Prenatal Genetic Diagnosis for Pediatricians

Committee on Genetics

The pediatrician who cares for a child with a birth defect or genetic disorder may be in the best position to alert families to the possibility of a recurrence of a genetic disorder in future offspring. The family may wish to know and may benefit from methods that convert probability statements about recurrence risks into more precise knowledge about a specific genetic disorder in the fetus. Many families will find knowledge and choice preferable to chance. Knowledge in the field of genetics is expanding very rapidly. The purpose of this statement is to update the pediatrician about the status of prenatal genetic diagnosis as it relates to genetic counseling in clinical practice.

The pediatrician can assist in addressing questions about the natural history of the disorder under consideration, the possibility of prenatal diagnosis, or the plan for immediate care of an affected newborn. The information gained from prenatal diagnosis may be helpful to the obstetrician in the management of the pregnancy, labor, and delivery and in some instances this may improve the outcome of pregnancy. The availability of prenatal diagnosis gives couples options they might not otherwise have, including preparation for the birth of a child with an abnormality, termination of an affected pregnancy, or use of fetal treatment, which though now experimental, may be an option in the future. This enables couples at increased genetic risk to have children, when without this information they might have been unwilling to attempt a pregnancy.

The techniques currently in use or under investigation for prenatal diagnosis include the following: (1) fetal tissue sampling—amniocentesis, chorionic villi sampling, percutaneous umbilical blood sampling (PUBS), percutaneous skin biopsy, and other organ biopsies including muscle and liver biopsy; (2) fetal visualization—ultrasound, fetal echocardiography, embryoscopy, fetoscopy, magnetic resonance imaging, and radiography; (3) screening for neural tube defects (NTDs) by measuring maternal serum α-fetoprotein (MSAFP); (4) screening for fetal Down syndrome by measuring MSAFP, unconjugated estriol (uE₂), and human chorionic gonadotropin (hCG); (5) separation of fetal cells from mother’s blood; and (6) preimplantation biopsy of blastocysts obtained by in vitro fertilization.

INDICATIONS FOR FETAL CELL OR TISSUE SAMPLING

Chromosomal Analysis
Fetal chromosomal analysis should be offered when any of the following apply:

1. The mother is 35 years old or older at delivery.
2. There is a previous offspring with one of the trisomic syndromes or other chromosome aberrations.
3. A chromosome abnormality is present in either parent, e.g., aneuploidy, balanced translocation, or clinically significant inversion. This does not include normal variations such as pericentric inversion of chromosome 9.
4. There is a fetus at risk for a serious X-linked condition and specific prenatal diagnosis is not available or, in cases in which specific diagnosis of the X-linked disorder is available to determine the fetal sex as an initial step prior to performing other genetic studies.
5. A parent is a fragile X carrier. Women are usually identified as carriers by molecular testing prompted by a family history of a mentally retarded child, brother, or uncle who has the fragile X syndrome. Fragile X prenatal diagnostic studies on amniocytes and/or chorionic villi are best accomplished using molecular studies, although cytogenetic tests continue to be used as an adjunct to diagnosis in some laboratories.
6. There is an increased risk for Down syndrome based on results of maternal serum screening (utilizing either MSAFP alone or MSAFP in conjunction with uE₂ and hCG) in a woman who is younger than those ordinarily offered prenatal diagnosis on the basis of maternal age alone.
7. Ultrasound has identified an anatomic abnormality in the fetus, e.g., omphalocele, hydrocephalus, or renal defects, that might indicate an increased risk for a chromosome abnormality. Even when these abnormalities are found in the third trimester of pregnancy, chromosome analysis of amniotic fluid may be indicated. Alternatively, PUBS or transabdominal chorionic villi sampling may be used for chromosome analysis in the late second and third trimesters when diagnosis is needed more rapidly than can be accomplished by amniocentesis. Information obtained from these studies can influence decisions regarding the advisability of further fetal evaluation. The findings may also be important to the obstetrician in the management of labor...
and delivery and to the pediatrician, neonatologist, and geneticist in the immediate management of the newborn. This information can also help parents prepare for the birth of a child with a specific disorder.

Biochemical Studies

Biochemical studies are indicated when there is an increased risk of genetic transmission of an inborn error of metabolism. Often, this increased risk is ascertained when (1) the diagnosis of an inborn error of metabolism is made in a previous child; (2) both members of a couple are found to be carriers of an autosomal recessive disorder by screening based on ethnic origin, eg, Tay-Sachs disease in Ashkenazi Jews; or (3) carrier testing is performed based on the history of a disorder in a close relative with a specific disorder. Biochemical studies may be performed on amniocytes or chorionic villi. Study of other specific tissues, eg, fetal white blood cells, liver biopsy, etc, may be indicated when expression of the gene product, protein, or enzyme is confined to that tissue.

Measurement of amniotic fluid a-fetoprotein (AFAFP) can detect open NTDs, other open defects in the fetus such as omphalocele, and congenital nephrosis. Measurement of AFAFP and acetylcholinesterase may be indicated when there is an increased risk for NTDs. In some instances ultrasound examination of the fetus in conjunction with MSAFP measurement is considered adequate evaluation, based on the level of risk and the results of these studies. Measurement of amniotic fluid AFP to detect NTDs should be considered in pregnancies (1) in women with a family history of NTDs, particularly in a parent or sibling of the fetus; (2) of women with insulin-dependent diabetes mellitus; (3) during which there has been exposure to a drug associated with NTDs, eg, valproic acid; (4) in which there is an elevated MSAFP level; and (5) with ultrasound findings consistent with an NTD, other defect associated with an NTD, eg, hydrops, or other defect associated with elevated AFAFP value.

Routine and Electron Microscopy

Routine and electron microscopy of biopsies and cells have been used to diagnose rare genetic disease, eg, epidermolysis bullosa, for which specific genetic tests are not available.

Molecular Genetic Studies

The use of molecular biologic techniques in prenatal diagnosis is increasing rapidly. The clinical application of these methods is based upon the fact that the DNA complement is generally identical in every cell of the body, and therefore a hereditary defect detectable at the DNA level should be found in any nucleated cells from that individual. Enzymes (restriction endonucleases) that cleave DNA within a specific base sequence recognition site are used to identify mutant genes, deletions within the gene, or to characterize DNA polymorphisms that are linked to the gene in question. The polymerase chain reaction (PCR) is commonly employed to rapidly amplify these DNA samples for study. The DNA extracted from amniocytes, chorionic villi, and fetal blood cells can be used for diagnosis of genetic mutations or deletions within a gene that cause specific genetic disease. Restriction fragment length polymorphisms (RFLPs) and other molecular markers may be used to assess the risk of transmission of a closely linked genetic mutation, to assess uniparental disomy (UPD), when there is an apparently balanced translocation in the fetus, or in the situation of confined placental mosaicism involving a trisomy. Increasingly in situ hybridization of fluorescent DNA probes (FISH) to specific chromosomes is being used to define chromosome rearrangements including deletions and microdeletions that may be below the sensitivity of detection by standard cytogenetic analysis. Hybridization of fluorescent DNA probes to interphase nuclei is being investigated as a rapid screening method for aneuploidy.

Studies of DNA are currently applicable for the prenatal diagnosis of hemoglobinopathies, hemophilia A, Duchenne and Becker muscular dystrophy, cystic fibrosis, fragile X, myotonic dystrophy, neurofibromatoses, etc. Two kinds of probes can be used for genetic analysis: (1) When the DNA sequence of a specific gene or mRNA is known, it can be used to synthesize an oligonucleotide probe that is only a few nucleotides long (such as allele-specific oligonucleotide probes) and that will hybridize directly with the gene in question. (2) However, when the gene in question has not yet been identified, it is possible to use DNA sequences surrounding the location of the gene (such as for RFLPs and VNTRs). These are known as linked markers.

ASOs provide a direct method for screening for a mutation. These probes can be used for any disorders in which the nucleotide sequence of the mutant and normal alleles are known. Under the right conditions (known as hybridization stringency), a complementary oligonucleotide probe that exactly matches the mutated sequence will anneal with that sequence, while a probe for the normal sequence will not, and vice versa. Heterozygotes for a particular mutation can be identified because both probes anneal with the DNA to an equal extent.

In addition to restriction fragment length polymorphisms (RFLPs), another kind of DNA polymorphism is found at hypervariable loci throughout the genome, comprised of a variable number of identical sequences repeated in tandem. The function and mechanism of formation of these repeats are uncertain. When DNA from this type of variable number tandem repeat (VNTR) locus is digested with a restriction endonuclease that cuts outside of VNTR, the lengths of the DNA fragments produced in different individuals depend on the number of repeats at that locus. These repeated sequences can be anywhere from 2 to 60 nucleotides long, and the number of different alleles can vary from 2 to more than 20. The variability at a number of different VNTR loci can generate enough diversity to allow identification of a particular individual. Although different individuals may have some fragments in common, the chance that two individuals might have all fragments in common is low.

UPD, a mechanism of genetic disease that has only recently been understood, may cause reproductive loss, specific genetic disorders due to single genes, multiple developmental defects similar to those observed with chromosome aneuploidy, or defects related to lack of inheritance from the parent of a particular sex (imprinting effects). UPD occurs when both members of a chromosome pair in a conception or offspring are inherited from one parent, rather than one member of each chromosome pair being contributed by each parent. In some instances two different chromosomes may be inherited from the same parent. In other instances both members of the chromosome are copies of the same parental chromosome. In the latter situation a child is homozygous for loci for which that parent is heterozygous. This explains how a child may be homozygous for a recessive trait when only one parent is a carrier. These children may have multiple other problems related to being homozygous at many loci. UPD probably occurs most often when there is a trisomic conception and then a chromosome contributed by one parent is lost during mitosis. Therefore, mosaicism, cell lines with different chromosome constitutions in the same individual, may provide a clue to the presence of UPD in the cell line with the normal chromosome number. UPD may also occur when there is an apparently balanced translocation in the fetus.
matosis, and a number of other disorders. They are being applied experimentally to the prenatal diagnosis of many other disorders. Because of the rapid developments occurring in this field, the pediatrician may wish to contact a genetic center to determine whether molecular testing has become available for a given genetic disorder. Genetic centers can also provide advice on the use of these tests in specific clinical circumstances, particularly those in which manifestations may not occur until adulthood.

TECHNIQUES FOR CELL OR TISSUE SAMPLING

Amniocentesis

Transabdominal amniocentesis at 15 to 16 menstrual weeks of pregnancy is a well-established, safe, reliable, and accurate procedure and is the most commonly used technique for obtaining fetal cells and amniotic fluid.11-13 Amniocentesis is performed under ultrasound guidance, which at this gestational age also affords the opportunity for evaluation of fetal anatomy. Samples that are adequate for testing can be obtained by experienced operators in the majority of cases. Initiation and processing of the cultures can take 2 to 4 weeks, but results of cytogenetic analysis are available in 2 weeks or less in an increasing number of laboratories. In addition, supernatant amniotic fluid can be used for measurement of substances such as AFAFP, hormones, enzymes, etc. The results of laboratory studies on amniotic cell cultures are highly accurate (more than 99% for most biochemical and cytogenetic studies). Significant maternal injury from amniocentesis is rare. The major maternal risk, Rh factor sensitization, is largely preventable by administration of Rh immune globulin with the procedure. Because of the high potential rate of fetal to maternal bleeding, pre-existing Rh factor sensitization is considered a relative contraindication to CVS. When compared to amniocentesis, higher rates of maternal cell contamination and confined placental mosaicism with CVS may result in diagnostic ambiguity, leading to the need for additional invasive diagnostic tests.24-26 The inability to diagnose NTDs by CVS and to evaluate the structural anatomy of the fetus with ultrasound at the time of CVS can be circumvented by MSAFP screening at 16 weeks and second trimester ultrasound in the majority of cases.

Transabdominal chorionic villi sampling may also be performed in the second and third trimesters. This is used when amniocentesis is precluded by severe oligohydramnios or more rapid diagnosis is needed.

Sampling of Fetal Blood and Other Tissues

Fetoscopy for fetal blood sampling has been largely replaced by PUBS, also known as cordocentesis.27 A technique for percutaneous sampling of fetal blood from the hepatic vein is also available. When fetal blood sampling is done under ultrasonic guidance, there is a lower rate of pregnancy loss following the procedure than after fetoscopy. However, the risk of fetal blood sampling has not been determined by large prospective controlled studies. An increased risk of fetal loss has been observed with common indications for fetal blood sampling such as multiple anomalies or intrauterine growth retardation (IUGR) in the fetus. Fetal blood sampling is a technique that allows rapid fetal chromosome analysis. It is also useful for studying other fetal blood constituents such as platelets, hemoglobin, blood gases, etc.

A number of serious skin disorders, eg, epidermolysis bullosa letalis and serious genetic forms of ichthyosis, may be diagnosed histologically by skin biopsy specimens obtained percutaneously under ultrasonic guidance, with or without fetoscopy.28 Liver and fetal muscle biopsies have also been performed.29 However, for many genetic conditions, fetal biopsy has been replaced by molecular genetic studies of amniocytes or chorionic villi.

TECHNIQUES FOR FETAL IMAGING OR VISUALIZATION

Ultrasound

Ultrasound has become the primary method for imaging fetal anatomy. This technique may be used throughout pregnancy, when there is a clinical indi-
cation to monitor fetal growth, movement, position, and morphology; to assess amniotic fluid volume; and to establish gestational age and placental location. In some countries it has become standard practice to perform routine fetal ultrasound at least once between 15 and 20 weeks. In many other countries, such as the United States, ultrasound is often performed during this same time period, but the performance of the test is based on a wide range of specific indications. During the early second trimester, ultrasound is used to date the pregnancy, identify twins, diagnose some fetal structural anomalies, and examine the placenta and the amount of amniotic fluid. Many centers have ultrasound imaging equipment that provides high resolution of fetal anatomy. Many fetal organ systems and anatomic lesions can be visualized by ultrasound, including some genitourinary, gastrointestinal, skeletal, and central nervous system abnormalities. Fetal echocardiography, as described in a later section, may also identify many cardiac lesions. When a structural abnormality is found in the fetus by ultrasound, further diagnostic tests may be indicated. Ultrasound is also used to guide invasive sampling such as amniocentesis, CVS, cordocentesis, and various fetal biopsies.

Transvaginal ultrasound has allowed improved resolution of the anatomy of the embryo during the first trimester. The utility of this approach for earlier detection of certain fetal anomalies, eg, anencephaly, limb, and some cardiac anomalies, is being evaluated.

Fetoscopy

Fetoscopy, a procedure in which a fine-caliber endoscope is inserted into the uterus, was developed for fetal visualization and fetal blood sampling. Because of the risk of spontaneous abortion associated with fetoscopy, it is rarely used today and has largely been supplanted by improved ultrasound resolution of fetal anatomy and ultrasound-guided fetal blood sampling or needle biopsy.

Magnetic Resonance Imaging

As a fetal imaging technique, magnetic resonance imaging is being actively investigated, but its clinical application has been limited by fetal movement.

Radiography

Although radiography has been largely replaced by ultrasonography for the detection of anatomic lesions, it may still be indicated for specific disorders in selected cases, particularly osteochondrodysplasias, in the late second and third trimesters.

Fetal Echocardiography

Fetal echocardiography may be performed from 15 weeks and beyond to term. When used together with duplex and/or color flow Doppler, it can identify a significant number of major structural cardiac defects and rhythm disturbances. Fetal echocardiography should be considered when any of the following lead to an increased risk for congenital heart disease: (1) an extracardiac malformation identified by ultrasound; (2) exposure to potentially teratogenic agent; (3) family history of congenital heart defects, particularly in a parent or sibling; (4) suspected fetal chromosome abnormality or genetic disease associated with heart defects; (5) maternal diseases associated with fetal structural heart defects, such as diabetes or PKU, or maternal diseases associated with fetal cardiac arrhythmia, particularly heart block, such as lupus or other immune disorders; or (6) suspected cardiac defects based on the findings of routine ultrasound.

Embryoscopy

Embryoscopy is an experimental technique used in the first trimester of pregnancy. A rigid endoscope inserted via the cervix into the space between the amnion and chorion is used to visualize the embryo and offers the potential for diagnosis of structural malformations.

MATERNAL SERUM STUDIES

MSAFP Detection of NTDs

Please refer to the statement on MSAFP screening by the Committee on Genetics.

Maternal Serum Screening for Trisomy 21

A number of studies have demonstrated that low MSAFP concentrations are associated with an increased risk for trisomy 21 and perhaps other chromosomal aneuploidies in the fetus. Results of MSAFP screening may be used along with maternal age to revise an individual patient’s risk of having a child with Down syndrome. More recently, decreased maternal serum levels of uE3 and increased hCG have also been associated with Down syndrome in the fetus. Of these, hCG is the most sensitive marker for detection of Down syndrome. A combination of laboratory tests (MSAFP, estriol, and hCG), in conjunction with maternal age, has been used to evaluate the risk of Down syndrome and other chromosome abnormalities such as trisomy 18 and 45,X. The utility of this approach has been evaluated by several prospective studies. Screening programs using MSAFP alone or in combination with other maternal serum markers commonly report pregnancies at high risk of Down syndrome if a patient’s revised risk is equal to or greater than the risk of a 35-year-old woman. Adjustment of Down syndrome risk using MSAFP and other serum markers substantially increases detection of Down syndrome among women who are less than 35 years old and who ordinarily would not have been offered amniocentesis.

FETAL CELL SEPARATION FROM MATERNAL BLOOD SAMPLES

Separation of fetal cells from maternal blood samples obtained during early pregnancy is being actively investigated. In the future, these samples may provide a source of material for screening or diagnosis of chromosome abnormalities, particularly using fluorescent in situ hybridization (FISH) or other genetic disorders by amplifying DNA using PCR.

PREIMPLANTATION GENETIC DIAGNOSIS

Techniques to perform testing of cells obtained from biopsy of early cleavage stages or blastocysts of
pregnancies conceived through in vitro fertilization are currently being developed. These techniques may allow selective transfer into the uterus and implantation of those pregnancies that are not affected by a specific genetic disorder. Both the laboratory techniques for testing and the biopsy are considered experimental at this time.

CONCLUSION

Pediatricians may be called upon to counsel a family in which prenatal diagnosis is being considered or in which there is a fetus with a genetic disorder. In some settings, the pediatrician may be the primary resource for counseling the family. More frequently, counseling may already have been provided by a clinical geneticist and/or obstetrician. However, because of a previous relationship with the family, the pediatrician may be called upon to review this information and to assist the family in the decision-making process. The pediatrician should be familiar with the principles of prenatal genetic diagnosis and know how to apply them to specific problems in genetic counseling, diagnosis, and management in clinical practice. At the same time, pediatricians should be familiar with resources available in their region for obtaining information about whether and how a specific disorder can be diagnosed and when and where to refer patients for prenatal genetic diagnosis. The technology of prenatal diagnosis is changing rapidly, and genetic consultants can assist pediatricians in the appropriate utilization and interpretation of the diagnostic tests that are available.

Glossary of Abbreviations

Amniotic fluid a-fetoprotein (AFAFP)—the level of this specific fetal protein is usually elevated in amniotic fluid when the fetus has an open NTD, or other open defects, such as omphalocele or gastroschisis.

Allele-specific oligonucleotides (ASO)—probes that are designed with the nucleotide sequence of the mutant or of the normal alleles in order to directly identify whether the mutation is present in a DNA sample.

Chorionic villus sampling (CVS)—technique in which fragments of placenta are aspirated through a catheter or needle for genetic testing of the placental cells.

Fluorescent in situ hybridization (FISH)—hybridization of a DNA probe that is tagged with a fluorescent marker to DNA in interphase nuclei or to metaphase chromosomes.

Human chorionic gonadotropin (hCG)—placental hormone that may be increased in maternal serum when the fetus has Down syndrome.

Intrauterine growth retardation (IUGR)—delayed fetal growth that is less than 10th percentile expected for gestational age. This is assessed by sequential ultrasound measurements.

Maternal serum a-fetoprotein (MSAFP)—screening test that measures this specific fetal protein in mother’s serum. This protein is often increased in maternal serum when there is an open NTD in the fetus or other open fetal defects, such as omphalocele or gastroschisis. The level of this protein may be decreased in maternal serum when the fetus has Down syndrome.

Neural tube defect (NTD)—abnormality in the development of the neural tube, such as anencephaly or spina bifida.

Polymerase chain reaction (PCR)—a molecular genetic laboratory technique used to synthesize multiple copies of a specific piece of DNA. The PCR is used to increase the amount of DNA from a sample that is available for laboratory testing.

Percutaneous umbilical blood sampling (PUBS)—a method of obtaining fetal blood samples by withdrawing fetal blood from the umbilical cord using ultrasound guidance.

Restriction fragment length polymorphism (RFLP)—a type of molecular marker produced when specific enzymes (restriction enzymes) are used to cut the DNA. Measurement of the fragment length to which a DNA probe will hybridize can be used to track the transmission of a variation in the DNA (polymorphism) through a family. Linkage of an RFLP to the gene of interest can be used for genetic diagnosis.

Uniparental disomy (UPD)—describes a situation in which both members of a chromosome pair in a conception or offspring are inherited from one parent, rather than one member of each chromosome pair being contributed by each parent.

Unconjugated estril (uE)—pregnancy-related estrogen produced by the fetal adrenal gland, converted by the placenta, and conjugated by the maternal liver.

Variable number tandem repeats (VNTR)—DNA polymorphs found in certain areas of the human genome that are comprised of a variable number of identical sequences repeated in tandem. The number of repeats vary from individual to individual but are inherited and can therefore be traced through the family.

REFERENCES


1014 PRENATAL GENETIC DIAGNOSIS FOR PEDIATRICIANS.
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