REVIEW

Prenatal Diagnosis of Inherited Skin Diseases

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Abstract. Significant advances in the prenatal diagnosis of hereditary skin disorders, including severe forms of epidermolysis bullosa (EB) and tyrosinase-negative oculocutaneous albinism (OCA1A), have been reviewed. Fetal skin biopsy during the second trimester of pregnancy has been utilized successfully for the prenatal diagnosis of EB and OCA1A. Recently, elucidation of the specific gene mutation in affected individuals allowed us to perform DNA-based prenatal diagnosis during the first trimester of pregnancy. Over the last 5 years, we have established several new strategies for prenatal diagnosis for EB and OCA1A at the Special Clinic for Inherited Skin Disorders at Keio University Hospital. (Keio J Med 45 (1): 28–36, March 1996)

Key words: epidermolysis bullosa, albinism, fetal skin biopsy, DNA-analysis, basement membrane

Introduction

There are a large number of severe inheritable skin disorders that have been considered indications for prenatal diagnosis. Until 1980's when the first prenatal diagnosis for inherited skin diseases was introduced, however, most of the severe genetic disorders of the skin has not been able to be diagnosed prenatally. Many parents, who once experience the severity and course of one of these severe genodermatoses, are inclined to interrupt a new pregnancy to prevent its repeat, even though they wish to have an another child. Thus, many healthy fetuses have been aborted because of the unavailability of the prenatal diagnosis.

Fetal skin biopsy has played an important part in the development of prenatal diagnosis of certain genetically determined skin disorders during the past decade, include epidermolysis bullosa, oculocutaneous albinism (OCA), Harlequin ichthyosis lamellar ichthyosis, bullous congenital ichthyosiform erythroderma, anhidrotic ectodermal dysplasia and incontinentia pigmenti Bloch Sulzberger.1-3 Fetal skin sample can be examined by light and electron microscopy for morphological, biological and immunohistochemical abnormalities. Nevertheless, skin biopsy has the disadvantages of being performed only in the second trimester of pregnancy and of requiring a long waiting period for test results. By this time too, an at-risk woman may have already experienced prolonged anxiety about the status of her baby. Thus, a number of investigators have sought a method by which to perform prenatal diagnosis in the first trimester. Advances in molecular biology have elucidated the specific mutant gene responsible for some of the genodermatoses, allowing for DNA-based prenatal diagnosis of certain inherited skin disorders that can introduce chorionic villi sampling or amniocentesis in the earlier stage of pregnancy.

In this review, two genodermatoses were focused in particular; epidermolysis bullosa in which prenatal diagnosis has been most commonly performed and oculocutaneous albinism in which rapid technological development has been achieved. Our own experience with the prenatal diagnosis of these genodermatoses at the Special Clinic for Genetic Counseling on Inherited Skin Diseases at Keio University Hospital for the last 5 years and several new technical strategies we have successfully established are also summarized.

Prenatal Diagnosis of Epidermolysis Bullosa (EB)

Epidermolysis bullosa is an inherited skin disease that encompasses more than twenty subtypes having the com-
mon characteristic of marked skin fragility and blister formation after seemingly minor or insignificant trauma to the skin (Fig 1). There are three major types of epidermolysis bullosa: simplex, junctional and dystrophic type. These are classified based on where the blisters form ultrastructurally within the skin following mechanical trauma (Table 1). In the epidermolytic form, referred to as EB simplex, skin cleavage or blister formation occurs within the lower portion of the epidermis. In junctional EB, blisters arise within the lamina lucida. As a result, the intact epidermis forms the roof of the blister while the lamina densa remains along its base. In the dermolysis form of inherited EB, referred to as dystrophic EB, skin cleavage occurs beneath the lamina densa; therefore, the epidermis and intact basement membrane form the roof of the blister while the denuded dermis forms its base (Table 1).

Technologic advances of the study of normal human skin has led to a greater understanding of the pathogenesis of various forms of inherited EB. In particular, the generation of monoclonal antibodies specific for skin basement membrane has led to the discovery of several selective antigenic defects in the skin from patients with various forms of EB (Fig 2). These antibodies provide additional confirmation that certain subtypes have similar or identical phenotypes during the neonatal period. Clinical features of EB variants greatly differ from one another. Prenatal diagnosis of three of most severe forms of EB variants with autosomal recessive inheritance, including lethal junctional EB of Herlitz type, recessive dystrophic EB, and pyloric atresia-junctional EB syndrome, has been the standard practice in dermatology for the last 15 years (Table 1).

Recessive dystrophic EB (RDEB)

Recessive dystrophic EB, inherited as an autosomal recessive trait, includes the generalized mutilating form (Hallopeau-Siemens type) which is the most severe RDEB, the localized non-mutilating type (mitis), and

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Fig 1 Clinical features of severe subtype of EB, including recessive dystrophic EB with fusion of toes and fingers (A,B), lethal junctional of Herlitz EB (C) and pyloric atresia – junctional EB syndrome (D).
Table 1 Ultrastructural and Immunohistochemical Features of Epidermolysis Bullosa and Their Candidate Genes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Ultrastructural Findings</th>
<th>Immunohistochemistry</th>
<th>Candidate Genes</th>
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<tbody>
<tr>
<td>EB-simplex</td>
<td>clumped tonofilaments</td>
<td>absence or marked reduction of laminin 5</td>
<td>KRT5, KRT14</td>
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<tr>
<td>EB-Herlitz Junctional</td>
<td>tissue separation at the level of lamina lucida and dystrophy of hemidesmosomes</td>
<td></td>
<td>LAMA3, LAMB3, LAMC2</td>
</tr>
<tr>
<td>PA-JEB</td>
<td>tissue separation at the level of lamina lucida and dystrophy of hemidesmosomes</td>
<td>absence of ucin, β4 integrin, α6 integrin</td>
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<tr>
<td>EB-dominant Dystrophic</td>
<td>scarcity of anchoring fibrils; sublamina densa blistering</td>
<td>reduction of type VII collagen</td>
<td>COL7A1</td>
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<tr>
<td>EB-recessive Dystrophic</td>
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Malnutrition, anemia, growth retardation, and squamous cell carcinoma are frequent complications of HS-RDEB.9 The morphologic criteria to recognize HS-RDEB in utero are based on observations of the skin from affected fetuses and postnatal individuals.10 The first prenatal diagnosis of HS-RDEB was made by fetal skin biopsy.11 Electron microscopy reveals separation of the epidermis from the dermis in the plane below the lamina densa and absent or a markedly reduced number of mature anchoring fibrils. Identification of type VII collagen, as the major component of anchoring fibrils, was a critical step in investigating the pathogenesis of RDEB.12 It allowed the development of a monoclonal antibody against the protein, such as LH7.2.13 The epitope of LH7.2 was found to be localized within the NC1 domain of type VII collagen at both ends of the anchoring fibrils.14-16 The majority of the HS-RDEB patients display the absence of staining of the epidermal basement membrane with LH7.2 (Fig 2A, 2B).13 Thus, LH7.2 has been applied as a diagnostic probe for HS-RDEB.5

Recently, we accomplished the first prenatal diagnosis of HS-RDEB for a Japanese family by fetal skin biopsy using the LH7.2 as a immunohistochemical probe for detection.17 In this family, electron microscopy of the skin from the proband demonstrated dermo-epidermal separation below the lamina densa and no mature anchoring fibrils. LH7.2 staining was completely negative at the epidermal basement membrane zone. The parents sought prenatal diagnosis for their next pregnancy. The fetal skin biopsy was performed and electron microscopy of the fetus revealed no dermo-epidermal separation and numerous mature anchoring fibrils. In addition, the fetal skin showed positive LH7.2 staining by indirect immunofluorescence (Fig 2A). This suggested that the fetus was unaffected resulting in the birth of a healthy newborn.17

Fig 2 Indirect immunofluorescence with monoclonal antibodies LH7.2(A,B), GB3(C,D) and 19-DEJ-1(E,F) in the skin of recessive dystrophic EB (B), gravis junctional EB of Herlitz type (D) and pyloric atresia-junctional EB syndrome (F). Normal fetal skin (A,C,E) shows positive linear staining at the epidermal basement membrane zone, while the severe subtype of EB lack reactivity with that antibody (B,D,F).
Recent cloning of COL7A1, encoding type VII collagen, and the identification of gene mutations provide a new means for direct DNA-based prenatal diagnosis. We succeeded in achieving DNA-based prenatal diagnosis for a Japanese family with RDEB both by amniocentesis and by chorionic villus sampling. The proband was a 6-month-old Japanese male with typical clinical, histological, and ultrastructural features of RDEB with severe fusion of toes. Molecular analysis of COL7A1 gene revealed the proband and his father to be heterozygous for a 1 bp deletion of a C in exon 70 (5818delC), while the maternal mutation has not been identified. In addition, four microsatellite markers (D3S1029, D3S1235, D3S1076, and D3S1573) located in the DNA flanking the COL7A1 locus were shown to be informative for genotyping. In December 1994, the mother was in her second pregnancy and hoped prenatal diagnosis. Analysis of the fetal DNA extracted from amniotic cells obtained at 14 weeks of gestation indicated that the fetus was affected. The parents chose the termination of the pregnancy and the abortus was confirmed to be affected with RDEB. In July 1995, the parents had their third pregnancy and wished the DNA based prenatal diagnosis again. The analysis of the fetal DNA confirmed that the fetus has two normal alleles of COL7A1 and the pregnancy has been continued. Genotype analysis with the COL7A1 mutation in families at risk for RDEB represents an early and rapid diagnostic alternative to second-trimester evaluation of fetal skin samples offering a major advancement in prenatal diagnosis.

Lethal junctional EB of Herlitz type (Herlitz EB)

Clinical features of Herlitz EB, autosomal recessive inherited genodermatoses, are widespread blisters, erosions and the development of large, non-healing areas of granulation tissue. The prognosis is very poor and the majority of patients die prior to one-year of age, although there have been a few exceptions. The prenatal diagnosis of this condition with autosomal recessive inheritance was first performed in 1980 using skin samples from an 18-week estimated gestational age fetus. Herlitz EB is the most commonly diagnosed genetic skin disease in utero using the fetal skin biopsy and is the first variant among EB whose prenatal diagnosis was performed in Asia.

Although structural abnormalities of the hemidesmosomes and specific antigen expression occur in the earlier weeks of gestation, the procedure is postponed until the second trimester due to difficulty in sampling and handling of the tissue at the earlier weeks. Until the mid 1980s, the primary criteria for diagnosis of the affected fetal skin was the separation of the epidermis in the plane of the lamina lucida and the hypoplastic development of the hemidesmosomes. The GB3 monoclonal antibody, directed against nicein/kalinin/epiligrin or laminin 5 in the new nomenclature, has been shown to be specifically absent in the skin of Herlitz EB and can be used as a prenatal diagnostic probe (Fig 2C, 2D).

In 1990, the Special Clinic for Genetic Counseling on Inherited Skin Diseases at Keio University was opened and in 1991, we have performed the first successful trial of prenatal detection of EB in Asia. In this Japanese family with Herlitz EB, the proband, who died a few months after birth, showed the formation of generalized bullae. Electron microscopy of the skin of the proband showed dermo-epidermal separation at the lamina lucida and complete negative staining with the GB3 monoclonal antibody against laminin 5. The parents opted for prenatal diagnosis with the next pregnancy, and the fetal skin biopsy was performed at 19 week's of gestation under the ultrasound guidance. Of the four small skin samples (<1mm³), two were processed for electron microscopy and the rest were used for immunohistochemistry. Electron microscopy showed no dermo-epidermal separation with mature hemidesmosomes. Indirect immunofluorescence revealed a normal bright basement membrane staining of GB3 monoclonal antibody, indicating that the fetus was not affected (Fig 2C, 2D).

GB3 antigen or laminin 5 in the new nomenclature, known to be absent in Herlitz EB, consists of three polypeptide subunit chains, α3 (150kDa), β3 (125kDa) and γ2 (100kDa), encoded by the distinct gene LAMA3, LAMB3, and LAMC2, respectively. Specific mutations in both the LAMB3 and LAMC2 genes were recently reported in patients with Herlitz EB. Cloning of the full-length cDNAs encoding the three chains of laminin 5 and identification of the mutations in the corresponding genes provide a new means for direct DNA-based prenatal diagnosis using chorionic villus sample at 10-week gestation. DNA-based prenatal diagnosis was reported recently, and expected to be further applicable for certain Herlitz EB families in the future. In September 1995, we indeed performed DNA based prenatal diagnosis for a Japanese fetus at risk of Herlitz EB, and diagnosed that the fetus was not affected (Shimizu H et al; paper in preparation).

Pyloric atresia-junctional EB syndrome (PA-JEB)

Pyloric atresia (PA) associated with epidermolysis bullosa (EB) is a distinct entity inherited as an autosomal recessive trait. A recent review of this condition disclosed more than 40 such cases, including 6 pairs of siblings. Most of these infants died in the first few months of life despite a surgical correction of the pyloric abnormality. PA is probably the primary event, rather than the result of scarring secondary to junctional EB. Therefore, all associated cases should be categorized
as junctional EB termed “PA-junctional EB syndrome (PA-JEB)”.

Previously prenatal diagnosis of PA-JEB was based on the ultrastructural findings within fetal skin of the intra-lamina lucida cleavage and hypoplasia of the hemidesmosomes. Electron microscopy seems to be the only reliable method for prenatal diagnosis, because laminin 5, detected by GB3 monoclonal antibody, is expressed normally in PA-JEB and cannot be used as a diagnostic probe.

To overcome this technical problem, we have applied the 19-DEJ-1 monoclonal antibody as an immunohistochemical probe for prenatal diagnosis. In this Japanese family, both the first and second child was affected with PA-JEB and died within 2 weeks after being delivered. In her third pregnancy, the prenatal diagnosis was performed by fetal skin biopsy and electron microscopy demonstrated no apparent sign of dermo-epidermal separation nor hypoplasia of hemidesmosomes. The 19-DEJ-1 monoclonal antibody showed bright linear staining at the epidermal basement membrane that was completely absent in the skin of the affected siblings (Fig 2E, 2F). The fetus at risk was diagnosed as unaffected. The 19-DEJ-1 monoclonal antibody should be applied as a diagnostic probe for the prenatal diagnosis of PA-JEB.

More recently, absence of β4 integrin, a component of hemidesmosome, was found in the skin of a patient of PA-JEB. Subsequent study proved the presence of mutation of β4 integrin gene in PA-JEB. On the contrary, we recently reported a Japanese patient with PA-JEB showing absence of detectable α6 integrin (manuscript in submission). These latest findings would indicate the genetic and phenotypic heterogeneity of PA-JEB.

Prenatal Diagnosis of Oculocutaneous Albinism (OCA)

Oculocutaneous albinism (OCA) is characterized by reduction of melanin pigment biosynthesis in the skin, hair and eyes. The reduction of melanin pigment in the skin results in an increased sensitivity to UV radiation and a predisposition to skin cancer. Reduction of melanin in eyes results in reduced visual acuity due to foveal hypoplasia associated with nystagmus and abnormal routing of the nerve fibers from the eye to the brain resulting in strabismus and loss of binocular vision. The tyrosinase-negative variant, the severest subtype of OCA in which no tyrosinase activity is seen in patients, is caused by mutation in tyrosinase gene and inherited as an autosomal recessive trait. The patient may be severely and socially handicapped, especially in non-Caucasian countries. Parents with an albino child usually do not risk another pregnancy because of the 25 percent risk that the fetus will be similarly affected. Since there is a 75 percent chance of a normal fetus, parents might continue the pregnancy if a reliable prenatal test were available.

Prenatal diagnosis by ultrastructural examination of fetal hair bulb melanocytes

The theoretical possibility of OCA prenatal diagnosis using the electron microscopy was proposed in 1981 by Haynes et al. They reported that mature stage IV melanosomes were always present in hair follicle melanocytes at the bulbous peg stage in the scalp of the 16-week gestational fetus, but rarely in the epidermal cells. They predicted that prenatal diagnosis of OCA would be possible by ordinary electron microscopic examination of fetal hair bulb melanocytes in the second trimester. Eady et al were the first to perform prenatal diagnosis of OCA demonstrating a lack of stage IV melanosomes in fetal hair bulb melanocytes at 20 weeks’ gestation.

Prenatal diagnosis by electron microscopic DOPA reaction test of fetal skin

There are several technical difficulties in the previous method for OCA prenatal diagnosis. First, it could be difficult to perform a fetal skin biopsy from the scalp depending upon the fetal placental position, and the risk of an inadvertent biopsy from the face or eyes. Second, it might not be possible to identify the hair bulb melanocytes by serial sectioning of small fetal skin samples. Melanocytes with mature melanosomes appear at 16 weeks of gestation, but 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. If the tyrosinase activity of each fetal melanocyte in the interfollicular epidermis could be evaluated accurately at the electron microscopic level, then fetal scalp biopsy would be unnecessary. In 1992, we succeeded detecting the presence of tyrosinase in fetal skin using electron microscopic dihydroxyphenylalanine (DOPA) reaction test.

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 OCA. Preliminary examination demonstrated that the electron microscopic DOPA reaction test was useful as a prenatal detection of tyrosinase negative OCA. Melanocytes from the patient’s epidermis contained mature stage IV melanosomes. The skin was incubated in DOPA solution as described previously and embedded in epon. Ultrathin sections were examined by electron microscopy. Results demonstrated that even after incubation with DOPA solution, further melanization was observed in the skin of the proband with tyrosinase negative OCA. In the skin obtained from three normal Japanese control fetuses, melanosomes of stages I, II
and III were observed in conjunction with a few stage IV melanosomes. Stage I melanosomes are round membrane-bound vesicles usually with an amorphous content. Stage II melanosomes are oval and about 300 nm in length and 100 in width. Often there is a matrix consisting of parallel filaments or sheets traversing the long axis of the organelle. The filaments exhibited a periodic beading which appear as cross-striations in a few sections. Stage III and IV melanosomes are moderately or heavily pigmented melanosomes. After incubation with the DOPA solution, most of the premature melanosomes were melanized to stage IV (Fig 3B).51

Prenatal diagnosis from a skin biopsy at 20th week of gestation obtained from the upper back of the fetus at risk of tyrosinase negative OCA was examined by electron microscopy. The melanocytes in the ordinary epidermis revealed 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 3A). These results suggested the absence of the tyrosinase activity in the fetal melanocyte. The parents decided to terminate the pregnancy at 21 weeks of gestation and abortus was confirmed to be affected with tyrosinase negative OCA. The skin demonstrated no tyrosinase activity51 with the retinal melanocytes containing immature melanosomes.52 This new strategy establishes that the, electron microscopic DOPA reaction test of fetal skin provides a reliable prenatal diagnosis of tyrosinase negative OCA in the second trimester.49

Prenatal diagnosis by analysis of the fetal tyrosinase gene

Tyrosinase negative OCA is caused by pathologic mutations in the tyrosinase gene. Tyrosinase, a key enzyme in the biosynthesis of melanin in pigment cells, catalyzes the conversion of tyrosine to DOPA. Since the first reported mutation that produces OCA1A,44 more than 25 alleles, each with a different mutation, have been found in patients with OCA1A.43 Of these, only two major tyrosinase gene mutations have been found in Japanese patients,44,53 a single base insertion in exon 2 shifting the reading frame and introducing a premature termination codon (TGA) following amino acid residue 298 (codon 316),44 and a single base mutation in exon 1, causing an arginine to glutamine substitution at position 59 (codon 77).53 Previously the prenatal diagnosis of OCA had been possible only by analyzing the fetal skin biopsy. Not only is the fetal skin biopsy more invasive than amniocentesis, it cannot be performed earlier than 19 weeks.54 Therefore, we attempted to establish a new strategy for the prenatal diagnosis of OCA at an earlier gestational age with a less invasive procedure to analyze the fetal tyrosinase gene.55

The proband is a 9-year-old Japanese boy with OCA1A, whose mother was in her second pregnancy. Polymerase chain reaction amplification and allele-specific oligonucleotide hybridization revealed that the child was homozygous and the parents heterozygous for a mutation of the tyrosinase gene in exon 2 (single base insertion) but not exon 1 (Fig 4). Prenatal diagnosis was performed by analyzing the tyrosinase gene in fetal cells obtained by amniocentesis at 14 weeks of gestation, demonstrating the fetus to be heterozygous for the mutant tyrosinase gene (Fig 4). The pregnancy, was therefore continued, and a normal male infant was born.55 The analysis of the fetal genomic tyrosinase DNA is a rapid and reliable approach to the prenatal diagnosis of tyrosinase negative OCA. It can be performed safely at an earlier gestational age and is less invasive than previous methods.
Fig 4  Family pedigree and results of PCR amplification and allele-specific oligonucleotide hybridization. Individual 13 is the proband, a 9-year-old Japanese boy with tyrosinase negative OCA. Number 16 is the fetus examined for prenatal diagnosis, and 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 sample examined (not all data shown). The mutant probe (M) for exon 2 hybridized with the amplified DNA of the proband (individual 13), his parents (4 and 10), his grandmothers (2 and 9), his three aunts (5, 11, and 12) and his cousin (15), but not with that of his grandfathers (1 and 8), his uncle (3), and his three cousins (6, 7, and 14). The normal probe (N) for exon 2 hybridized with the amplified DNA of all samples except that from the proband (13). On prenatal diagnosis, no nucleotide mutation was found on exon 1 of the fetal tyrosinase DNA (16). Amplified DNA of the fetus (16) hybridized with both mutant and normal probes for exon 2 of the tyrosinase gene, confirming that fetus was phenotypically normal and not affected with OCA, but was a heterozygous carrier of the mutant tyrosinase gene.

Discussion

Several genetic skin diseases are life threatening or have consequences that are so significant that the quality of life of affected individuals is severely compromised. For many families in which these disorders are expressed, there is a desire to recognize the condition of a fetus at risk. Some of these disorders can be detected through the use of cultured amniotic fluid cells, chromosomal analysis, assays of amniotic fluid, or blood. For other diseases, samples of fetal skin are obtained between the 18 to 19 weeks of pregnancy in utero and evaluated using morphologic, immunohistochemical, and biochemical techniques. Linkage analysis and identification of mutations for some of these diseases have permitted prenatal diagnosis to be accomplished in specific families using fetal DNA obtained from chorionic villi or amniotic fluid cells. Molecular analysis of fetal DNA is certainly the method of choice for prenatal diagnosis because it can be performed early in gestation and the results can be obtained in a day or two. However, this method is available for only a few of the diseases. In sampling the fetal cells for DNA analysis, the possibility of the contamination of the maternal cells should always be considered. To exclude this possibility, culture of the fetal cells for the later back up analysis is always desired. On the contrary, the experience in prenatal diagnosis using fetal skin samples is internationally extensive, thus evaluation of fetal skin samples still remains a valid and an important procedure for distinguishing normal from affected fetuses in utero.

For example, until we have introduced DNA based prenatal diagnosis for OCA1A, fetal skin biopsy had been the only available method to distinguish normal and affected fetus. Similarly in epidermolysis bullosa, until recent successful introduction of DNA based prenatal diagnosis, all the previous fetal detection had been made by fetal skin biopsy. Further advances in molecular study of each inherited skin disorder should replace fetal skin sampling by DNA based prenatal diagnosis in the near future. Furthermore, elucidation of detailed genotype/phenotype correlation in respective condition would enable future introduction of gene therapy not only for the patients but also for the affected fetuses during pregnancy.

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