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Prenatal Diagnosis for Inherited Skin Diseases

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The last 20 years have seen major advances in developing methods for prenatal testing of inherited skin disorders. Initially, the techniques involved fetal skin biopsy sampling through direct visualization of the fetus using fetoscopy. Subsequent improvements in ultrasonography with real-time high-resolution scanning led to accurate tissue biopsying without the need for a fetoscope. The usefulness of this approach was, however, limited to a small number of disorders and could only be undertaken as a second-trimester procedure. More recently, elucidation of specific gene abnormalities in a number of genodermatoses has resulted in the development of DNA-based diagnostic screening using fetal DNA from chorionic villi or amniotic fluid samples. These tests are applicable to a wider range of disorders and can be undertaken earlier in the pregnancy, usually in the first trimester. Advances in in vitro fertilization protocols and embryo manipulation technology have further led to the feasibility of even earlier assessment through preimplantation genetic diagnosis. This article reviews some of the significant advances in prenatal diagnosis of genodermatoses, one of the major translational benefits of basic skin research endeavors.

Fetal Skin Biopsy

Fetal skin biopsy samples are usually obtained at about 16 to 20 weeks' gestation. Assessment typically involves light microscopy and transmission electron microscopy. The first example of examination of fetal skin to assess risk of an inherited skin disorder was reported in 1980. In that case, microscopic examination of fetal skin sampled at 18 weeks' gestation led to a diagnosis of Herlitz junctional epidermolysis bullosa. Soon after, testing for other severe forms of epidermolysis bullosa was also reported. Subsequently, the characterization of antibodies to structural components of the dermal-epidermal junction during the 1980s also contributed to fetal skin biopsy analysis in such cases. Specifically, indirect immunofluorescence microscopy using antibodies to laminin 5 (junctional epidermolysis bullosa) and type VII collagen (dystrophic epidermolysis bullosa) provided valuable diagnostic support to complement transmission electron microscopy.

The global experience of fetal skin biopsy prenatal diagnosis was published in the proceedings of a Workshop of the World Congress of Dermatology (New York, 1992). More than 450 cases were reported, the major indications being junctional epidermolysis bullosa, dystrophic epidermolysis bullosa, non-bullous ichthyosiform erythroderma (or lamellar ichthyosis), and harlequin ichthyosis. Also assessed were pregnancies at risk for epidermolytic hyperkeratosis (bullous congenital ichthyosiform erythroderma), tyrosine-negative oculocutaneous albinism, epidermolysis bullosa simplex, Sjogren-Larsson syndrome, X-linked hypohidrotic ectodermal dysplasia, restrictive dermopathy, and Chediak-Higashi syndrome.

Since 1995, the indications for fetal skin biopsy in the prenatal diagnosis of inherited skin diseases have become less as discoveries of the molecular pathology of certain diseases have led to the development of DNA-based testing. Nevertheless, fetal skin biopsy remains the only method currently available for some disorders such as harlequin ichthyosis or restrictive dermopathy.

Fetal DNA Analysis

The first reports of fetal DNA analysis in dermatology involved mutation assessment in the keratin 10 gene in a case at risk for epidermolytic hyperkeratosis/bullous congenital ichthyosiform erythroderma. Initial reports for junctional and dystrophic epidermolysis bullosa were published in 1995. These tests involved assessment of fetal DNA at 10 to 12 weeks' gestation (extracted mostly from chorionic villi), considerably earlier compared to fetal skin biopsy sampling. For dystrophic epidermolysis bullosa, it is evident that all cases involve pathogenic mutations in a single gene, COL7A1 at 3p21, which encodes type VII collagen, the major component of anchoring fibrils at the dermal-epidermal junction. This means that prenatal testing can either be
based on direct mutation analysis or the use of indirect linkage markers.12

Conversely, junctional epidermolysis bullosa has emerged as a heterogeneous disorder with specific gene pathology in six different genes comprising the three genes encoding laminin 5 (LAMA3, LAMB3, and LAMC2), the 180-kDa bullous pemphigoid antigen (also known as type XVII collagen gene) BPAG2/COL17A1, and the genes for α6β4 integrin (ITGA6 and ITGB4).13 The Herlitz form of junctional epidermolysis bullosa, for which prenatal testing is most indicated, may involve nonsense mutations on both alleles of either LAMA3, LAMB3, or LAMC2.14–16 Thus, junctional epidermolysis bullosa should only be tested for using mutation analysis and should not be based on linkage studies.

DNA-based prenatal analysis has also been performed for Dowling-Meara epidermolysis bullosa simplex,17 other forms of junctional epidermolysis bullosa,18 ocularcutaneous albinism,19 lamellar ichthyosis,20 some mucopolysaccharidoses and other selected genodermatoses.1

Recent Gene Pathology Discoveries

The rapid advances in detection of specific gene pathology in a number of genodermatoses has led to the feasibility of DNA-based prenatal testing for several further disorders. Of note, a recent paper illustrated how more than 300 inherited single-gene diseases affecting the skin now have pathogenic mutations defined.21 Clearly, not all of these are of relevance in considering prenatal testing, but in many cases the option is now available. Such disorders might include Sjogren-Larsson syndrome (fatty aldehyde dehydrogenase gene, FALDH, on 17p11), Wiskott-Aldrich syndrome (WASP gene on Xp11.23), Chediak-Higashi syndrome (LYST gene on 1q42), pachyonychia congenita (keratin 6a, 6b, 16, and 17 genes, KRT6a, KRT6b, KRT16 or KRT17 on 12q11-q13 or 17q21-22), and Fabry’s disease (angiokeratoderma corporis diffusum, α-galactosidase gene on Xq21). Other recent additions to this list might include Netherton’s syndrome (SPINK5 gene on 5q32), Darier’s disease (ATP2A2 gene on 12q23), Hailey-Hailey disease (ATP2C1 gene on 3q21), dyskeratosi congenita (dyskerin gene, DKC1, on Xq28), and EEC syndrome (p63 gene on 3q27). To these could be added several photosensitivity and DNA-repair disorders and cancer-susceptibility syndromes. Overall, it is clear that the mutation data and new genetic discoveries are emerging rapidly, and therefore careful consideration should be given to the relevance and ethical acceptability of testing for certain disorders.

Workup for DNA-Based Testing

Compared to fetal skin biopsy sampling, DNA-based diagnosis has several practical advantages, including earlier and more widely available testing. However, an important prerequisite to undertaking any DNA-based prenatal test procedure is the prior delineation of informative genetic markers. In most cases, the first step is to obtain DNA samples from the parents and the affected individual (usually extracted from peripheral blood samples) to search for pathogenic mutations. This in itself depends on the accuracy of the original clinical diagnosis, which may often involve skin biopsy of the proband. Availability of DNA from the nuclear family will also enable other considerations such as the occurrence of de novo mutations, nonpaternity, uniparental disomy, and germline mosaicism to be addressed more fully. In most instances, molecular markers are family-specific and are best determined before pregnancy is contemplated as it may take several weeks to complete the DNA screening analysis. This is most important given the usually short window between confirming pregnancy and arranging a suitable time for chorionic villus sampling. The success of all DNA-based prenatal diagnostic tests depends, for the most part, on a clear understanding of the molecular pathology of the disorder in question. To date, the most experience has been obtained in assessing junctional and dystrophic epidermolysis bullosa, and some specific guidelines for prenatal testing of these disorders have been published.12,22 Such reports help set a framework for other genodermatoses under consideration for prenatal diagnosis.

Importance of Accurate Skin Biopsy Diagnosis in Proband

In neonates, skin biopsy is often crucial in establishing a precise diagnosis. In some inherited diseases light microscopy may be helpful—for example, in demonstrating eosinophilic spongiosis in suspected incontinentia pigmeniti. In other disorders, such as epidermolysis bullosa, further assessment is necessary because light microscopy is unable to make distinctions between clinically similar cases of neonatal epidermolysis bullosa, which often have profoundly different prognoses. The ideal biopsy consists of a sample of gently rubbed nonblistered skin. Biopsying blisters is inadvisable because any early re-epithelialization will distort tissue assessment and could lead to an erroneous diagnosis. The skin biopsy should be divided for electron microscopy and immunohistochemical analysis.13 Electron microscopy is useful in determining the plane of blister formation, and immunohistochemistry may provide specific clues to the abnormal candidate gene/protein system. Immunohistochemical analysis should include labeling with antibodies to laminin-5, the 180-kDa bullous pemphigoid antigen (also known as type XVII
collagen), and type VII collagen. Further antibodies (eg, to keratins 5/14; plectin, or α6β4 integrin) may also be indicated in certain cases. All these antibodies may show altered, attenuated, or absent basement membrane labeling in different subtypes of epidermolysis bullosa.

These skin biopsy data are a fundamental prerequisite to undertaking appropriate molecular investigations that may be relevant to genetic counseling and the approach to prenatal diagnosis in subsequent pregnancies at risk for recurrence.

**Heterogeneous Disorders**

Junctional epidermolysis bullosa represents one example of a genetically heterogeneous disorder. Nevertheless, provided that the precise mutations are known, DNA-based prenatal testing is feasible. In other disorders, such as lamellar ichthyosis, the situation is more complex. In this condition, it is important to first try to identify keratinocyte transglutaminase abnormalities in the keratinocyte transglutaminase gene (TGM1 on 14q11). This is because other cases of lamellar ichthyosis do not involve TGM1 mutations and may harbor pathogenic mutations in other genes. Indeed, some cases have shown linkage to at least three other loci on 2q, 3p, and 19p. Similar comments also apply to hypohidrotic ectodermal dysplasia. Some cases are X-linked and involve mutations in the EDA gene, whereas some autosomal inherited cases arise from mutations in the DL gene. Additional patients with this condition may have mutations in other, as yet uncharacterized, genes. Inherited skin diseases such as these emphasize the clear need for delineation of specific gene pathology in individual cases before the possibility of DNA-based prenatal diagnosis is ever contemplated.

**Experience of DNA-Based Prenatal Diagnosis**

In the last 4 years our laboratory has carried out DNA-based prenatal diagnosis in pregnancies at risk for recurrence of severe forms of epidermolysis bullosa. Thirty-five tests have been performed: 21 for Hallopeau-Siemens recessive dystrophic epidermolysis bullosa and 14 for junctional epidermolysis bullosa. The dystrophic epidermolysis bullosa cases have involved direct mutation analysis of COL7A1 in 17 of 21 cases and mutation analysis in combination with linkage (for an undisclosed mutation on one allele) for the remaining four. All tests involved chorionic villus sampling between 10 to 12 weeks' gestation (Fig 1). Testing for dystrophic epidermolysis bullosa revealed six genotypically normal fetuses, seven heterozygous carriers, and eight affected. By contrast, all junctional epidermolysis bullosa tests were based on mutation analysis (Fig 2). Most cases involved assessment of laminin-5 genes (9 tests: 5 genotypically normal, 3 carriers, 3 affected), but three cases involved β4 integrin mutations (1 genotypically normal, 2 carriers) and two cases involved screening the type XVII collagen gene (both carriers). All analyses were carried out within 72 hours, and all predictions were shown to be 100% accurate. In only one case was the pregnancy lost following the procedure.

One-half of the tests were performed locally, whereas in the remainder the chorionic villus samples were taken at other sites and sent to the laboratory in transport medium. Samples were either sent in RPMI (Roswell Park Memorial Institute) medium or, if maternal decidua was first cleaned from the villi, in proteinase K digestion buffer. The specimens included one from Spain and one from India, as well as other centers around the UK.

**Ethical Considerations**

Several important issues have emerged from the advent of the advances leading to DNA-based testing. For example, in epidermolysis bullosa, technical limitations meant that fetal skin biopsy assessment could only be
mortality and prolonged morbidity. Should these disorders be included in the list of suitable diseases to screen for? Perhaps there is no easy answer, and each case may necessitate individual discussion about the ethical rationale. The rapid accumulation of new genetic information has led to an increased demand for widening the scope for prenatal disease screening, all of which requires careful, formal, ethical consideration.

Preimplantation Genetic Diagnosis

Although DNA-based prenatal diagnosis through chorionic villus sampling represents a significant advance, using this approach in the diagnosis of an affected fetus often leads to consideration of termination of pregnancy. Preimplantation genetic diagnosis, however, represents an alternative approach. It offers distinct advantages for several groups of patients—for example, those at risk of passing on an X-linked disorder for which there is, as yet, no specific molecular diagnosis (eg, incontinentia pigmenti). Others who might benefit from this approach include those who have had to suffer repeated terminations of pregnancy and those with moral or religious objections to pregnancy termination.

Preimplantation genetic diagnosis involves in vitro fertilization and sampling of one or two cells from the embryo at the cleavage stage or blastocyst (Fig 3). The cells are then lysed in a buffer (alkaline lysis or proteinase K) and the DNA is amplified using a nested polymerase chain reaction (PCR) protocol. Amplified DNA can then be screened by heteroduplex analysis, restriction endonuclease digestion, or sequenced directly. Potential for misdiagnosis includes contamination by extraneous DNA (eg, from additional sperm attached to the embryo). This risk is reduced by carrying out fertilization using intracytoplasmic sperm injection with a single sperm. A further problem is assessment of DNA sequences from a single chromosome, either because the cell taken from the embryo is haploid or because of the phenomenon of allele dropout, when one allele fails to amplify in the initial PCR. This risk may be reduced by optimizing lysis buffers before the test is performed and also by sampling two cells in cases at risk for dominant diseases. Preimplantation genetic diagnosis has been applied to a range of autosomal dominant and recessive disorders. Autosomal dominant conditions include Marfan syndrome, myotonic dystrophy, Huntington's chorea, familial adenomatous polyposis coli, and Charcot-Marie-Tooth 1A disease. Autosomal recessive diseases tested for include cystic fibrosis, Tay-Sachs' disease, Lesch-Nyhan syndrome, β-thalassemia, spinal muscular atrophy, and adrenoleukodystrophy. Embryo sexing has also been used in preimplantation genetic diagnosis approaches, either using PCR probes specific for the X or Y chromosomes.
or using interphase fluorescent chromosomal in situ hybridization labeling. Disorders assessed in this way include Duchenne's muscular dystrophy, fragile X syndrome, and incontinentia pigmenti.

Conclusions

In the absence of specific treatments for patients with severe inherited skin diseases, prenatal testing in pregnancies at risk for recurrent disease now represents an option for many families. Recent gene analyses have disclosed pathogenic mutations in several disorders, and application of these findings to DNA-based prenatal diagnosis represents one of the major current translational benefits of disease-related genetic research. Technical advances have led to the feasibility of first-trimester diagnosis or even preimplantation genetic diagnosis in a range of genodermatoses. Careful consideration needs to be given to both diagnostic scientific rigor in each case and, perhaps even more fundamentally, to the ethical implications of which diseases and which other clinical, social, and moral factors need to be taken into account in pursuing prenatal diagnosis.

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