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A Frequent Tyrosinase Gene Mutation Associated with Type I-A (Tyrosinase-negative) Oculocutaneous Albinism in Puerto Rico

William S. Oetting,* Carl J. Witkop, Jr.,† Stephanie A. Brown,* Rosa Colomer,# James P. Fryer,* Kenneth E. Bloom,‡ and Richard A. King*'§'‖

Departments of *Medicine, †Dentistry, ‡Dermatology, and §Pediatrics and ‖Institute of Human Genetics, University of Minnesota, Minneapolis; and #Servicio Medico de Empressa de la ONCE, Canary Islands, Spain

Summary

We have determined the mutations in the tyrosinase gene from 12 unrelated Puerto Rican individuals who have type I-A (tyrosinase-negative) oculocutaneous albinism (OCA). All but one individual are of Hispanic descent. Nine individuals were homozygous for a missense mutation (G47D) in exon I at codon 47. Two individuals were heterozygous for the G47D mutation, with one having a missense mutation at codon 373 (T373K) in the homologous allele and the other having an undetermined mutation in the homologous allele. One individual with negroid features was homozygous for a nonsense mutation (W236X). The population migration between Puerto Rico and the Canary Islands is well recognized. Analysis of three individuals with OCA from the Canary Islands showed that one was a compound heterozygote for the G47D mutation and for a novel missense mutation (L216M), one was homozygous for a missense mutation (P81L), and one was heterozygous for the missense mutation P81L. The G47D and P81L missense mutations have been previously described in extended families in the United States. Haplotypes were determined using four polymorphisms linked to the tyrosinase locus. Haplotype analysis showed that the G47D mutation occurred on a single haplotype, consistent with a common founder for all individuals having this mutation. Two different haplotypes were found associated with the P81L mutation, suggesting that this may be either a recurring mutation for the tyrosinase gene or a recombination between haplotypes.

Introduction

Tyrosinase (E.C.1.14.14.1) is the key enzyme in melanin pigment formation in animals (Lerner and Fitzpatrick 1950). This copper-containing bifunctional enzyme catalyzes the first two steps of melanin synthesis, including the hydroxylation of tyrosine to 3,4-dihydroxylananine (dopa) and the oxidation of dopa to dopaquinone. Tyrosinase activity reduction (or loss) resulting from mutations of the tyrosinase gene is associated with type I (tyrosinase-related) oculocutaneous albinism (OCA) (King and Summers 1988; Witkop et al. 1989). The phenotype of type I OCA varies from hypopigmentation to a complete absence of melanin pigment in melanocyte-containing tissues (skin, hair, and eyes), along with abnormalities of the ocular system that include nystagmus, strabismus, reduced retinal pigment, foveal hypoplasia, and misrouting of the optic nerve (King and Summers 1988). Among the many alleles of type I OCA that have been identified, the type I-A OCA alleles are the most severe, resulting in complete absence of melanin pigment formation in all pigment-producing tissues.

Our laboratory and others have reported over 27 different mutations in the tyrosinase gene that are responsible for type I OCA, including missense, nonsense, and frameshift mutations (Tomita et al 1989; Giebel et al. 1990, 1991*a*, 1991*b*, 1991*c*; Kikuchi et al. 1990; Spritz et al. 1990, 1991; Takeda et al. 1990; King et al. 1991; Oetting et al. 1991*a*, 1991*b*; Oetting and King, in press-*b*). The majority of families that have been studied have been Caucasian, and many of the affected individuals have been compound heterozygotes with different maternal and paternal mutations.

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Address for correspondence and reprints: Dr. Richard A. King, Department of Medicine, Box 485 UMHC, University of Minnesota, 420 Delaware Street S.E., Minneapolis, MN 55455.

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Field work in Puerto Rico by our laboratory has shown that Hermansky-Pudlak syndrome (HPS) is the most common type of OCA in this country, but a disproportionate number of individuals with type I-A OCA rather than HPS exist in the northwestern region of this island (Witkop et al. 1989, 1990). We were interested in determining the variety of mutations in the tyrosinase gene in this population and expected to find several different mutations. It is surprising that we found that the majority of studied individuals with type I-A OCA in Puerto Rico were homozygous for a single missense mutation.

Material and Methods

Study Sample

Blood samples from individuals and families with type I-A OCA were obtained in Puerto Rico. Individuals had typical features of type I-A OCA, including white skin and hair, translucent irides, foveal hypoplasia, nystagmus, and strabismus. Control individuals from the same region of Puerto Rico had normal pigmentation and no family history of albinism. All but one individual (PR 9) was of Hispanic descent. Individual PR 9 and her parents had negroid features and were felt to be of African descent. Several individuals remembered having distant relatives from the Canary Islands, Spain. Blood was also obtained from three individuals with type I-A OCA who were from the Canary Islands. Two families from the United States, each having at least one child with type I OCA, were analyzed for haplotypes; the mutation analysis of both families has been published previously (Oetting et al. 1991*a*).

Genomic DNA Extraction

Peripheral blood was collected, the lymphocytes were isolated, and the DNA was extracted by methods described elsewhere (Oetting et al. 1991*a*).

Exon Amplification and Direct Dideoxy Sequencing

DNA sequencing of the tyrosinase gene was performed according to a method described elsewhere (Oetting et al. 1991*a*, 1991*b*). In brief, the entire coding region, part of the flanking intron sequences for each of the five exons, and 500 bp of the 5′ promoter region of the tyrosinase gene were individually amplified using PCR (Saiki et al. 1985). The amplified product was directly sequenced using the dideoxy method of Sanger, the reaction products were separated on an 8% urea-acrylamide gel, and the pattern was visualized by autoradiography (Sanger et al. 1977).

Haplotype Analysis

Four polymorphic sites of the tyrosinase gene were used for haplotype analysis. The first polymorphism [$(GA)_n$] consists of a GA cluster found in the 5′ promoter region of the gene (Morris et al. 1991). The cluster was amplified using the primers GGAAAAACAATATGGCTACA and TGGGCGATTTGTTCATTGTG. Before amplification, one of the primers was radiolabeled with [γ-^{32}P]-ATP and T4 polynucleotide kinase. The amplified products were separated on a urea-acrylamide sequencing gel, and the polymorphism was visualized with autoradiography. At least five alleles of this polymorphism have been recognized. When these primers are used for amplification, our 298-bp allele is the same as the 290-bp allele (allele A4) of Morris et al. (1991).

The second polymorphism (– 199) is located next to the CCAATT box in the 5′ promoter region at nucleotide – 199 (Oetting et al. 1991*c*). This polymorphism (CCAATTA or CCAATTC) affects a *Taq*I restriction-endonuclease site (TCAG). The third polymorphism (192) is located at codon 192 within exon I (Giebel and Spritz 1990; Johnston et al. 1992). This polymorphism (TAT or TCT) affects an *Mbo*I restriction-endonuclease site (GATC). Products of PCR amplification of the 5′ promoter region (for the – 199 polymorphism) or exon I (for the 192 polymorphism) were digested with either *Taq*I or *Mbo*I, respectively, and subsequently were separated on a 1.5% agarose gel. The resulting bands were then visualized with ethidium bromide staining.

The fourth polymorphism (*Bgl*II) consists of a *Bgl*II RFLP within the first intron and consists of either a 5.6- or 5.8-kb fragment (Spritz and Strunk 1991). Genomic DNA was digested with *Bgl*II restriction endonuclease, separated on a 0.7% agarose gel, and transferred to a nitrocellulose membrane by the method of Southern (1975). The RFLP was then detected by hybridization with the tyrosinase cDNA probe pMel 34 (Kwon et al. 1987).

Results

The initial sequence analysis of two individuals with type I-A OCA who were from Puerto Rico showed that both were homozygous for a base substitution at codon 47 in exon I (fig. 1). This mutation consisted of a G→A nucleotide substitution resulting in a missense mutation of glycine to aspartic acid (G47D; fig. 2*A*). This mutation also disrupts a *Hae*III restriction-endonuclease site (GGCC→GACC). By means of *Hae*III endonuclease cutting of amplified exon I, nine

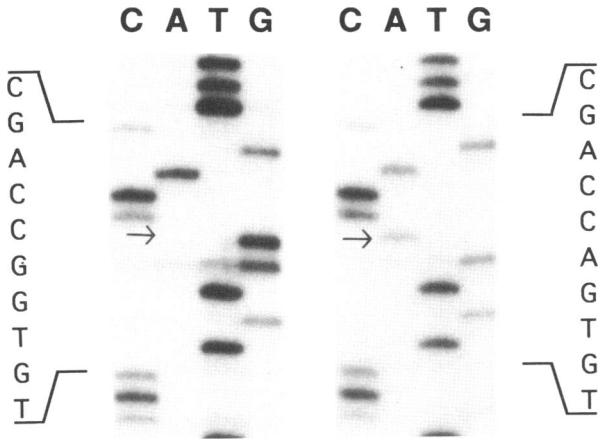

Figure 1 Sequencing autoradiogram showing codon 47. The arrows mark the location of the substituted nucleotide. *Left,* Normal sequence for tyrosinase. *Right,* Sequence of individual PR 1, showing the G47D mutation.

additional unrelated Puerto Rican individuals with type I-A OCA were found to be homozygous for this mutation, and two were found to be heterozygous. A total of 17 individuals including sibs were found to be homozygous for the G47D mutation in the nine families studied. Sequence analysis of the two heterozygotes revealed that one also had a nucleotide substitution at codon 373 on the homologous chromosome (C→A; fig. 2*B*), which results in a missense mutation of threonine to lysine (T373K). The second mutation of the other heterozygous individual was not detected within the coding region, the flanking intron sequences, or in the proximal 500 bp of the 5′ promoter region.

Individual PR 9 was found to be homozygous for a nonsense mutation at codon 236 (fig. 2*C*). This mutation consisted of a base substitution of G→A, resulting in a nonsense mutation of tryptophan to a termination codon (W236X).

Sequence analysis of three individuals with type I-A OCA who were from the Canary Islands showed that one (CI 2) was a compound heterozygote for the G47D mutation and for a missense mutation at codon 216 (fig. 2*D*), resulting in a leucine substituted by a methionine (L216M). The second individual (CI 1) was homozygous for a mutation at codon 81 (fig. 2*E*), resulting in a proline substituted by a leucine (P81L), and the third individual (CI 3) was heterozygous for the P81L mutation, with an unknown mutation on the homologous allele.

Haplotypes were determined for all individuals by using the four polymorphic sites of the tyrosinase gene (table 1). For several individuals, the correct haplotype could not be determined without further family analysis. Haplotype analysis of the affected individuals showed that all individuals from Puerto Rico who were homozygous for the G47D mutation were also homozygous for haplotype 1 (table 2). The individuals from the United States who had the G47D mutation

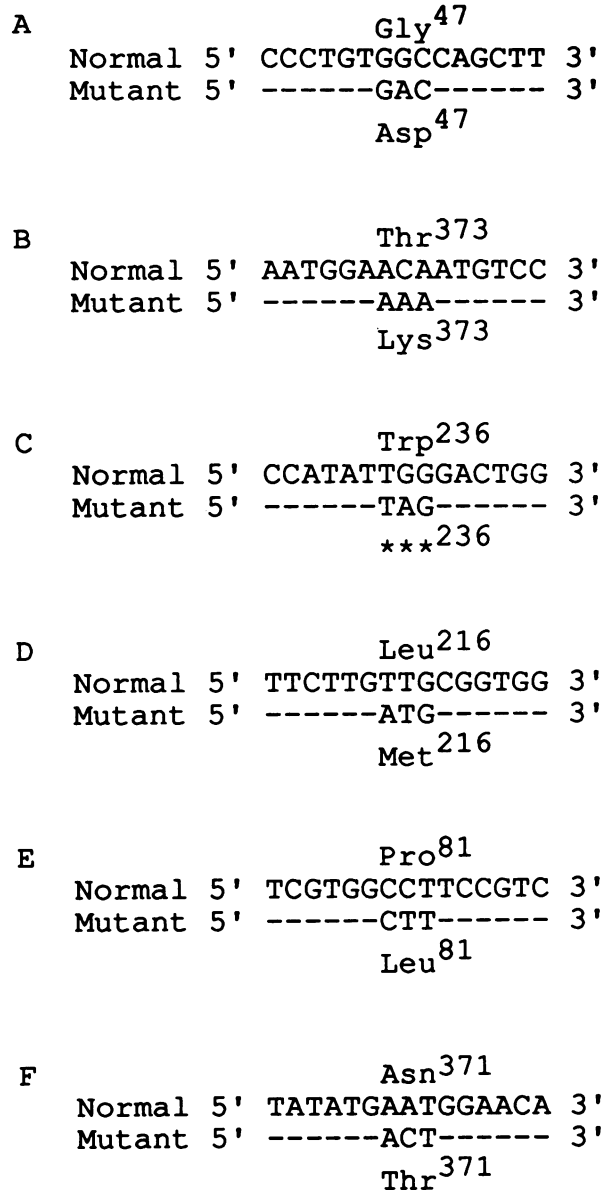

Figure 2 Mutations found in the populations of Puerto Rico and the Canary Islands. *A,* Most common mutation, also shown in fig. 1. The mutation occurs at codon 47, resulting in an amino acid substitution of glycine to aspartic acid. *B,* Missense mutation at codon 373, of threonine to lysine. *C,* Nonsense mutation at codon 236, of tryptophan to the termination codon TAG. *D,* Missense mutation at codon 216, of leucine to methionine. *E,* Missense mutation at codon 81, of proline to leucine. *F,* Missense mutation at codon 371, of asparagine to threonine.

Table 1

Haplotype Analysis of the Tyrosinase Gene Mutation

Polymorphism	Status of Haplotype[a]																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
$(GA)_n$	298	298	298	298	298	298	298	298	306	306	306	306	306	306	306	306	322
−199	+	+	+	+	−	−	−	−	+	+	+	+	−	−	−	−	+
192	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
*Bgl*II	+	+	−	−	+	+	−	−	+	+	−	−	+	+	−	−	+

[a] A plus sign (+) denotes presence of a restriction-endonuclease site, and a minus sign (−) denotes absence of a restriction-endonuclease site.

Table 2

Mutation and Polymorphic Analysis of Unrelated Individuals

Population and Individual	Mutation	$(GA)_n$	Polymorphism			Haplotype
			−199	192	*Bgl*II	
Puerto Rico:						
PR 1	G47D/G47D	298/298	+/+	+/+	+/+	1/1
PR 2	G47D/G47D	298/298	+/+	+/+	+/+	1/1
PR 3	G47D/G47D	298/298	+/+	+/+	+/+	1/1
PR 4	G47D/G47D	298/298	+/+	+/+	+/+	1/1
PR 5	G47D/G47D	298/298	+/+	+/+	+/+	1/1
PR 6	G47D/G47D	298/298	+/+	+/+	+/+	1/1
PR 7	G47D/G47D	298/298	+/+	+/+	+/+	1/1
PR 8	G47D/T373K	298/298	+/+	+/−	+/−	1/4 or 2/3
PR 9	W236X/W236X	306/306	−/−	+/+	−/−	15/15
PR 10	G47D/G47D	298/298	+/+	+/+	+/+	1/1
PR 11	G47D/G47D	298/298	+/+	+/+	+/+	1/1
PR 12	G47D/?	298/298	+/+	+/−	+/−	1/4 or 2/3
Canary Islands:						
CI 1	P81L/P81L	298/298	+/+	−/−	−/−	4/4
CI 2	G47D/L216M	298/298	+/+	+/+	+/−	1/3
CI 3	P81L/?	298/298	+/+	−/−	−/−	4/4
United States:						
US 1 (father)	P81L/+	298/298	+/+	+/−	+/−	1/4
US 2 (mother)	G47D/+	298/298	+/+	+/−	+/−	1/4
US 3 (proband)	G47D/P81L	298/298	+/+	+/+	+/+	1/1
US 4 (father)	N371T/+	298/298	+/+	−/−	−/−	4/4
US 5 (mother)	P81L/+	298/298	+/+	+/−	+/−	1/4
US 6 (proband)	P81L/N371T	298/298	+/+	+/−	+/−	1/4
Normal pigmentation—Puerto Rico:						
PR 13	+/+	298/298	+/+	−/−	−/−	4/4
PR 14	+/+	306/322	+/+	+/+	+/+	9/17
PR 15	+/+	298/306	+/−	+/+	−/−	3/15 or 7/11
PR 16	+/+	306/306	−/−	+/+	+/−	15/15
PR 17	+/+	298/298	+/+	+/−	+/−	1/4 or 2/3
PR 18	+/+	306/306	−/−	+/+	+/−	13/15
PR 19	+/+	298/306	+/+	+/−	+/−	?
PR 20	+/+	298/306	+/+	+/−	+/−	?
PR 21	+/+	298/298	+/+	+/+	+/−	11/3
PR 22	G47D/+	298/306	+/+	+/+	+/−	1/11 or 3/9

also showed linkage between the G47D mutation and haplotype 1. Individual CI 2 has both the G47D mutation and haplotype 1, yet linkage between the two cannot be inferred without analysis of additional family members. In contrast to this are the results for the P81L mutation. The P81L mutation was found on haplotype 4 in the two individuals from the Canary Islands, while the P81L mutation was found to be on haplotype 1 in the extended family from the United States. Haplotype analysis of the normally pigmented control individuals from Puerto Rico showed that both haplotypes associated with the mutations (i.e., haplotypes 1 and 4) were present, as were several different haplotypes not observed with any of the type I-A OCA mutations.

Discussion

We have analyzed individuals with type I-A OCA who are from the northwestern region of Puerto Rico, an area that includes the cities of Rincon, Aguadilla, and Arecibo, or from the Canary Islands, Spain. All individuals studied had a classic type I-A OCA phenotype, with white skin and hair, blue translucent irides, and no evidence of pigment in these tissues. Table 3 shows the distribution of mutations found in these

Table 3

Mutations Found in Puerto Rico and the Canary Islands

Population and Mutation	No.
Puerto Rico:	
G47D/G47D	17
G47D/T373K	1
G47D/?	1
W236X/W236X	1
Canary Islands:	
G47D/L216M	1
P81L/P81L	1
P81L/?	1

affected individuals. Clearly, the G47D mutation is the dominant mutation associated with type I-A OCA in the northwestern area of Puerto Rico, and it is also found in the Canary Islands. This mutation is identical to the G47D mutation found in a previously described non-Hispanic extended family from the United States (Oetting et al. 1991*a*). In Puerto Rico and the United States, the G47D mutation is on haplotype 1 and potentially is linked to haplotype 1 from individual CI 2 from the Canary Islands, providing strong evidence for a common founder for this mutation. Historically, there is a connection between the populations of Puerto Rico and the Canary Islands, and it is very possible that the G47D mutation was carried with the migration of these people. The family in the United States is also likely to be related (however remotely) to the same individuals who were among the founders of these two island populations, although family history revealed no suggestion of this linkage.

Coincidentally, the same extended U.S. family also had the P81L mutation found in individuals with OCA in the Canary Islands. In this case, there were two different haplotypes associated with this mutation. The P81L mutation has also been described by Giebel et al. (1990) in a large extended family in Wisconsin and was reported as a frequent tyrosinase gene mutation associated with type I OCA in the Caucasian population. They also found the P81L mutation to be associated with two different haplotypes. There are two potential explanations. First, this could be a very old mutation that has been distributed to the two haplotypes by recombination. Only one recombination is needed to exchange the P81L from haplotype 1 to haplotype 4. Second, this mutation could have occurred at least twice on two different haplotype backgrounds.

Two of these mutations, W236X and L216M, have not been previously reported. The nonsense mutation W236X, located in exon I, would result in the production of a truncated protein with over half the expected amino acids missing. The L216M mutation is located in the putative copper A–binding region of the enzyme (see below).

We have found that the missense mutations associated with type I OCA cluster in three distinct areas of the tyrosinase-coding region (King et al. 1991). These clusters are thought to represent important functional domains of the enzyme necessary for catalytic activity and therefore would be sensitive to amino acid substitutions. Currently, six different missense mutations, including G47D and P81L, have been found within a cluster in the 5′ end of the gene. We hypothesize that this region contains a substrate (tyrosine)- or cofactor (dopa)-binding site for the enzyme (Oetting and King, in press-*b*). The second and third clusters are located at the putative copper A– and copper B–binding regions. The L216M mutation is located at the putative copper A–binding region, and both the T373K mutation and the N371T mutation (fig. 2*F*) from the extended family are found in the putative copper B–binding region (Oetting and King, in press-*a*). These two regions are thought to form a coupled binuclear copper complex (Ross and Solomon 1991). The copper atoms complex with oxygen and tyrosine in the first step of melanin biosynthesis within this site. We hypothesize that any alterations—whether in the copper-copper distance of the coupled binuclear copper complex, the stability of the copper-protein interaction, or the ability of oxygen to bind the copper complex—that are caused by these mutations could affect the catalytic properities of tyrosinase and result in a lack of melanin biosynthesis (Oetting and King, in press-*a*). Further correlation between mutation location and tyrosinase activity will contribute to a better understanding of how alterations in enzyme structure affect phenotype-genotype relationships.

Haplotype analysis of mutations of the tyrosinase gene that are associated with type I OCA will now allow us to follow the distribution of specific mutations in populations. The analysis of additional individuals with type I OCA who are from various regions of the world will provide insight into the population origin and distribution of mutations of this important gene.

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