Inherited Skin Diseases: DNA-Based Diagnoses and Prenatal Diagnoses

Hiroshi SHIMIZU
Department of Dermatology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan

ABSTRACT Among a range of inherited skin disorders, some of markedly severe phenotypes have been widely considered as indications for prenatal diagnoses. Recent studies have achieved significant progress in understanding the molecular basis of these heritable skin diseases. These include epidermolysis bullosa (EB), a group of mechanobullous genodermatoses, ichthyosis, a heterogeneous condition of generalized hyperkeratosis, and oculocutaneous albinism, in which an abnormal melanin synthesis in the skin and eyes is a hallmark. The responsible gene for the dystrophic forms of EB has been identified as a gene encoding type VII collagen, for lethal Herlitz EB the genes encoding the $\alpha_3$, $\beta_3$, or $\gamma_2$ chain of laminin 5, for pyloric- atresia juctional EB syndrome the genes encoding the $\alpha_6$ or $\beta_4$ integrins, and for tyrosinase negative oculocutaneous albinism the tyrosinase gene. Specific mutations in the responsible gene or genes have implications for understanding the structure–function relationship and phenotype/genotype correlation of each disorder, and also provide the basis for DNA-based diagnoses as well as prenatal diagnoses for families at risk of a recurrence of the disease. Furthermore, understanding the genetic basis of each inherited skin disease sets the stage for gene therapy approaches for the treatment of the respective condition.

Key words: prenatal diagnosis, epidermolysis bullosa, albinism, ichthyosis, fetal skin biopsy

1) Introduction
In dermatology, there are a range of inherited skin diseases, or genodermatoses, in which the responsible genes have been recently identified. Of these, some conditions with severe clinical features have been considered to be indications for prenatal diagnoses (PND). The majority of severe genodermatoses are

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Abbreviations: EB, epidermolysis bullosa; OCA, oculocutaneous albinism; OCA1A, tyrosinase-negative oculocutaneous albinism; PA-JEB, pyloric atresia- junctional EB syndrome; PND, prenatal diagnosis.
Correspondence to: Hiroshi Shimizu, M.D., Ph.D.
Fax: +81-3-3351-6880, e-mail: shimizu@med.keio.ac.jp
transmitted in an autosomal recessive mode, and people usually do not realize that they are heterozygote carriers of certain responsible genes until they have become the parents of a new born affected with a disorder.

PND of inherited skin diseases were first introduced in the 1980s with a fetal skin biopsies. Fetal skin biopsies were undertaken under fetoscopy or ultrasound guidance using small forceps (Suzumori and Kanzaki, 1991). The biopsied specimen could be examined with light and electron microscopy for morphological abnormalities. The subsequent development of monoclonal antibodies or biochemical techniques made it possible to evaluate abnormalities in the protein level of fetal skin samples (Heagerty et al., 1986b).

One of the inevitable disadvantages of fetal skin biopsies is that they can be performed only during the second trimester of pregnancy at around 18–20 weeks of gestation. Biopsies can only be taken when the fetal

<table>
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<th>Disease at risk</th>
<th>Pt. No.</th>
<th>Results</th>
<th>Year</th>
<th>Reference</th>
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EB: epidermolysis bullosa
PA-JEB: pyloric atresia-junctional epidermolysis bullosa syndrome
skin is morphologically and biochemically established sufficiently enough for PND. With this technique, the parents of a fetus at risk thus remain anxious for a prolonged period regarding the status of their baby (Shimizu, 1996).

Applications of molecular biological techniques have allowed the identification of specific mutant genes responsible for a number of inherited skin diseases. Genes have been discovered and DNA-based diagnoses have already been introduced in dermatology. Based on the same approach, DNA-based PND has become possible for a range of severe inherited skin diseases, including recessive dystrophic EB (Christiano et al., 1996), Herlitz junctional EB (Christiano et al., 1997), Dowling-Meara EB simplex (Rugg et al., 1997), tyrosinase negative oculocutaneous albinism (Shimizu et al., 1994c), bullous congenital ichthyosiform erythroderma (Rothnagel et al., 1994), Sjogren-Larsson syndrome (Sillen et al., 1997), lamellar ichthyosis (Bichakjian et al., 1998), Fabry’s disease (Caggana et al., 1997), and type II Hunter’s mucopolysaccharidosis (Bunge et al., 1994). A chorionic villus sampling at 10 weeks’ gestation or amniocentesis at 13 weeks’ gestation during the first trimester can be carried out to obtain fetal DNA for prenatal testing. The diagnosis can be made in 24 to 48 hours through DNA analysis, and the results obtained before the pregnant mother feels the first fetal movement. The elucidation of precise gene defects in families has led to the development of DNA-based PND (Shimizu, 1996). Although fetal skin biopsies are gradually being superseded by gene analysis, the fetal skin biopsy still remains the sole strategy for the PND of lethal skin diseases such as Harlequin ichthyosis for which the responsible gene-protein complex has not yet been identified.

Since we started the Special Clinic for Genetic Counseling in Dermatology at Keio University Hospital in Tokyo, we have conducted PND for certain severe genodermatosis by analysis of fetal DNA or by fetal skin biopsy as shown in the Table 1 (Shimizu et al., 1994c; Ishiko et al., 1994; Christiano et al., 1996; Shimizu et al., 1995; Takizawa et al., 1998a, 1998b; Shimizu et al., 1992, 1994a, 1994d, 1996a, 1996b, 1998; Akiyama et al., 1999).

2) Epidermolysis bullosa (EB)

Among fetuses with a range of inherited skin diseases, those at risk of having severe forms of epidermolysis bullosa (EB) have been the most frequent indications for PND in dermatology. EB encompasses more than twenty subtype conditions with the common characteristic of skin fragility and blister formation after seemingly minor or insignificant trauma to the skin (Fine et al., 1991). Based on the ultrastructural location of blister formations, EB is divided into three major categories, simplex, junctional and dystrophic. In EB simplex, blister formation occurs within the basal cells or lower epidermis. In junctional EB, separations arise within the lamina lucida between the plasma membrane of the basal cell and the lamina densa. In dystrophic EB, skin cleavages occur in the dermis just beneath the lamina densa. It has become widely accepted to diagnose prenatally for a few severe subtypes of EB phenotypes inherited recessively. These subtypes include lethal Herlitz junctional EB (HJEB) and the pyloric atresia-junctional EB syndrome (PA-JEB) in which death of the patient usually occurs before one year of age, as well as recessive dystrophic EB (RDEB) in which there is marked fragility of the skin associated with severe mutilation, a fusion of fingers and toes, and the later onset of squamous cell carcinoma.

Morphological abnormalities of the epidermal basement membrane of the fetal skin were the sole PND hallmark of EB in the early 1980s. Therefore, electron microscopy played a crucial role in the classification as well as PND of EB (Anton-Lamprecht and Arnold, 1987; Holbrook et al., 1993; Eady et al., 1994). Specific ultrastructural abnormalities of epidermal basement membrane components were found in the skin of patients
with severe forms of EB, including immature hypoplastic hemidesmosomes in HJEB and PA-JEB, and the absence, or markedly perturbed anchoring fibrils in RDEB (Hashimoto et al., 1976). These structural abnormalities were found to be already present in the skin of affected fetuses at 17 to 20 weeks of gestation. Based on this morphological hallmark, the PND of HJEB (Rodeck et al., 1980), PA-JEB (Nazzaro et al., 1990) and RDEB (Anton-Lamprecht et al., 1981) were first successfully introduced in clinical practice with electron microscopic observation of fetal skin specimen.

The production of monoclonal antibodies specific to skin basement membranes led to the discovery of several selective molecular defects in the skin of patients with various forms of EB (Heagerty et al., 1986b). Type VII collagen, laminin 5 or α6β4 integrins were found to be specifically absent or markedly reduced in the skin of patients with RDEB, HJEB or PA-JEB, respectively. These phenomena were observed as occurring in the skin of affected fetuses as early as the second trimester, making it possible to diagnose the conditions using these monoclonal antibodies as diagnostic probes (Heagerty et al., 1986a, 1986c; Fine et al., 1990; Shimizu et al., 1994a, 1996a).

Based on the morphological and molecular defects specific to EB subtypes, responsible genes for severe subtypes of EB have been elucidated. For example, all subtypes of dystrophic EB, including severe generalized and milder forms of RDEB, were found to be caused by mutations of genes encoding type VII collagen, COL7A1 (Uitto et al., 1994). Accordingly, the DNA-based PND of EB was first introduced for identifying the RDEB subtype (Christiano et al., 1996). Absence of laminin 5 in the HJEB leads to the elucidation of specific mutations in each of the LAM3A, LAMB3 and LAMC2 genes (Uitto et al., 1994) encoding three polypeptide subunit chains, α3 (150 kDa), β3 (125 kDa) and γ2 (100 kDa) (Burgeson et al., 1994). R635X and R42X mutations in the LAMB3 gene have been found to be hot spot mutations in European and American patients with HJEB (Kivirikko et al., 1996). This finding contributed to making it possible for mutation detection in families that underwent DNA-based PND (Christiano et al., 1997). However, R635X and R42X mutations were found to be rare in Japanese counterparts (Shimizu et al., 1997). Instead, W610X and Q166X were frequently found in Japanese families with HJEB, and were thus used for their DNA-based PND (Takizawa et al., 1998). In PA-JEB, the absence or markedly reduced expression of both α6 and β4 integrins (Gil et al., 1994; Shimizu et al., 1996a), a component of hemidesmosome, led to the elucidation of responsible genes for this unique syndrome. It was found that mutations of either the α6 integrin gene (Pulkkinen et al., 1997; Ruzzi et al., 1997) or β4 integrin gene (Vidal et al., 1995; Takizawa et al., 1997) lead to the absence of α6β4 integrins and cause pyloric atresia during the development of the fetus as well as the JEB phenotype due to an abnormal formation of hemidesmosomes. Such molecular information has made it possible to diagnose PA-JEB prenatally (Ruzzi et al., 1997).

3) Harlequin ichthyosis

Ichthyosis is a heterogeneous condition in which hyperkeratosis is the common hallmark. Harlequin ichthyosis (HI) is a severe and usually fatal congenital ichthyosis with an autosomal recessive inheritance pattern. The clinical features of the condition include thick, plate-like scales with ectropion, eclabium and flattened ears (Williams and Elias, 1987; Akiyama et al., 1998). Because the responsible gene for HI has not yet been identified, DNA-based tests are not available for the condition at the present time. Fetal skin biopsies and ultrastructural detection of abnormal keratinization in the fetal skin still remain the sole reliable strategies for the PND of HI, although structural abnormalities of amniotic fluid cells provide additional information (Akiyama et al., 1994). The characteristic ultrastructural phenotype of HI in the interfollicular fetal epidermis
does not occur in the fetus until 20 weeks of gestation. Although the necessity of fetal skin biopsies for the PND of inherited skin disorders has become underestimated, HI is a clear example for which DNA-based testing is not effective, and fetal skin biopsies are indispensable (Blanchet-Bardon et al., 1983; Suzumori and Kanzaki, 1991). We recently confirmed that the HI phenotype can be identified through an observation of keratinizing hair canals of the fetus at 19 weeks of gestation. The PND of this condition is thus also feasible at this stage (Akiyama et al., 1998 in press).

4) Oculocutaneous albinism (OCA)

Absence or reduced production of melanin pigments in melanocytes leads to oculocutaneous albinism (OCA). OCA, inherited in an autosomal recessive mode, is characterized by a reduction of melanin pigment biosynthesis in the skin, hair and eyes (King and Summers, 1988; Tomita, 1993; Shimizu, 1997). It results in a predisposition to skin cancer and reduced visual acuity associated with nystagmus. Tyrosinase negative OCA, or OCA1A, is the severest subtype in which the activity of tyrosinase, the key enzyme for melanin synthesis, is completely absent. In the 1980s, PND of OCA were first carried out using electron microscopic examinations of hair bulb melanocytes in the fetal scalp skin at 20 weeks’ gestation (Eady et al., 1983). From 1992 with the introduction of electron microscopic DOPA reaction tests of fetal skin, the PND of OCA1A became possible through fetal skin biopsies from all parts of the body (Shimizu et al., 1992). Although interfollicular epidermal melanocytes have only premature melanosomes at 16 weeks of gestation, they already possess tyrosinase activity (Holbrook, 1983). The tyrosinase activity of each fetal melanocyte in the interfollicular epidermis can thus be evaluated accurately at the electron microscopic level, making fetal scalp biopsies unnecessary (Shimizu et al., 1994b).

Based on the evidence of the lack of tyrosinase activity in OCA1A, specific mutations of the tyrosinase gene were identified in patients with this condition (Tomita et al., 1989). Accordingly, the first successful DNA-based PND of OCA1A was performed in a Japanese family at risk through an analysis of the fetal tyrosinase gene (Shimizu et al., 1994c). DNA-based prenatal tests of this condition were subsequently carried out in other countries (Falik Borenstein et al., 1995). Further molecular studies of other subtypes of OCA have also progressed.

5) Future prospects of the prenatal diagnosis

What developments can be expected in PND for severe inherited skin diseases in the year 2000s A preimplantation diagnosis is an alternative to conventional PND. In this process the genetic defects of an embryo are identified before the embryo is implanted and pregnancy is established (Handyside and Delhanty, 1997). Preimplantation diagnosis basically includes ovarian stimulation, egg retrieval and in vitro fertilization. In this new method, in vitro fertilization techniques consisting of a blastomere biopsy of the 8 cell embryo and a DNA analysis of single blastomeres are utilized for testing. Disease-free embryos are selected for transfer to the uterus, thereby avoiding the need to terminate of fetuses diagnosed as affected by PND.

In single gene disorders, affected embryos are usually detected by carrying out a mutation analysis at the single cell level, achieved through using nested PCR to amplify short target sequences containing the region of interest. Preimplantation diagnoses have been applied in identifying cystic fibrosis, Tay-Sachs disease, Lesch-Nyhan syndrome, β-thalassemia, spinal muscular atrophy, adrenoleukodystrophy, Marfan syndrome, Huntington’s chorea, familial adenomatous polyposis coli, and Charcot-Marie-Tooth 1A (McGrath and Handyside, 1998). In the case of X-linked recessive disorders such as Duchenne muscular dystrophy, a female
embryo which is not affected is selected. Preimplantation diagnosis should also be a viable means to prevent the development of severe hereditary skin diseases since most of these conditions follow Mendelian inheritance patterns. Recently, trials have been carried out of preimplantation genetic diagnoses against the risk of HJB, and the selection of unaffected embryos was reported to have been successful (Cserhalmi-Friedman et al., 1998).

Another alternative is the application of fetal blood cells which appear in the maternal blood. Recent studies have clarified the presence of various fetal cells, such as trophoblasts, erythrocytes, and leukocytes, which circulate in maternal blood. Among these fetal cells, nucleated erythrocytes appear to be suitable for PND because they are uncommon in the peripheral blood of normal adults and are most abundant in the blood of the mother (Sekizawa et al., 1996a, 1996b). Technical advances enable the isolation of fetal cells from maternal blood using a discontinuous density gradient method with Percoll and a micromanipulator (Takabayashi et al., 1995). PCR and DNA analyses of single cells permit non-invasive prenatal genetic diagnoses. The PND of fetal RhD blood types and Duchenne muscular dystrophy have been reported using this method (Sekizawa et al., 1996a, 1996b). The difficult technical problem that must be overcome with this method, however, is to identify whether selected cells are fetal or maternal in origin, especially when the fetus is female and does not carry the Y chromosome.

In summary, recent studies have clarified the morphological, immunohistochemical and molecular abnormalities of a range of inherited skin disorders. Based on these data, responsible genes have been identified in many genodermatoses. In association with scientific achievements, strategies for diagnosis as well as PND, and the content of gene counseling have been changed. In the year 2000s, it can be expected that DNA-based diagnoses and PND will be applied in a much wider range of inherited skin disorders.

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