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OCULOCUTANEOUS ALBINISM AND ANALYSIS OF TYROSINASE GENE IN JAPANESE PATIENTS

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ABSTRACT

Oculocutaneous albinism (OCA) is a heterogeneous group of autosomal-recessive genetic disorders. The molecular pathogenesis of several types of OCA have been clarified in the ten years since our first report in 1989 on a pathologic mutation of the tyrosinase gene. In this article, a new classification of OCA based on genetic evidence is briefly reviewed, and our study on Japanese patients with tyrosinase-negative OCA is summarized.

Key Words: tyrosinase, oculocutaneous albinism

INTRODUCTION

The variation of skin color in human mainly depends on the amount of melanin pigment in the epidermis. Congenital disorders of melanin formation in all parts of the involved tissue causes hypopigmentation, called albinism. These are clinically separated into two types, ocular albinism involving the eyes alone, and oculocutaneous albinism (OCA) involving the skin and the hair as well as the eyes.

OCA is a heterogeneous group of autosomal recessive genetic disorders. The molecular bases of several types of OCA have been clarified during the past ten years and OCA is now classified according to the molecular pathogenesis as follows: tyrosinase-related OCA (type I OCA), P gene-related OCA (type II OCA), tyrosinase related protein-1 (TRP-1) OCA (type III OCA), and Unclassified.[1-3]

This article reviews briefly recent advances in our knowledge of the molecular pathogenesis of OCA and describes our study on Japanese patients with tyrosinase-negative OCA.

TYROSINASE-RELATED OCA (TYPE I OCA)

Tyrosinase-related OCA develops from a mutation of the tyrosinase gene, resulting in a dysfunction of the tyrosinase enzyme. Tyrosinase is a key enzyme which catalyzes the first and second step in the melanin synthetic pathway, that is, tyrosine to dopa and dopa to dopaquinone. Mutations of the tyrosinase gene cause impairments of the enzyme activity to various extents according to the site of the mutation, resulting in the various clinical types of OCA such as tyrosinase-negative (type I-A), yellow-mutant (type I-B), and temperature-sensitive OCA type

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I-TS). All types of OCA caused by tyrosinase gene mutations are categorized as tyrosinase-related OCA.[1-2)]

TYROSINASE-NEGATIVE OCA (TYPE I-A OCA)

In a patient with tyrosinase-negative OCA, type I-A, tyrosinase activity is completely lacking due to homozygously mutated genes of tyrosinase, and melanin formation never occurs throughout the patient's life. Its phenotype is white skin and hair, and red eyes. Photophobia, nystagmus and foveal hypoplasia accompany this hypopigmentation. We actually found, for the first time, a pathologic mutation of the tyrosinase gene in a type I-A patient in 1989.[4)] Since then more than 40 mutations causing OCA have been reported from several research groups.[2)]

YELLOW-MUTANT OCA (TYPE I-B OCA)

Patients with yellow-mutant OCA, type I-B completely lack detectable pigment at birth and are initially indistinguishable from patients with tyrosinase-negative OCA. However, such patients rapidly develop yellow hair pigment in the first few years of life and then continue to slowly accumulate pigment in the hair, eyes and skin with time.[1,2)] The tyrosinase activity of such patients is greatly decreased but not completely abolished. The point mutation in the patient gene causes a small change in the tyrosinase conformation, which must occur for a great decrease in the enzyme activity.[5)]

The mutated alleles found in type I-B and type I-A patients are termed *y* and *t-*, respectively in this review. The genotype of the type I-B OCA patient who has apparent melanin pigment is homoallelic for *y*, whereas patients who have less pigment are compound heterozygotic for *y* and *t-*. This amount of melanin pigment in such patients, therefore, appears to correlate well with the genotypes.[12)]

TEMPERATURE-SENSITIVE OCA (TYPE I-TS OCA)

A patient with temperature-sensitive OCA, type I-TS, has white hair and skin, and blue eyes at birth. At puberty, the patient develops progressively darker hair in the cooler areas (extremities) but retains white hair in the warmer areas (scalp and axilla).[2,7)] A missense mutation in the tyrosinase gene of the patient introduces one amino acid replacement which changes the enzyme into a temperature-dependent one, ie, very low activity at 35°C and loss of activity above 35°C.[8)]

AS the mutated allele found in the type I-TS patient is termed *ts* in this review, the patient's genotype is *tsxt-*; the ts gene produces temperature-sensitive tyrosinase, whereas the *t-*gene produces inactive tyrosinase. A patients with homozygous *ts* may also be diagnosed as having type I-TS OCA, but a patient with *tsxy* would probably be diagnosed as suffering from yellow-mutant OCA. However, there have been no reports of such cases to date.

P-GENE RELATED OCA (TYPE II OCA)

The human P gene is the homologue of the mouse *p* locus, a mutation of which causes a reduction of eumelanin (black-brown pigment), ie, mouse pink-eyed dilution. The P gene

encodes an integral membrane transport protein that may be a component of the melanosomal membrane and, therefore, possibly involved in the transport of tyrosine, the primary precursor to melanin synthesis.[9-11]

The phenotypes of type II OCA range from patients who are extremely hypopigmented in a manner similar to that of type I OCA to those whose mild depigmentation is appreciated only in comparison with normal family members. With time, pigmented nevi and lentigines may develop, and pigmented freckles are seen in exposed areas with repeated sun exposure. The hair slowly turns darker through the first two or more decades of llfe. Patients with Angelman syndrome and Prader-Willi syndrome also show hypopigmentation in addition to various mental and growth retardations, since both syndromes have a defect or a deletion of chromosome of 15q11-13 in which p gene is located.[2,3]

TYROSINASE RELATED PROTEIN-1 OCA (TYPE III OCA)

Tyrosinase related protein-1 (TRP-1) is now known to exhibit the activity of DHICA oxidase which catalyzes 5,6-dihydroxyindole-2-carboxylic acid (DHICA) to indole-5,6-quinone-carboxylic acid in the pathway of melanin synthesis. A defect of DHICA oxidase causes type III OCA, originally called Brown and/or Rufous albinism. Phenotypically, type III OCA shows minimal hypopigmentation. In African and African-American patients, the hair and skin color are light brown at birth but they turn darker with time.[2,3] In Caucasian individuals, the hair color is golden blond and the skin is white. We do not know the phenotype of Asiatic or Oriental type II patients because no case has been reported.

TYROSINASE MUTATION IN JAPANESE PATIENTS WITH TYPE I-A OCA

The tyrosinase gene localized in the long arm of chromosome 11 in region 11q14-q21 consists of five exons as shown in Fig. 1.[2] The amino acid sequence is composed of 529 amino acids. The first 18 amino acid residues at the N-terminal region constitute a signal peptide, which is removed just after crossing the membrane of the rough endoplasmic reticulum. The

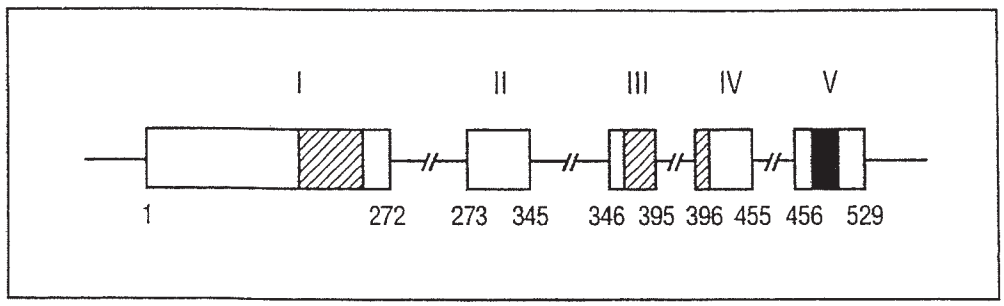

Fig. 1. Structure of the human tyrosinase gene
The tyrosinase gene consists of five exons. Numbers under the boxes represent the codon number of the first or the last codon of each exon. Putative copper-binding regions (residues 172 through 238 and 361 through 403) assumed to partly construct the active site of the enzyme are indicated by boxes with diagonal lines. The transmembrane segment (residues 474 through 499) buried in the melanosomal membrane is indicated by black.

melanosome-bound form of mature tyrosinase that removes the 18 amino acids of the signal peptide is thus composed of 511 amino acids with a molecular weight of 58,000.[1,12]

The mutation of the tyrosinase gene causing OCA that we reported for the first time in 1989 is a single-base insertion (C) into codon 310 that shifts the reading frame and introduces a stop codon (TGA) at 317.[4] In 1990 we then found a single-base mutation at codon 77 that changes CGG (Arg) to CAG (Gln).[13] We subsequently identified two different point mutations.[14] One is a nonsense mutation, codon 278CGA (Arg) to TGA (stop codon), and the other is a

Table 1. Mutations of the tyrosinase gene in Japanese patients.

a. point mutation

1) R77Q
CGG (Arg) to CAG (Glu)

2) R239W
CGG (Arg) to TGG (Trp)

3) R278X
CGA (Arg) to TGA (Stop codon)

4) D383N
GAT (ASp) to AAT (Asn)

5) P431L
CCA (Pro) to CTA (Leu)

b. insertion mutation

6) + C310
Insertion at 310 introduces stop codon at 317.

Table 2. Genotype analysis of 16 Japanese cases with type I-A OCA

Patient Number	Genotype		Native Place	
	Paternal	Maternal	Father	Mother
1	R77Q	R77Q	Akita	Akita
2	R77Q	R77Q	Fukushima	Fukushima
3	R77Q	R77Q	Gunma	Gunma
4	ND	R77Q	Niigata	Niigata
5	ND	R77Q	Nagano	Nagano
6	+ C310	+ C310	Fukushima	Fukushima
7	+ C310	+ C310	Kanagawa	Tokyo
8	+ C310	+ C310	Shizuoka	Shizuoka
9	+ C310	+ C310	Shizuoka	Shizuoka
10	+ C310	+ C310	Nagano	Nagano
11	+ C310	+ C310	Mie	Mie
12	+ C310	+ C310	Gifu	Aichi
13	P431L	+ C310	Fukushima	Fukushima
14	D383N	+ C310	Tokyo	Ibaragi
15	R239W	+ C310	Tokyo	Oita
16	R278X	+ C310	Osaka	Osaka

substitution mutation, codon 431CCA (Pro) to CTA (Leu). These two mutations seem to be localized in Asia, because they were also observed in Indo-Pakistani patients. Recently, two mutations have been added, ie, codon 239CGG (Arg) to TGG (Trp), and codon 383GAT (Asp) to AAT (Asn) (Table 1.).

We developed two improved techniques for detecting tyrosinase mutations in type I-A OCA patients. One is allele-specific amplification based on the specific amplification of the target allele by a PCR with the normal and mutant allele-specific modified primers inhibiting unfavorable amplification.[15] The other is successful sequence analysis of all amplified exons of the tyrosinase gene by PCR from fairly limited samples of blood spots dried on filter paper.[16]

Until now, we have examined 16 cases with type I-A OCA (Table 2.).[4,13-18] Nine cases were homozygous for mutations at codon 77 or codon 310. The other 7 cases harbored mutations heterozygously at codon 310 or codon 77. None of the mutations detected in Japanese patients have been reported in Caucasians. We therefore think that the mutations at codon 77 and 310 might be the major ones in Japanese patients with type I-A OCA.

All of the homozygous cases were unrelated, and their family histories indicated no consanguineous marriages. But in each homozygous case, the parent's native place was the same city or prefecture. Therefore, we postulated that the people in Japan have inhabited an area without moving for many generations.

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