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Technical Advances in Prenatal Diagnosis of Tyrosinase-negative Oculocutaneous Albinism

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Technical advances in the prenatal diagnosis of tyrosinase-negative oculocutaneous albinism, the most severe subtype of this disease, are reviewed. Ultrastructural examination of hair bulb melanocytes in fetal skin during the second trimester of pregnancy was the first successful method for the prenatal diagnosis of oculocutaneous albinism. Subsequent introduction of the electron microscopic DOPA reaction test in fetal skin provided safer, more practical, and reliable information for diagnosing tyrosinase-negative oculocutaneous albinism prenatally. The recent elucidation of the specific gene mutation of tyrosinase in the affected individuals now allows the DNA-based prenatal diagnosis of tyrosinase-negative oculocutaneous albinism in the first trimester of pregnancy. ***Key words: fetal skin biopsy; DNA; electron microscopy; DOPA.***

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Oculocutaneous albinism (OCA) is characterized by a reduction in biosynthesis of melanin in the skin, hair and eyes (1–3). The reduction of melanin in the skin leads to an increased sensitivity to ultraviolet radiation and a predisposition to skin cancer (4, 5). Reduction of melanin in the eyes causes a reduction in visual acuity due to foveal hypoplasia associated with nystagmus and an abnormal routing of the nerve fibers from the eye to the brain (6, 7). The tyrosinase-negative variant, the most severe subtype of OCA in which the affected individuals lack tyrosinase activity, is caused by a mutation in the tyrosinase gene and is inherited as an autosomal recessive trait (3, 8). The patient may be severely handicapped socially, especially in certain non-Caucasian communities, where albinos are treated as outcasts (4). In these countries parents of an albino child usually do not wish to risk another pregnancy, because of the 25% chance that the next fetus will be similarly affected. Since there is a 75% chance for a normal fetus, couples might decide to continue the pregnancy if a reliable prenatal test were available.

Fetal skin biopsy has been important in the prenatal diagnosis of OCA. A sample of fetal skin can be examined by light and electron microscopy to detect morphological and immunohistochemical abnormalities. However, fetal skin biopsy can be performed only in the second trimester of pregnancy, and there is a long waiting period for test results, prolonging the parents' anxiety. A method for making a prenatal diagnosis in the first trimester is therefore desirable. Advances in molecular biology have elucidated the tyrosinase gene mutation in OCA and allow a DNA-based prenatal diagnosis using samples of chorionic villi or amniocentesis at 10 weeks of gestation in the first trimester.

METHODS OF PRENATAL DIAGNOSIS OF OCA

1) Ultrastructural examination of fetal hair bulb melanocytes

Melanosomes develop in four stages, gradually moving from the cytoplasm of the melanocytes into the dendritic processes. As the melanosomes mature, their melanin content increases, while their concentration of melanogenic enzyme, as measured by the electron microscopic dihydroxyphenylalanine (DOPA) reaction, decreases. Stage I melanosomes are round, about 300 nm in size, and contain no melanin. Stage II melanosomes are ellipsoid, contain longitudinal filaments that are cross-linked with one another, and have a slight deposition of melanin. In stage III melanosomes, melanin deposition is increased. In stage IV melanosomes, deposition of the melanin obscures the internal structure.

The possibility of a prenatal diagnosis of OCA via electron microscopy was proposed in 1981 by Haynes & Robertson (9). In tyrosinase-negative OCA, development of the melanosomes does not proceed beyond stage II, corresponding to a total lack of melanin synthesis. In contrast, mature stage IV melanosomes are always present in hair follicle melanocytes at the bulbous peg stage in the scalp of the 16-week-gestation normal fetus but are rarely found in the epidermal cells. Haynes & Robertson predicted that prenatal diagnosis of OCA would be possible by examining the ultrastructure of the fetal hair bulb melanocytes in the second trimester using electron microscopy (9).

In 1983, Eady et al. (10) performed the first prenatal diagnosis of tyrosinase-negative OCA by biopsy of fetal skin from the scalp. Two of the four fetuses at risk that they examined were affected with OCA; only stage I and II melanosomes were observed in hair bulb melanocytes (11). In one of the two affected fetuses, skin samples were obtained from the eyebrows instead of the scalp for technical reasons and these samples turned out to be equally satisfactory for diagnosis. The other two fetuses were normal, as numerous stage IV melanosomes were detected in the fetal hair bulb melanocytes. They encountered considerable difficulty in locating hair bulb melanocytes in one of the two normal fetuses, despite an extensive search of serial sections. However, the demonstration of large numbers of stage IV melanosomes in the hair cortex was considered sufficient to exclude OCA.

As the experiences of Eady demonstrate, there are several technical difficulties with the ultrastructural examination of fetal hair bulb melanocytes for prenatal diagnosis (11). First, it can be difficult to perform a fetal skin biopsy of the scalp, depending on the position of the fetus and placenta. There is a risk of an inadvertent biopsy of the face, eyes, or eyebrows. Second, in some cases there may be considerable difficulty in locating hair bulb melanocytes, despite an extensive search in serial sections of small samples of fetal skin.

2) Electron microscopic DOPA reaction test of fetal skin

Melanocytes with mature melanosomes appear at 16 weeks of gestation in the fetal scalp hair bulb. At this point, there are

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fewer less mature melanocytes in the interfollicular epidermis. It was speculated that the interfollicular epidermal melanocytes with premature melanosomes possessed tyrosinase activity at 16 weeks of gestation (12). If the tyrosinase activity of fetal melanocytes in the interfollicular epidermis could be evaluated accurately by electron microscopy, biopsy of the fetal scalp would be unnecessary.

In 1992, the electron microscopic DOPA reaction test was successfully utilized to detect the presence of tyrosinase in fetal skin for prenatal diagnosis of OCA (13). A Japanese family, whose first son suffered from typical tyrosinase-negative OCA, decided to continue the second pregnancy only if a prenatal examination confirmed no evidence of the disease. Preliminary examination was carried out to evaluate whether the electron microscopic DOPA reaction test could accurately identify the presence of tyrosinase activity in ordinary fetal skin, not necessarily from the fetal scalp. In the skin obtained from the trunk of three normal Japanese control fetuses, melanosomes of stages I, II and III were observed in conjunction with a few stage IV melanosomes. For the electron microscopic DOPA reaction test, the skin sample was incubated in DOPA solution and embedded in epon (14). After incubation with the DOPA solution, most of the premature stage I and II melanosomes of normal fetus were melanized to stage IV (Fig. 1*a*) (15). However, even after incubation with DOPA solution, the patients' melanocytes showed only stage I and II melanosomes, and no evidence of further melanization was observed.

These preliminary results justified the use of the electron microscopic DOPA reaction test for prenatal diagnosis of tyrosinase-negative OCA. A skin biopsy was obtained at the estimated gestation age of 20 weeks from the upper back of the fetus at risk of tyrosinase-negative OCA. Electron microscopy revealed that the melanocytes in the ordinary interfollicular epidermis contained stage I and II melanosomes, but no stage III and IV melanosomes. After incubation with the l-DOPA solution, no further melanization of premature melanosomes was seen (Fig. 1*b*). These results suggested the absence of tyrosinase activity in the fetal melanocytes.

The parents decided to terminate the pregnancy at 21 weeks of gestation. The skin of the abortus demonstrated no tyrosinase activity (15). The retinal melanocytes contained immature melanosomes (16), further confirming the diagnosis. The electron microscopic DOPA reaction test of fetal skin provides a reliable prenatal diagnosis of tyrosinase-negative OCA in the second trimester (13, 15).

3) Analysis of the fetal tyrosinase gene

Studies in molecular biology have shown that tyrosinase-negative OCA is caused by pathologic mutations in the tyrosinase gene (8). Tyrosinase, a key enzyme in the biosynthesis of melanin in pigment cells, catalyzes the conversion of tyrosine to DOPA. Since the first reported mutation of the tyrosinase gene in OCA (8), more than 25 alleles, each with a different mutation, have been found in patients with tyrosinase-related OCA (7). Two common tyrosinase gene mutation sites have been found in Japanese patients (8, 17): a single base insertion in exon 2, shifting the reading frame and introducing a premature termination codon (TGA) after amino acid residue 298 (codon 316) (8); and a single base mutation in exon 1, causing substitution of an arginine by glutamine at position 59 (codon 77) (17). These data suggest the possibility of DNA-based prenatal diagnosis of OCA at an earlier gestational age by a less invasive procedure than the previous approach with fetal skin biopsy (18).

The proband was a 9-year-old Japanese boy with tyrosinase-negative OCA, whose mother was in her second pregnancy. The fetal DNA was obtained by amniocentesis performed at the estimated gestation age of 14 weeks. Polymerase chain reaction amplification and allele-specific oligonucleotide hybridization were used to examine the presence of the common mutations (8, 17) of exon 1 and exon 2 in the tyrosinase gene in Japanese patients with this condition. The results indicated that the child was homozygous and the parents heterozygous for a mutation of the tyrosinase gene in exon 2 (single base insertion) but not in exon 1 (Fig. 2). Prenatal diagnosis was performed by analyzing the tyrosinase gene in fetal cells obtained by amniocentesis at 14 weeks of

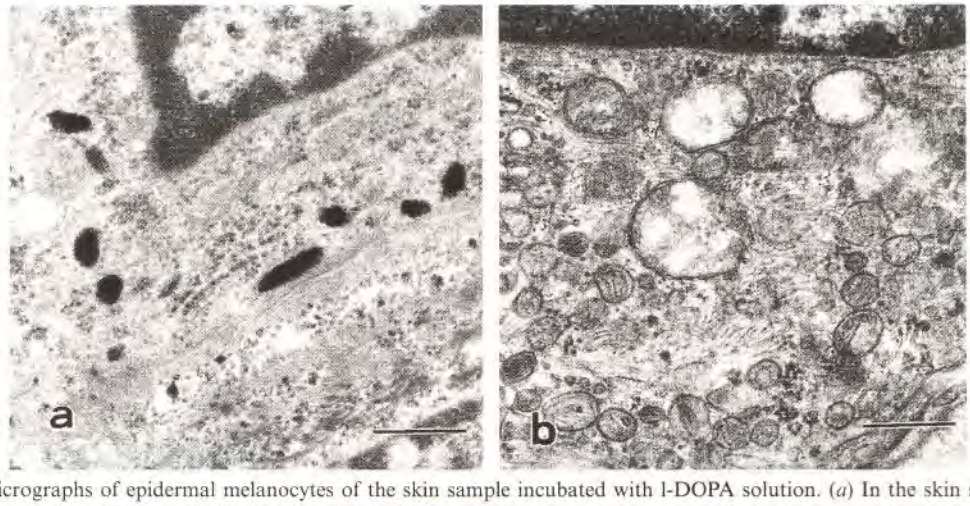

Fig. 1. Electron micrographs of epidermal melanocytes of the skin sample incubated with l-DOPA solution. (*a*) In the skin sample of a normal fetus with tyrosinase-positive OCA, after incubation with l-DOPA solution, most premature melanosomes in melanocytes have melanized to stage IV, demonstrating the presence of tyrosinase activity. (*b*) Melanocytes of the fetus affected with tyrosinase-negative OCA contain only stage I and II melanosomes, even after incubation with l-DOPA solution, demonstrating a lack of tyrosinase activity (the figure was originally published elsewhere (15)).

gestation. The fetus was found to be heterozygous for the mutant tyrosinase gene (Fig. 2). The pregnancy was therefore continued, and a normal male infant was born (18).

More recently, we introduced a new system with 8 sets of primers and heteroduplex analysis that can cover mutations in entire exons of the tyrosinase gene. Using this system, DNA-based prenatal diagnosis was performed on another fetus at risk of tyrosinase-negative OCA. Fetal DNA was shown to have a homozygous mutation of a single base insertion in exon 2, shifting the reading frame and introducing a premature termination codon (TGA) after amino acid residue 298 (codon 316). The parents decided to terminate the pregnancy. Analysis of the DNA obtained from the abortus further confirmed the diagnosis (unpublished data). The DNA-based prenatal diagnosis of this condition was subsequently reported by another group (19). This technique should become the method of choice for the prenatal diagnosis of tyrosinase-negative OCA.

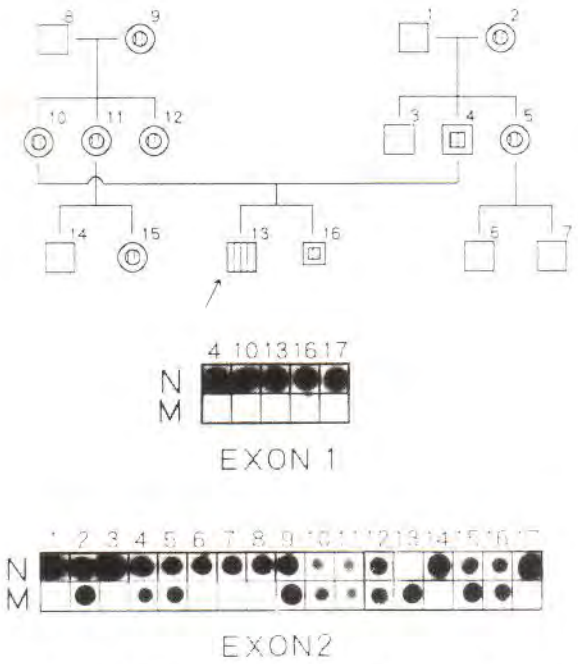

Fig. 2. Family pedigree and results of polymerase chain reaction amplification and allele-specific oligonucleotide hybridization. No. 13 is the proband, a 9-year-old Japanese boy with tyrosinase-negative OCA. No. 16 is the fetus examined for the prenatal diagnosis, and No. 17 is a normal healthy adult serving as a control. No nucleotide mutation was found in exon 1 of the tyrosinase gene in any family member, including the proband. The normal probe (*N*) for exon 1 but not the mutant probe (*M*) thus hybridized to all the samples examined (not all data shown). The mutant probe (*M*) for exon 2 hybridized with the amplified DNA of the proband (No. 13), his parents (No. 4 and No. 10), his grandmothers (No. 2 and No. 9), his three aunts (No. 5, No. 11, and No. 12) and his cousin (No. 15), but not with that of his grandfathers (No. 1 and No. 8), his uncle (No. 3), and his three cousins (No. 6, No. 7, and No. 14). The normal probe (*N*) for exon 2 hybridized with the amplified DNA of all samples except that of the proband (No. 13). On prenatal diagnosis, no nucleotide mutation was found in exon 1 of the fetal tyrosinase DNA (No. 16). Amplified DNA of the fetus (No. 16) hybridized with both mutant and normal probes for exon 2 of the tyrosinase gene, confirming that the fetus was not affected with OCA but was a heterozygous carrier of the mutant tyrosinase gene (the figure was originally published elsewhere (18)).

DISCUSSION

Prenatal diagnosis of tyrosinase-negative OCA was first achieved by analyzing skin biopsied from the fetal scalp. Introduction of the electron microscopic DOPA reaction test enabled prenatal diagnosis by fetal skin biopsy from any site of the body, not necessarily from the scalp. The more recent introduction of DNA-based analysis provides for rapid and reliable prenatal diagnosis at an earlier stage in the first trimester of the pregnancy by chorionic villus sampling at 10–11 weeks or amniocentesis at 13–16 weeks of estimated gestation age. Chorionic villus sampling offers a great advantage in prenatal diagnosis that can be performed earlier than any other methods. The disadvantage of chorionic villus sampling is the higher rate of pregnancy loss associated with it. In experienced hands, the rate of fetal loss after the procedure is 3% to 5% while that attributed to amniocentesis is 0.5% or less (20). In experienced centers, the incidence of fetal loss from skin biopsy is not more than 5% (21).

Many families with a history of OCA want to know the condition of a fetus at risk. The molecular analysis of fetal DNA is certainly the method of choice for prenatal diagnosis. Until we introduced the DNA-based prenatal diagnosis for OCA (18), fetal skin biopsy was the only available method for detecting a fetus affected with tyrosinase-negative OCA. Analysis of fetal genomic tyrosinase DNA is a rapid and reliable approach to the prenatal diagnosis of this condition. It is less invasive than previous methods, can be performed at an early stage of pregnancy, and the results can be obtained in a day or two. Elucidation of a detailed genotype/phenotype correlation may eventually lead to earlier diagnosis through preimplantation blastocyte analysis, and to gene therapy for patients with OCA as well as for affected fetuses during gestation.

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