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Prenatal Diagnostic Testing for Genetic Disorders

Prenatal genetic diagnostic testing is intended to determine, with as much certainty as possible, whether a specific genetic disorder or condition is present in the fetus. In contrast, prenatal genetic screening is designed to assess whether a patient is at increased risk of having a fetus affected by a genetic disorder. Originally, prenatal genetic testing focused primarily on Down syndrome (trisomy 21), but now it is able to detect a broad range of genetic disorders. Although it is necessary to perform amniocentesis or chorionic villus sampling (CVS) to definitively diagnose most genetic disorders, in some circumstances, fetal imaging with ultrasonography, echocardiography, or magnetic resonance imaging may be diagnostic of a particular structural fetal abnormality that is suggestive of an underlying genetic condition.

The objective of prenatal genetic testing is to detect health problems that could affect the woman, fetus, or newborn and provide the patient and her obstetrician–gynecologist or other obstetric care provider with enough information to allow a fully informed decision about pregnancy management. Prenatal genetic testing cannot identify all abnormalities or problems in a fetus, and any testing should be focused on the individual patient's risks, reproductive goals, and preferences. It is important that patients understand the benefits and limitations of all prenatal screening and diagnostic testing, including the conditions for which tests are available and the conditions that will not be detected by testing. It also is important that patients realize that there is a broad range of clinical presentations, or phenotypes, for many genetic disorders and that results of genetic testing cannot predict all outcomes. Prenatal genetic testing has many benefits, including reassuring patients when results are normal, identifying disorders for which prenatal treatment may provide benefit, optimizing neonatal outcomes by ensuring the appropriate location for delivery and the necessary personnel to care for affected infants, and allowing the opportunity for pregnancy termination.

The purpose of this Practice Bulletin is to review the current status of prenatal genetic diagnostic testing and the evidence supporting its use. For information regarding screening for fetal aneuploidy, refer to Practice Bulletin No. 163, Screening for Fetal Aneuploidy.

Background

Fetal genetic disorders are abnormalities in structure or function caused by differences in the genome that are distinct from those primarily caused by environmental or other disruptive factors. Increasingly, it is recognized that these distinctions are not always clear. A genetic predisposition may increase a person's susceptibility to environmental influences, and some genetic abnormalities may be symptomatic or apparent only under specific

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environmental conditions or circumstances. Some disorders have an epigenetic basis; that is, genes can be activated or silenced by modifications that may depend on the parent of origin or other influences. It is increasingly appreciated that inheritance and genetics are complex and that the current understanding of them is imperfect. Therefore, prenatal diagnosis can be complex, and it is not always possible to predict clinical outcome based on a prenatal genetic test. Additionally, prenatal diagnostic testing is available for some, but not all, genetic disorders.

In general, chromosomal abnormalities and single-gene disorders can be identified by analysis of fetal tissue. These conditions are most often the target of prenatal diagnostic testing. Chromosomal abnormalities in pregnancy are relatively common. Approximately 1 in 150 live births involves some type of chromosomal abnormality that results in an abnormal fetal or neonatal phenotype (1). Chromosomal aberrations are more common early in pregnancy; about two thirds of occult spontaneous abortions (ie, early embryonic death in an unrecognized pregnancy), one half of recognized miscarriages in the first trimester, and 5% of stillbirths are the result of a cytogenetic abnormality (2). An estimated 5–7% of infant and childhood deaths are the result of chromosomal abnormalities (3). Chromosomal abnormalities are also more common in the setting of multiple miscarriages and structural fetal abnormalities (4).

Chromosomal abnormalities include aberrations in chromosome number or structure. The most common abnormality of chromosome number is aneuploidy, in which there is an extra or missing chromosome or chromosomes. It is also possible to have one or more extra sets of chromosomes (eg, triploidy or tetraploidy). Abnormalities in chromosome number can be mosaic, which means that the abnormal number of chromosomes is not present in all cell lines.

In addition to abnormalities of chromosome number, aberrations in chromosome structure, such as deletions, duplications, translocations, and other rearrangements, also can occur. Although not all deletions and duplications are pathologic, some can be quite large and are easily identified with karyotype analysis; others are small microdeletions or duplications that are detectable only via chromosomal microarray, fluorescence in situ hybridization (FISH), or other specialized methods. In some cases, chromosomal translocations are present but balanced, meaning that the normal genomic content is preserved but rearranged. In other cases, translocations or other rearrangements can result in pieces of chromosomes being duplicated or missing entirely. Balanced chromosomal translocations most often are associated with a normal phenotype, especially if they are inherited, although they can lead to recurrent miscarriage or an increased risk of a genetic abnormality in offspring. Some translocations, though, may be missing genetic material that can be clinically significant. This is especially true for translocations that are new mutations rather than those that are inherited from a parent.

In contrast with larger rearrangements, some genetic disorders are caused by mutations in single genes. Diseases caused solely by abnormalities in a single gene are relatively rare. The phenotype of many single-gene disorders is influenced by modifying genes or by the independent actions of a combination of additional genes, often with environmental influences. Examples of single-gene disorders include sickle cell anemia, cystic fibrosis, hemophilia, and Tay–Sachs disease. Single-gene disorders can be detected by targeted genetic testing of fetal cells if the disorder has been diagnosed with certainty and the particular mutation in the affected family has been identified.

Even more common than chromosomal abnormalities are isolated structural birth defects such as congenital heart defects, neural tube defects, and facial clefts. These traits generally are determined by multiple genes along with environmental factors and usually are isolated (not associated with a genetic syndrome or diagnosis). Because a genetic component can exist, however, congenital anomalies occur more commonly within an affected family than in the general population. Isolated structural birth defects are caused by a complex interplay of genetic and environmental factors, so prenatal diagnostic genetic testing is usually not available using specific DNA methods; rather, diagnosis usually is made by ultrasonography or other imaging techniques.

Although most genes are encoded in the nuclear genome, the mitochondria contain their own distinct genome. Mitochondria are all maternally inherited from the cytoplasm of the oocyte. Mutations can occur in mitochondrial DNA and also cause disease. Because mitochondria are essential for aerobic metabolism, mitochondrial diseases commonly affect tissues with high energy requirements, such as the central nervous system, heart, and muscle. Prenatal diagnosis for mitochondrial diseases can be complex, and clinical outcomes are difficult to predict because of variation in the number of abnormal mitochondria as well as variability in association with a predicted phenotype.

Prenatal Diagnostic Laboratory Techniques

Several laboratory techniques can be used to test fetal samples for prenatal diagnosis. Each test provides different information, and the choice of test depends on the relevant abnormality and the preferences of the patient.

The leading indication for prenatal diagnostic testing is for diagnosis of fetal chromosomal abnormalities. Testing is most commonly done with cells obtained by amniocentesis or CVS using traditional karyotype analysis. This method is adequate for identification of all aneuploidies, including the trisomies, 45,X (Turner syndrome), other sex chromosome aneuploidies such as 47,XXY (Klinefelter syndrome), and large rearrangements. Mosaicism in the fetus may not be detected by karyotype analysis if the mosaicism is not present in the specific line of fetal cells obtained for testing. Because karyotype analysis relies on metaphase analysis of cultured cells, results usually are not available until 7–14 days after sampling. Culture failure is rare when testing cells obtained by CVS or amniocentesis, but it is more common when testing cells from a fetal death or stillbirth. The diagnostic accuracy of karyotype analysis is greater than 99% for aneuploidy and chromosomal abnormalities larger than 5–10 megabases (5).

Fluorescence in situ hybridization analysis uses fluorescent-labeled probes for specific chromosomes or chromosomal regions to identify the number of those chromosome regions that are present in a specimen. Fluorescence in situ hybridization can be performed on uncultured cells collected by amniocentesis or CVS to provide an assessment of the common aneuploidies. Results obtained by FISH analysis are available more rapidly than results obtained by conventional karyotype, usually within 2 days. The most common FISH panel is a screening test for chromosomes 13, 18, 21, X, and Y. Probes for other abnormalities such as 22q11.2 deletion syndrome are available but must be requested specifically. Fluorescence in situ hybridization also can be performed on metaphase cells after cell culture to assess for specific microdeletions or duplications when requested. Although FISH analysis has been shown to be accurate for the chromosomes in the panel, it should be considered a screening test. False-positive and false-negative results have been reported with FISH (6–8), and an abnormal FISH result should not be considered diagnostic. Therefore, clinical decision making based on information from FISH should include at least one of the following additional results: confirmatory traditional metaphase chromosome analysis or chromosomal microarray, or consistent clinical information (such as abnormal ultrasonographic findings or a positive screening test result for Down syndrome or trisomy 18) (9).

Chromosomal microarray analysis is a technique that can identify major chromosomal aneuploidy as well as submicroscopic changes that are too small to be detected by conventional karyotyping. Duplicated or deleted sections of DNA often are referred to as "copy number variants" (10). Chromosomal microarray analysis can identify nearly all abnormalities that are detectable with karyotype (except for balanced translocations and triploidy), but as with karyotype analysis, some cases of low-level mosaicism may not be identified. Like FISH, chromosomal microarray analysis can be performed either directly on uncultured tissue or on cultured cells. An advantage of direct chromosomal microarray analysis of uncultured cells is the fast turnaround time (approximately 3–7 days). Also, this technique can yield results from nonviable cells that would not grow in culture or provide a conventional karyotype. Thus, microarray is preferred over karyotype for cases of fetal death or stillbirth (10).

Chromosomal aberrations that are smaller than the resolution of conventional karyotype also can result in phenotypic anomalies; these copy number variants can be detected in the fetus using chromosomal microarray analysis. When structural abnormalities are detected by prenatal ultrasound examination, chromosomal microarray will identify clinically significant chromosomal abnormalities in approximately 6% of the fetuses that have a normal karyotype (11, 12). For this reason, chromosomal microarray analysis should be recommended as the primary test (replacing conventional karyotype) for patients undergoing prenatal diagnosis for the indication of a fetal structural abnormality detected by ultrasound examination (10). If a structural abnormality is strongly suggestive of a particular aneuploidy in the fetus (eg, duodenal atresia or an atrioventricular heart defect, which are characteristic of trisomy 21), karyotype with or without FISH may be offered before chromosomal microarray analysis.

Chromosomal microarray analysis has been found to detect a pathogenic (or likely pathogenic) copy number variant in approximately 1.7% of patients with a normal ultrasound examination and a normal karyotype (11), and it is recommended that chromosomal microarray analysis be made available to any patient choosing to undergo invasive diagnostic testing.

Other tests that can be performed include measurement of enzyme activity or other biomarkers, when indicated, to determine the presence of biochemical and other disorders, such as Tay–Sachs disease and Canavan disease. However, as DNA testing for specific mutations has become increasingly available and high-resolution ultrasonography has improved diagnostic accuracy, such testing is used less often (13).

Invasive Prenatal Diagnostic Testing Techniques

A variety of techniques are available to obtain fetal cells for diagnosis, including analysis of preimplantation

embryonic cells, CVS, and amniocentesis. Fetal blood and tissue are rarely required for prenatal diagnosis, and umbilical cord blood sampling and fetal biopsy are rarely performed for this indication. Analysis of cell-free DNA from maternal plasma has been used for prenatal testing for a number of DNA abnormalities or traits, such as Rh type, but cell-free DNA testing still is considered to be a screening method and is not sufficiently accurate to be considered diagnostic for any indication (14, 15).

Preimplantation Genetic Diagnosis

Preimplantation genetic diagnosis refers to the testing of an embryo for a specific genetic disorder before implantation. Preimplantation genetic testing is performed on polar bodies from the oocyte and zygote, a single blastomere from a cleavage-stage embryo, or a group of cells from the trophectoderm at the blastocyst stage. Preimplantation genetic diagnosis can be performed using either cytogenetic or molecular techniques on early embryos created by in vitro fertilization and can be used to test for most genetic conditions in which a mutation has been identified in the family. Because preimplantation genetic diagnosis uses only one or a few cells from the early embryo and errors are possible, confirmation of results with CVS or amniocentesis is usually recommended.

Chorionic Villus Sampling

Chorionic villus sampling for prenatal genetic diagnosis generally is performed between 10 weeks and 13 weeks of gestation. Placental villi may be obtained through transcervical or transabdominal access to the placenta. Using continuous ultrasonographic guidance, the tip of a needle or specialized catheter is placed in the placenta without entering the amniotic sac. Negative pressure with a syringe is used to aspirate a small amount of placental villi. Although data comparing the risks of transcervical and transabdominal CVS are limited, there appears to be no significant difference between the two approaches (5, 16).

The primary advantage of CVS over amniocentesis is that the procedure can be performed earlier in pregnancy and the viable cells obtained by CVS for analysis allow for shorter specimen processing time (5–7 days versus 7–14 days), so the results are available earlier in pregnancy. After an abnormal first-trimester ultrasound examination or screening test, the earlier CVS results allow for more management options, although amniocentesis also is an option for diagnosis.

The pregnancy loss rate from CVS has decreased over time (17). The most recent meta-analysis of studies that included a control group, including 8,899 women who had CVS and 37,388 who had no procedure, calculated a procedure-related loss rate of 0.22% (1 in 455) (18).

Although there have been reports of an association between CVS and limb-reduction defects, the risk of these anomalies appears to be very low, and the anomalies are more significant with procedures performed earlier than 10 weeks of gestation (19). In an analysis by the World Health Organization, an incidence of limb-reduction defects after CVS of 6 per 10,000 was reported, which is not significantly greater than the incidence in the general population (20). Women who are considering CVS and are concerned about the possible association of CVS with limb defects can be reassured that when the procedure is performed at or after 10 weeks of gestation, the risk is low and appears to be no greater than the risk among the general population (21). Another complication of CVS is vaginal spotting or bleeding, which may occur in up to 32% of patients after transcervical CVS (22); the incidence after transabdominal CVS is lower. The incidence of culture failure, amniotic fluid leakage, or infection after CVS is less than 0.5% (16, 22, 23).

Amniocentesis

Amniocentesis for the purpose of genetic diagnosis usually is performed between 15 weeks and 20 weeks of gestation, but it can be performed at any later gestational age. Many large, multicenter studies have confirmed the safety of genetic amniocentesis as well as its cytogenetic diagnostic accuracy (5). Typically, amniocentesis is performed using a sterile technique, a 22-gauge spinal needle, and continuous ultrasonographic guidance. An amniotic fluid sample of 20–30 mL is obtained from a pocket free of fetal parts and umbilical cord. If technically feasible, transplacental passage of the needle usually is avoided, especially in cases involving alloimmunization, although the data suggest that the procedure-related loss rate is not different with transplacental and nontransplacental approaches (24, 25). The procedure often is postponed if the amnion and chorion have not fused because there is a higher likelihood of failing to obtain amniotic fluid or requiring a second puncture.

The most significant risk of amniocentesis is pregnancy loss. As with CVS, the procedure-related loss rate of midtrimester amniocentesis has decreased over time, likely because of increasing experience and improvements in technique and imaging. Accurate data on miscarriage after amniocentesis are difficult to obtain because of the rarity of the outcome and the difficulty in comparing women who experience miscarriage after amniocentesis with an appropriate control group. Contemporary single-center procedure-associated loss

rates of 0.13% (1 in 769) to 0.27% (1 in 370) have been reported (17, 26). A recent meta-analysis of miscarriage risk after amniocentesis, including more than 42,000 women who underwent a procedure and 138,000 women who did not, estimated the loss rate due to the procedure to be approximately 0.11% (1 in 900) (18). The rate of procedure-related pregnancy loss that is attributable to a prenatal diagnostic procedure currently is estimated to be approximately 0.1–0.3% in procedures performed by experienced health care providers. The loss rates for amniocentesis and CVS are both very low. These data are calculated from reports from high-volume, experienced centers and may not apply to other situations. Also, when counseling patients about the possibility of miscarriage after amniocentesis, it is important to place the procedure-related risk in the context of the patient's background risk.

Minor complications from amniocentesis occur infrequently and include transient vaginal spotting or amniotic fluid leakage in approximately 1–2% of all cases (27). The perinatal outcomes of preterm premature amniotic membrane rupture are significantly better after amniocentesis than after spontaneous rupture of membranes at a similar gestational age; the perinatal survival rate in cases of amniotic fluid leakage after midtrimester amniocentesis is greater than 90% (28). Needle injuries to the fetus have been reported but are rare when amniocentesis is performed under continuous ultrasonographic guidance. Amniotic fluid cell culture failure occurs in 0.1% of samples (29, 30).

In the past, early amniocentesis has been performed between 10 weeks and 13 weeks of gestation using a technique similar to midtrimester amniocentesis (31). However, early amniocentesis has significantly higher rates of pregnancy loss and other complications than midtrimester amniocentesis. In a multicenter randomized trial, the spontaneous pregnancy loss rate after early amniocentesis was 2.5%, compared with 0.7% for traditional amniocentesis (27). Membrane rupture was more likely after early amniocentesis, and the incidence of clubfoot was 1.3%, compared with 0.1% after midtrimester amniocentesis. Significantly more amniotic fluid culture failures occurred after the early procedure, necessitating an additional invasive procedure for diagnosis (27). For these reasons, early amniocentesis (before 14 weeks of gestation) is not recommended.

Experience With Diagnostic Procedures

In early studies, it was shown that the incidence of pregnancy loss, blood-contaminated specimens, leaking of amniotic fluid, and the need for more than one needle puncture are related to the experience of the operator, the use of small-gauge needles, and ultrasonographic guidance (32–34). There also is a significant learning curve associated with the safe performance of CVS (35, 36), and most published data were collected in experienced, high-volume centers. Procedure-related loss rates may be different among health care providers with less cumulative experience.

Clinical Considerations and Recommendations

▶ *When should prenatal diagnostic testing be offered?*

All pregnant women should be offered prenatal assessment for aneuploidy by screening or diagnostic testing regardless of maternal age or other risk factors. Genetic testing should be discussed as early as possible in pregnancy, ideally at the first obstetric visit, so that first-trimester options are available. Pretest counseling should be a process of shared decision making and should include a discussion of the patient's risk of aneuploidy and other genetic diseases. The differences between screening and diagnostic testing also should be discussed.

▶ *Which patients are at increased risk of a fetal genetic disorder?*

Patients with an increased risk of a fetal genetic disorder include those in the following categories:

- Older maternal age—Although the risk of aneuploidy increases with increasing maternal age, age alone is not an effective screen for aneuploidy. In contrast, structural chromosomal abnormalities, including microdeletions and duplications, do not increase in frequency with maternal age (37).
- Older paternal age—Advanced paternal age is associated with an increased risk of having a child with a single-gene disorder such as achondroplasia, Apert syndrome, and Crouzon syndrome. Although there is no consensus, most studies have suggested age 40–50 years as a definition of advanced paternal age. The genetic risk is related mostly to an increased incidence of gene mutations that occur during spermatogenesis. Currently, there are no recommended screening or diagnostic panels that target the disorders that may be increased with advanced paternal age; pregnancies are managed with standard screening and diagnosis, including an ultrasound examination to evaluate fetal anatomy (38).
- Parental carrier of chromosome rearrangement—Women or men who carry balanced chromosome

rearrangements, such as translocations or inversions, typically have a normal phenotype themselves but are at risk of producing gametes with unbalanced chromosomes that result in genetic abnormalities in offspring. This may occur because of the loss or duplication of a small amount of genetic material, disruption of a gene, or alteration of gene function. For most rearrangements, the observed risk of an abnormal live-born child is less than the theoretic risk because some of these gametes result in nonviable conceptions and miscarriage. In general, carriers of chromosome rearrangements that are identified after the birth of a child with an abnormality have a 5–30% risk of having offspring with unbalanced chromosomes in the future, whereas those identified for other reasons (eg, during an infertility workup) have a 0–5% risk (2). Exceptions are some pericentric inversions, such as the one involving chromosome 9, which are seen as common variants in the general population and generally are considered to be of no clinical consequence (2).

- Parental aneuploidy or aneuploidy mosaicism—Women with trisomy 21, although subfertile, have an increased risk of having offspring with a trisomy (39). Women with 47,XXX and men with 47,XYY usually are fertile, and although limited data are available, they are not known to have a discernible increased risk of having offspring with a trisomy (40). The limited available data on men with Klinefelter syndrome (47,XXY) whose partners conceive by in vitro fertilization with intracytoplasmic sperm injection do not indicate an increased risk of aneuploidy in the offspring (41).
- Prior child with structural birth defect—Most birth defects, such as neural tube defects and congenital heart defects, are isolated and occur because of an interaction of multiple genes with environmental factors. Because there is a genetic component to such conditions, they have a tendency to recur in families. Although the recurrence risk of isolated structural abnormalities that are not associated with a recognized genetic syndrome varies by the anomaly and often by the sex of the affected child, it generally is in the range of 2–3%, but it may be higher depending on the number of affected individuals (42–44).
- Parental carrier of a genetic disorder—Parents who are affected by or are carriers of genetic disorders such as sickle cell disease, Tay–Sachs disease, and cystic fibrosis are at increased risk of having an affected child. Individuals who are affected by an autosomal dominant disorder such as neurofibromatosis have a 50% risk of transmission. Some autosomal dominant disorders seen in a previous child but with no other family history may have arisen as a new mutation. In such cases, there may be a small increased risk of recurrence, depending on the disorder (45). To ensure that any testing for recurrence is informative, a diagnosis established by molecular testing of the affected child usually is necessary. Such confirmation also will ensure that the risk for a future pregnancy has been assessed accurately.
- Previous fetus or child with autosomal trisomy or sex chromosome aneuploidy—The recurrence risk after one affected pregnancy is 1.6–8.2 times the maternal age risk of autosomal trisomies, depending on the type of trisomy, whether the index pregnancy was a spontaneous abortion, the maternal age at initial occurrence, and the maternal age at subsequent prenatal diagnosis (46, 47). The risk of a second autosomal trisomy appears to pertain to any chromosome, not just the trisomy occurring in the index pregnancy. The recurrence risk is less certain but also is elevated for 47,XXX and 47,XXY. The recurrence risk does not appear to be increased for 45,X or 47,XYY (46, 48).
- Structural anomalies identified by ultrasonography—The presence of a fetal structural abnormality increases the likelihood of aneuploidy, copy number variants such as microdeletions, and other genetic syndromes (11, 12, 49, 50). The risks are highly dependent on the number and nature of the structural abnormalities present in the fetus, and some anomalies (or combination of anomalies) are strongly associated with specific genetic abnormalities. For some structural abnormalities, the risk of genetic abnormality surpasses 50%, whereas other isolated malformations are only rarely associated with aneuploidy or other genetic conditions. The association of aneuploidy with ultrasonographic soft markers varies with different findings but generally is low in the presence of most of the minor markers (51, 52).

▶ *What laboratory tests are used to diagnose fetal genetic abnormalities?*

The laboratory testing performed to diagnose fetal genetic disorders depends upon the indication for the test, the gestational age at testing, and patient preferences. Patients at risk of aneuploidy should be offered CVS or amniocentesis for chromosomal analysis with karyotype. A patient at increased risk of having a pregnancy affected by a genetic disorder should be offered CVS or amniocentesis with DNA testing for the specific

Table 1. Tests Available for Prenatal Genetic Diagnosis

Test	Turnaround Time	Conditions Detected	Comments
Conventional karyotype	7–14 days	Chromosomal abnormalities > 5–10 Mb	Traditional method for diagnosis of chromosomal abnormalities
FISH —– Direct preparation (interphase)	24–48 hours	Rapid assessment of major aneuploidies (chromosomes 13, 18, 21, X, and Y)	FISH with direct testing of cells from CVS is less accurate than with cultured cells from CVS or amniocentesis. Results should be confirmed on cultured cells or have additional clinical features before acting on results.
FISH — Cultured cells (metaphase)	7–14 days	Microdeletions and duplications	Can be used to test for specific abnormalities when clinically suspected
Chromosomal microarray	3–5 days (direct testing); 10–14 days (cultured cells)	Copy number variants >50–200 kb	Whole genome screen for copy number variants. Detects major chromosomal abnormalities except balanced rearrangements and some triploidies. Detection varies with different microarray platforms.
Preimplantation genetic diagnosis	1–2 days	Genetic disorder in which familial mutation has been identified	Due to possibility of error, confirmation with CVS or amniocentesis is recommended
Molecular DNA testing	3–14 days (faster with direct testing than when cultured cells are required)	Genetic mutations previously demonstrated to be present in a family or suspected based on ultrasound or other findings in a fetus	Usually a targeted test focusing on a specific disorder (or category of disorders) suspected to be present in a fetus based on ultrasound findings or family history

Abbreviations: CVS, chorionic villus sampling; FISH, fluorescence in situ hybridization; IVF, in vitro fertilization.

mutation that causes the disease. Karyotype or microarray analysis should be offered in every case, although performing karyotype or microarray may not be necessary in a low-risk patient. Also, routine measurement of amniotic fluid alpha fetoprotein to screen for neural tube defects may not be necessary in all cases when amniocentesis is performed for other indications and the ultrasound examination is normal with good visualization of the fetal spine and head (Table 1).

In patients with a major fetal structural abnormality found on ultrasound examination, CVS or amniocentesis with chromosomal microarray should be offered (10). If a structural abnormality is strongly suggestive of a particular aneuploidy in the fetus (eg, duodenal atresia or an atrioventricular heart defect, which are characteristic of trisomy 21), karyotype analysis with or without FISH may be offered before chromosomal microarray analysis.

If a patient is at increased risk of having offspring with trisomy 13, 18, or 21 based on abnormal serum screening or cell-free DNA testing, amniocentesis with FISH plus karyotype or with karyotype alone should be offered. Additionally, chromosomal microarray analysis should be available to women undergoing invasive diagnostic testing for any indication. Because chromosomal microarray analysis does not require dividing cells, it is the best test for the assessment of fetal death or stillbirth (10).

Some structural malformations or patterns of malformations are characteristic of specific genetic disorders. Increasingly, molecular DNA testing for single conditions is available and may be appropriate. For other findings, such as a skeletal dysplasia, a panel of genes for common and similar conditions may be available.

▶ *What information should be provided to the patient before and after the diagnosis of a fetal genetic abnormality and how should this information be provided?*

Patients should be provided with general information about the disorders that are potentially detectable with genetic testing before making a decision to undergo the specific tests being offered. Although much of this counseling can be provided by the patient's obstetrician–gynecologist or other obstetric care provider, referral to a genetic counselor or other specialist with genetic training and expertise can be helpful in providing an

individualized determination of risk, especially in complex situations. In all cases where a fetal genetic abnormality is suspected, referral to a health care provider with genetics expertise can help with counseling, choosing the right test, and interpreting the test results.

Although prenatal testing historically has focused largely on Down syndrome, the range of clinically significant disorders that can be detected has expanded far beyond this one condition. Patients should be offered screening for structural defects with ultrasonography and maternal serum screening, carrier screening for single-gene disorders such as cystic fibrosis, and testing for chromosomal aneuploidy. When the diagnosis of a chromosomal abnormality or another genetic disorder in the fetus is made, the patient should receive detailed information, to the extent that information is available, about the natural history of the specific condition. With most fetal genetic or structural abnormalities, referral to specialists with expertise in the specific disorder is indicated because patient decision making requires accurate and detailed counseling. For many copy number variants identified by chromosomal microarray, interpretation requires consultation with a genetic counselor or specialist in prenatal genetic diagnosis. The option of pregnancy termination should be discussed when a genetic disorder or major structural abnormality is detected prenatally. Patients may benefit from additional testing, including ultrasonography or fetal echocardiography, and referral to appropriate obstetric and pediatric specialists or neonatologists to discuss pregnancy and neonatal management issues. Referral to parent support groups, counselors, social workers, or clergy may provide additional information and support for some patients.

▶ *What is the best test and what is the best tissue for genetic diagnosis in cases of fetal death or stillbirth?*

Genetic testing often is recommended in the evaluation of unexplained fetal death and stillbirth. The commonly available tests are conventional karyotype and chromosomal microarray analysis. Because a karyotype analysis can only be obtained with living tissue, it has a higher failure rate when used to test tissue from a stillbirth or fetal death. In contrast, chromosomal microarray analysis does not require viable cells and, therefore, is the preferred test for genetic analysis after fetal death or stillbirth. Also, the additional information that chromosomal microarray analysis provides can be beneficial in ascertaining a genetic cause for the fetal death (10).

Any type of fetal or placental tissue or amniotic fluid can be submitted for genetic testing by chromosomal microarray analysis. Care should be taken to avoid contamination with maternal tissue or blood.

If a conventional karyotype is the only test available to the patient and the timing of the fetal death is recent, amniotic fluid should be obtained by amniocentesis (53, 54). Amniocytes obtained in sterile fashion provide a greater likelihood of cell growth and an eventual result compared with tissue obtained after delivery.

▶ *How should women who have blood-borne infections, such as hepatitis B virus, hepatitis C virus, or human immunodeficiency virus, be counseled about prenatal diagnostic testing for fetal genetic disorders?*

Although somewhat limited, current data indicate that amniocentesis increases the risk of neonatal infection in women who are chronically infected with hepatitis B virus, and the rate of vertical transmission is dependent on viral load. The vertical transmission rate was not increased with amniocentesis in a group of women who had a low viral load, whereas women with a high viral load had a 21-fold higher rate of newborn infection (55). Also, it appears that women who are positive for hepatitis B e antigen have a higher risk of vertical transmission after amniocentesis (56).

The data regarding amniocentesis in women with hepatitis C are even more limited, but the risk of transmission appears to be low. A series of 22 pregnant women who were positive for hepatitis C virus and who underwent second-trimester amniocentesis included 16 women who had detectable hepatitis C RNA by polymerase chain reaction testing; only one of these 16 women had hepatitis C virus detected in the amniotic fluid. None of the resulting 10 newborns who were tested were positive for hepatitis C RNA, including the one with virus in the amniotic fluid (57).

Before the advent of multidrug therapy for human immunodeficiency virus (HIV) infection, amniocentesis in HIV-positive women was associated with an increased risk of vertical transmission (58). However, more recent small series of women taking combination antiretroviral therapy (CART) have suggested that the risk of newborn infection is not increased after amniocentesis, especially when maternal viral load is low or undetectable (59). Data from the French Perinatal Cohort included 81 HIV-positive patients who underwent amniocentesis and who were treated with CART using three or more drugs during their pregnancy; 94% of the patients initiated CART before their procedure (60). There was no difference in the rate of maternal-to-child transmission in this group compared with a CART-treated control group who did not have amniocentesis (0.0% [0 of 81] versus 1.2% [30 of 2,528]; P=1.0). Although data on maternal viral load were not reported in this study, it

is assumed that the low vertical transmission rate was related to low or undetectable viral loads in the CART-treated women and to the presence of antiretroviral medication in the amniotic fluid.

There are insufficient data to assess the risk of CVS in women with chronic viral infections. Also, there are no adequate data to define the degree of risk, but it is likely that transmission risk is higher in patients with multiple infections, such as simultaneous infection with HIV and hepatitis C virus.

Overall, pregnant women who are considering prenatal diagnostic testing and who have hepatitis B virus, hepatitis C virus, or HIV should be counseled about the possibility of an increased risk of transmission to the newborn that may come with CVS or amniocentesis. The potential risks of the procedure should be discussed in the context of the likelihood of detecting a fetal abnormality and the value that the test result might provide. In women with HIV infection, CART should be initiated and any procedure postponed until the viral load is undetectable (61). Transmission of HIV with amniocentesis does not appear to be increased in women treated with CART when the viral load is undetectable. Counseling is complex in these situations, and the advantages and disadvantages of invasive and noninvasive testing and screening options should be discussed.

▶ *How does prenatal diagnostic testing differ for women with multiple gestations?*

Counseling patients regarding the risk of aneuploidy and the risks of diagnostic testing in multiple gestations is more complex than for singleton pregnancies because of the presence of more than one fetus and because the data on multiple gestations are limited. In women who are pregnant with twins, formulas and tables have been used to estimate the risk of aneuploidy based on maternal age and ultrasonographic determination of zygosity (62, 63). Recent data, however, suggest that such models may overestimate the risk of aneuploidy in twins. Data from a large European population registry indicated that the adjusted relative risk of Down syndrome per fetus from multiple gestations is only approximately one half of that of singletons (64).

Counseling for multiple gestations should include a discussion of options for pregnancy management if only one fetus has aneuploidy. Such options include continuing the pregnancy, terminating the entire pregnancy, and selective second-trimester termination of the affected fetus.

There are limited data concerning the risk of fetal loss in twin gestations when amniocentesis or CVS is performed. Recent studies have estimated that the attributable loss rate of amniocentesis in twins is approximately 2% (65, 66). There are no data concerning loss rates after amniocentesis is performed in women with high-order multiple gestations.

Similar information for twin gestations from small, nonrandomized series exists for CVS (67, 68). In one recent systematic review, the procedure-related loss rate for CVS and amniocentesis in twin pregnancies was estimated at 1%. With CVS, there is the additional potential for cross-contamination, or inadvertent sampling of both fetuses that gives rise to misleading results; this risk has been estimated at approximately 1% (69).

Chorionicity is important in assessing risk in multiple gestations. A complex counseling issue arises with a monochorionic twin gestation, in which the likelihood of discordance in the karyotype is low, and patients may opt for having a karyotype analysis performed on a single fetus. In this situation, it is important to discuss the accuracy of determining chorionicity by ultrasonography. In rare circumstances, monochorionic twins can be discordant for chromosomal abnormalities; the rate of such discordance is unknown.

▶ *How should variants of uncertain significance be discussed with women after karyotype analysis or chromosomal microarray?*

Prenatal tests of all types, including ultrasonography, screening tests, and diagnostic tests, can provide results of uncertain significance. When so-called genetic "variants of uncertain significance" are detected with karyotype or chromosomal microarray analysis, the results should be discussed with the patient by a knowledgeable health care provider who has a good understanding of what is and is not known about the specific finding. Understanding of variants of uncertain significance is rapidly evolving, and referral to a genetics expert for consultation and counseling can assist the patient with informed decision making.

▶ *How often does chromosomal mosaicism occur in amniocentesis or chorionic villus sampling results and what does it mean?*

Chromosomal mosaicism, the presence of more than one cell line identified during cytogenetic analysis, occurs in approximately 0.25% of amniocentesis specimens and 1% of chorionic villus specimens (70–72). Mosaicism can be suggested when the fetal specimen contains maternal cell contamination, causing a false-positive mosaic result. These false-positive results can be minimized by discarding the first 1–2 mL of the amniocentesis specimen and by careful dissection of chorionic villi from maternal decidua. Mosaicism is higher in CVS samples that are tested directly, whereas the rate

of mosaicism is much lower in CVS testing of cultured trophoblasts.

When mosaicism is found by CVS, amniocentesis typically is offered to assess whether mosaicism is present in amniocytes. In approximately 90% of cases, the amniocentesis result is normal, and the mosaicism is assumed to be confined to the trophoblast, a condition called confined placental mosaicism (70). Although confined placental mosaicism is unlikely to cause defects in the fetus, it carries an increased risk of third-trimester growth restriction (73). Confined placental mosaicism also can be associated with so-called "trisomy rescue" of an originally trisomic conception. When this occurs, the fetus may be disomic but have uniparental disomy, a condition in which both chromosomes were inherited from the same parent. Trisomy rescue and uniparental disomy can involve potentially any chromosome, but if imprinted genes are present on the particular chromosome involved, this may have consequences for the fetus. Therefore, testing for uniparental disomy is indicated as a follow-up to confined placental mosaicism detected by CVS when a chromosome containing known imprinted genes is involved, such as those related to Prader–Willi syndrome or Angelman syndrome. If the chromosome involved in the original trisomy does not contain imprinted genes, the phenotype usually is normal.

After a mosaic CVS result with a normal karyotype on cultured amniocytes, there still is a possibility of the fetus having mosaicism in other cell lines. With true somatic mosaicism, clinical manifestations depend on the specific mosaic cell lines and may range from completely normal to findings consistent with the abnormal chromosome result. The counseling of patients with the finding of chromosomal mosaicism is complex, and referral for genetic counseling may be especially useful in these cases. In the past, cordocentesis often was performed to further evaluate chromosomal mosaicism discovered after CVS or amniocentesis; more recently, it has been recognized that this adds little to the prediction of outcome for the same reason that amniocentesis can be misleading.

▶ *What are the advantages, risks, and considerations for prenatal diagnostic testing for fetal genetic disorders for the patient who states that she would not pursue pregnancy termination for an abnormality?*

Prenatal diagnosis is not performed solely for assistance with the decision of pregnancy termination. Such testing provides other useful information for the physician and the patient. Counseling should be nondirective, informative, and respectful of any decision made by the patient. If a diagnosis of a genetic abnormality is made, counseling should include family education and preparation; obstetric management recommendations, including fetal surveillance, intrapartum monitoring, and mode of delivery; referral to pediatric specialists and a tertiary care center for delivery, if appropriate; availability of adoption or pregnancy termination; and perinatal palliative care services and comfort care for delivery of a child with a diagnosis or fetal presentation that is incompatible with long-term survival (74).

Summary of Recommendations and Conclusions

The following recommendations and conclusions are based on good and consistent scientific evidence (Level A):

- Chromosomal microarray analysis has been found to detect a pathogenic (or likely pathogenic) copy number variant in approximately 1.7% of patients with a normal ultrasound examination result and a normal karyotype, and it is recommended that chromosomal microarray analysis be made available to any patient choosing to undergo invasive diagnostic testing.
- Early amniocentesis (before 14 weeks of gestation) is not recommended.
- When structural abnormalities are detected by prenatal ultrasound examination, chromosomal microarray will identify clinically significant chromosomal abnormalities in approximately 6% of the fetuses that have a normal karyotype. For this reason, chromosomal microarray analysis should be recommended as the primary test (replacing conventional karyotype) for patients undergoing prenatal diagnosis for the indication of a fetal structural abnormality detected by ultrasound examination. If a structural abnormality is strongly suggestive of a particular aneuploidy in the fetus (eg, duodenal atresia or an atrioventricular heart defect, which are characteristic of trisomy 21), karyotype with or without FISH may be offered before chromosomal microarray analysis.

The following recommendations and conclusions are based on limited or inconsistent scientific evidence (Level B):

- An abnormal FISH result should not be considered diagnostic. Therefore, clinical decision making based on information from FISH should include at least one of the following additional results: confirmatory traditional metaphase chromosome analysis or chromosomal microarray, or consistent clinical information

(such as abnormal ultrasonographic findings or a positive screening test result for Down syndrome or trisomy 18).

- The rate of procedure-related pregnancy loss that is attributable to a prenatal diagnostic procedure currently is estimated to be approximately 0.1–0.3% in procedures performed by experienced health care providers. The loss rates for amniocentesis and CVS are both very low.
- Transmission of HIV with amniocentesis does not appear to be increased in women treated with CART when the viral load is undetectable.

The following recommendations and conclusions are based primarily on consensus and expert opinion (Level C):

- All pregnant women should be offered prenatal assessment for aneuploidy by screening or diagnostic testing regardless of maternal age or other risk factors.
- Prenatal genetic testing cannot identify all abnormalities or problems in a fetus, and any testing should be focused on the individual patient's risks, reproductive goals, and preferences.
- Genetic testing should be discussed as early as possible in pregnancy, ideally at the first obstetric visit, so that first-trimester options are available.

For More Information

The American College of Obstetricians and Gynecologists has identified additional resources on topics related to this document that may be helpful for ob-gyns, other health care providers, and patients. You may view these resources at http://www.acog.org/more-info/PrenatalGeneticTesting.

These resources are for information only and are not meant to be comprehensive. Referral to these resources does not imply the American College of Obstetricians and Gynecologists' endorsement of the organization, the organization's web site, or the content of the resource. The resources may change without notice.

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The MEDLINE database, the Cochrane Library, and the American College of Obstetricians and Gynecologists' own internal resources and documents were used to conduct a literature search to locate relevant articles published between January 1985–July 2014. The search was restricted to articles published in the English language. Priority was given to articles reporting results of original research, although review articles and commentaries also were consulted. Abstracts of research presented at symposia and scientific conferences were not considered adequate for inclusion in this document. Guidelines published by organizations or institutions such as the National Institutes of Health and the American College of Obstetricians and Gynecologists were reviewed, and additional studies were located by reviewing bibliographies of identified articles. When reliable research was not available, expert opinions from obstetrician–gynecologists were used.

Studies were reviewed and evaluated for quality according to the method outlined by the U.S. Preventive Services Task Force:

I	Evidence obtained from at least one properly designed randomized controlled trial.
II-1	Evidence obtained from well-designed controlled trials without randomization.
II-2	Evidence obtained from well-designed cohort or case–control analytic studies, preferably from more than one center or research group.
II-3	Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments also could be regarded as this type of evidence.
III	Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

Based on the highest level of evidence found in the data, recommendations are provided and graded according to the following categories:

Level A—Recommendations are based on good and consistent scientific evidence.

Level B—Recommendations are based on limited or inconsistent scientific evidence.

Level C—Recommendations are based primarily on consensus and expert opinion.

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