sibs of patient 2 are very darkly complected, whereas those of patient 3 are very lightly complected; therefore, in the absence of OCA, patient 2 might otherwise have been darkly complected, whereas patient 3 might otherwise have been lightly complected.

We have shown that the phenotypes of both type IA OCA and type IB OCA result from homozygosity or compound heterozygosity for a series of allelic mutations at the human tyrosinase locus. This will greatly enhance genetic counseling for the various forms of type I OCA. The occurrence of the codon 275 mutant allele in both proband 2 and proband 3, who are unrelated, suggests that this may be a frequent allele in patients with type IB OCA. Furthermore, the availability of carrier detection for the Amish codon 406 mutant allele offers obvious advantages for genetic counseling of this high-risk population.

Acknowledgments

References

Barton DE, Kwon BS, Francke U (1988) Human tyrosinase gene mapped to chromosome 11 (q14→q21), defines second region of homology with mouse chromosome 7. Genomics 3:17–24

Bouchard B, Fuller BB, Vijayasaradhi S, Houghton A (1989) Induction of pigmentation in mouse fibroblasts by expression of human tyrosinase cDNA. J Exp Med 169:2029–2042

Giebel LB, Musarella MA, Spritz RA. A nonsense mutation in the tyrosinase gene of Afghan patients with tyrosinase-negative (type IA) oculocutaneous albinism. J Med Genet (in press)

Giebel LB, Spritz RA (1990a) RFLP for Mbo I in the human tyrosinase (Tyr) gene detected by PCR. Nucleic Acids Res 18:3103

——— (1990b) Site-directed mutagenesis using double-stranded DNA fragment as a PCR primer. Nucleic Acids Res 18:4947

Giebel LB, Strunk KM, King RA, Spritz RA (1990) A frequent tyrosinase gene mutation in classic, tyrosinase-negative (type IA) oculocutaneous albinism. Proc Natl Acad Sci USA 87:3255–3258

Giebel LB, Strunk KM, Spritz RA (1991a) Organization and nucleotide sequence of the human tyrosinase gene and a truncated tyrosinase-related segment. Genomics 9:435–445

Giebel LB, Tripathi RK, King RA, Spritz RA (1991b) A tyrosinase gene missense mutation in temperature-sensitive type I oculocutaneous albinism. J Clin Invest 87:1119–1122

Gorman CM, Moffat LF, Howard BH (1982) Recombinant genomes which express chloromphenicol acetyltransferase in mammalian cells. Mol Cell Biol 2:1044–1051

Hu F, Hanifin JM, Prescott GH, Tongue AC (1980) Yellow mutant albinism: cytochemical, ultrastructural, and genetic characterization suggesting multiple allelism. Am J Hum Genet 32:387–395

Husain I, Vijayan E, Ramaiah A, Pasricha JS, Madan NC (1982) Demonstration of tyrosinase in the vitiligo skin of human beings by a sensitive fluorometric method as well as by $^{14}C(U)$-L-tyrosine incorporation into melanin. J Invest Dermatol 78:243–252

Kikuchi H, Hara S, Ishiguro S, Tamai M, Watanabe M (1990) Detection of point mutation in the tyrosinase gene of a Japanese albino patient by direct sequencing of amplified DNA. Hum Genet 85:123–124

King RA, Olds DP (1985) Hairbulb tyrosinase activity in

oculocutaneous albinism: suggestions for pathway control and block location. Am J Med Genet 20:49–55

King RA, Summers GC (1988) Albinism. Dermatol Clin 6: 217–228

Kogan SC, Gitschier J (1990) Genetic prediction of hemophilia A. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols. Academic Press, San Diego, pp 288–299

Kwon BS, Haq AK, Pomerantz SH, Halaban R (1987) Isolation and sequence of a cDNA clone for human tyrosinase that maps at the mouse c-albino locus. Proc Natl Acad Sci USA 84:7473–7477

Lerner AB, Fitzpatrick TB (1950) Biochemistry of melanin formation. Physiol Rev 30:91–126

Nance WE, Jackson CE, Witkop CJ Jr (1970) Amish albinism: a distinctive autosomal recessive phenotype. Am J Hum Genet 22:579–586

Ott J (1974) Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. Am J Hum Genet 26:588–597

Peterson GL (1977) A simplification of the protein assay method of Lowry et al., which is more generally applicable. Anal Biochem 83:346–356

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–507

Spritz RA, Strunk KM, Giebel LB, King RA (1990) Detection of mutations in the tyrosinase gene in a patient with type IA oculocutaneous albinism. N Engl J Med 322: 1724–1728

Spritz RA, Strunk KM, Hsieh C-L, Sekhon GS, Francke U (1991) Homozygous tyrosinase gene mutation in an American black with tyrosinase-negative (type IA) oculocutaneous albinism. Am J Hum Genet 48:318–324

Spritz RA, Strunk K, Oetting H, King R (1988) RFLP for Taq I at the human tyrosinase locus. Nucleic Acids Res 16:9890

Takeda A, Tomita Y, Matsunaga J, Tagami H, Shibahara S (1990) Molecular basis of tyrosinase-negative oculocutaneous albinism. J Biol Chem 265:17792–17797

Tomita Y, Takeda A, Okinaga S, Tagami H, Shibahara S (1989) Human oculocutaneous albinism caused by a single base insertion in the tyrosinase gene. Biochem Biophys Res Commun 164:990–996

Tripathi RK, Chaya Devi C, Ramaiah R (1988) pH-dependent interconversion of two forms of tyrosinase in human skin. Biochem J 252:481–487

Tripathi RK, Giebel LB, Strunk KM, Spritz RA. A polymorphism of the human tyrosinase gene is associated with temperature-sensitive enzymatic activity. Gene Expression (in press)

Witkop CJ Jr, Quevedo WC Jr, Fitzpatrick TB, King RA (1989) Albinism. In: Scriver CR, Beaudet RL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th ed. McGraw-Hill, New York, pp 2905–2947

Wittbjer A, Dahlback B, Odh G, Rosengren A-M, Rosengren E, Rorsman H (1989) Isolation of human tyrosinase from cultured melanaoma cells. Acta Derm Venereol (Stockh) 69:125–131

Zhang H, Scholl R, Browse J, Somerville C (1988) Double stranded DNA sequencing as a choice for DNA sequencing. Nucleic Acids Res 16:1220