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How to choose a test for prenatal genetic diagnosis: a practical overview

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Abstract

Establishing the diagnosis of a fetal genetic disease in utero expands decision-making opportunities for individuals during pregnancy and enables providers to tailor prenatal care and surveillance to disease-specific risks. The selection of prenatal genetic tests is guided by key details from fetal imaging, family and obstetrical history, suspected diagnoses and mechanisms of disease, an accurate understanding of what abnormalities each test is designed to detect, and, at times, the gestational age at which testing is initiated. Pre- and posttest counseling, by or in conjunction with providers trained in genetics, ensure an accurate understanding of genetic tests, their potential results and limitations, estimated turnaround time for results, and the clinical implications of their findings. As prenatal diagnosis and testing options continue to expand rapidly, it is increasingly important for obstetrical providers to understand how to choose appropriate genetic testing and contextualize the clinical implications of their results.

Keywords

chromosomal microarray; exome sequencing; fetal diagnosis; genome sequencing; karyotype; next-generation sequencing; prenatal diagnosis; prenatal genetic testing

Introduction

Rapid advances in genetic technology in recent years have substantially enhanced our ability to identify diseases in utero.[1–9] Several screening approaches exist primarily for common aneuploidies in a fetus and genetic disease carrier status in individuals or couples. In addition, diagnostic testing options have expanded to allow much earlier diagnosis of not only large chromosomal abnormalities but also small copy number variants (CNVs) and rare single-gene disorders. Chromosomal microarray (CMA) and next-generation sequencing (NGS) technologies, such as exome sequencing (ES) and genome sequencing (GS), have led

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to earlier identification of fetal diseases that historically were not diagnosed until after birth. Fetal imaging with ultrasound and, in some cases, magnetic resonance imaging enables detailed phenotyping to establish a differential diagnosis. The selection of genetic tests from the large array of available options is guided by key details from fetal imaging, family and obstetrical history, suspected diagnoses and mechanisms of disease, and, importantly, what abnormalities each test is designed to detect.

Establishing the diagnosis of a fetal genetic disease in utero expands decision-making opportunities for individuals and families during pregnancy, ranging from termination of pregnancy to mode of delivery, antenatal surveillance, and in utero interventions for that disease. Counseling by providers who are trained in genetics is key for pretesting counseling and in scenarios when a fetal genetic diagnosis is made, to understand the certainty of the diagnosis, expected prognosis, recurrence risk, and further testing that might be indicated.

This article has outlined key considerations concerning genetic counseling, genetic screening vs diagnostic genetic testing, choosing among diagnostic genetic tests, and incorporating results into clinical decision-making (Videos 1 and 2).

Pre- and posttest genetic counseling

Genetic counseling is essential for individuals who are considering genetic testing, those who are deciding between genetic screening and diagnostic testing, and those who have received the results of genetic testing.[8] Counseling performed by, or in conjunction with, providers trained in genetics ensures thorough discussions of genetic testing options, the clinical relevance of genetic testing results, and any further testing that might be recommended. Providers who conduct this counseling must be well versed in prenatal genetic testing, given unique considerations in pregnancy, such as fetal manifestations of genetic diseases and time constraints. Furthermore, these providers must consider the health and genetic literacy of the individuals considering genetic testing, to tailor discussions and educational materials to ensure optimal understanding. In the absence of thorough pre- and posttest counseling, there are risks of individuals not understanding the implications of testing, and receiving results that they do not understand, and diagnoses being missed. Table 1 outlines a summary of important genetic counseling points.

Pretest counseling should explore an individual’s goals for testing and desires regarding the level of information provided by testing. This includes discussions about genetic screening options, such as fetal aneuploidy screening and carrier screening for individuals or couples and exploration of genetic screening vs diagnostic genetic testing. Pretest counseling should clearly explain that no genetic test can detect all diseases; tests can yield positive, uncertain, or negative results; the interpretation of results can change over time or with additional phenotypic information; and secondary or incidental findings can be detected with some tests.[10] Pretest counseling should address the risk of psychological distress if a diagnosis is identified; the potential effects of results on health, life, or disability insurance; and the potential for health or further testing implications for other family members. Furthermore, individuals should be advised that most laboratories contribute deidentified data about

variants they identify to genomic databases to improve widespread understanding of disease-causing variants.

Nuances of each genetic test are also important to discuss ahead of time. For example, a realistic expectation should be provided about the chance that the selected test will yield a clear diagnosis given the diseases under consideration and the clinical scenario. Furthermore, the risk of uncertain results should be discussed. There is an approximately 1% to 2% risk of identifying a variant of uncertain significance (VUS) with CMA,[1,11–13] and a VUS should be contextualized on the basis of its size, gene content, inheritance, and other features when detected. Some laboratories have different thresholds for reporting VUS in the prenatal setting vs after birth, with, at times, a greater level of concern required for a variant to be reported during pregnancy. With gene panels or ES, a VUS may also be detected, and the chance of finding a VUS varies by the test and specific laboratory. With CMA, ES, and GS, individuals should also be counseled and provide consent for receiving or declining secondary findings in the fetus and/or biological parents, such as an increased risk of cancer that is unrelated to the reason for sending the test in the first place.[14]

Posttest counseling should review the findings of genetic testing, clinical relevance, impact on further reproductive decision-making, and potential for a future change in interpretation of the results.[15] For example, a likely pathogenic variant that did not meet the full criteria for pathogenicity can be contextualized on the basis of the clinical phenotype and relevant history. A pathogenic or likely pathogenic variant inherited from a parent may be tested for in the future through either preimplantation genetic testing or diagnostic testing during pregnancy. Genetic diseases can exhibit both reduced penetrance (some individuals show symptoms and signs of the disease, whereas others do not) and variable expressivity (individuals are affected to varying degrees), even within the same family. In addition, any finding with genetic testing that could be reclassified as additional data is published over time, and those reclassifications could either increase or decrease the degree of concern that a particular finding can lead to disease. Finally, individuals should be made aware based on specific laboratory policies that autoreanalysis of their genetic data may be performed in the future and that they may be contacted by the laboratory with the results of those reanalyses.[15]

Genetic screening vs diagnostic testing

Genetic screening is offered universally in the prenatal setting and is a noninvasive approach primarily to identify fetuses with common aneuploidies and genetic disease carrier status in individuals or couples. Several options for fetal aneuploidy screening are available, such as integrated screening, quadruple screening, and cell-free DNA.[16,17] Although some cell-free DNA platforms also offer screening for microdeletions, these disorders are very rare, the positive predictive value of cell-free DNA for these additional disorders has not been well established, and the use of cell-free DNA for this purpose is not recommended.[18] Each screening test has unique advantages and disadvantages, which are beyond the scope of this article but discussed in detail in existing society publications.[16,17] Importantly, genetic screening tests for fetal aneuploidy are not diagnostic, and karyotype or CMA using samples

obtained from invasive testing (such as chorionic villus sampling [CVS] or amniocentesis) is necessary to confirm abnormalities detected by screening.

In addition to fetal aneuploidy screening, genetic disease carrier screening may also be offered to identify individuals or couples at risk of having a child with an autosomal recessive or X-linked genetic disease.[9,19] Several approaches exist for carrier screening, ranging from ancestry-based risk assessments to panethnic approaches that assess hundreds of genes, highlighting the necessity of thorough genetic counseling to understand the individual's goals. These forms of screening will identify individuals or couples at risk of having a child with a genetic disease, but definitive testing with a sample obtained from CVS or amniocentesis is required for targeted testing when screening results are positive to identify if a fetus has the disease.

Compared with genetic screening tests, diagnostic genetic tests are used to confirm genetic abnormalities after a positive aneuploidy screen or detection of a fetal structural anomaly on imaging; to evaluate for a familial disease, such as after positive results of genetic carrier screening tests; or to provide information for individuals desiring greater accuracy in the setting of otherwise normal findings. Several diagnostic genetic tests are available, and considerations for how to select the most appropriate tests are outlined below in the "Diagnostic genetic testing options" section. Examples of diagnostic tests are karyotype, CMA, targeted testing for familial variants, targeted gene panels, and ES. Importantly, no 1 diagnostic test is perfect, and there are limitations in the range of diagnoses that each test can detect. When considering genetic screening vs diagnostic testing, shared decision-making is key, and an individual's goals of testing and value systems are central considerations. Risks and benefits of diagnostic testing must be weighed against those of screening, considering procedure-related risks, individual's desires for genetic information and accuracy, and risk of genetic disease based on family history, imaging findings, and other factors. A definitive diagnosis of a fetal genetic disease can also expand decision-making opportunities during pregnancy, not only in terms of whether to continue a pregnancy but also in terms of pregnancy management, such as antenatal surveillance, in utero interventions, and site and mode of delivery.

Diagnostic genetic testing options

When individuals elect to pursue diagnostic genetic testing, there are many considerations regarding the types of tests to send. These considerations are based on indications for diagnostic testing, results of genetic screening, findings on prenatal imaging, family and clinical history, individual preferences, and cost-effectiveness. For example, CMA has largely replaced karyotype for prenatal diagnosis of fetal anomalies and for elective diagnostic testing in the absence of abnormalities.[1] However, karyotype may still be most efficient and cost-effective in select clinical scenarios, such as a high pretest probability of Down syndrome when differentiation between trisomy vs a translocation is necessary for accurate recurrence risk counseling. Some individuals may choose minimal to no testing, whereas others choose broad NGS when applicable, with decisions centering around the desire for information, willingness to receive uncertain findings, anxiety with awaiting and coping with results, and concerns about results staying private.[20–22] Further studies are

needed to clarify the most efficient and cost-effective testing approaches for different clinical scenarios, and a clear understanding of genetic tests and their limitations is essential to avoid unnecessary testing that adds substantial time and cost.

The Figure provides a framework for considering which tests to send based on common clinical scenarios, and the most commonly used genetic tests in clinical practice today are reviewed in Table 2. CNVs refer to submicroscopic chromosome deletions and duplications ranging from under 1 kilobase to several megabases (Mb) in size, and NGS refers to any test using this methodology, including single-gene testing, gene panels, ES, and GS.

Karyotype

Karyotype displays a complete set of chromosomes as seen during the metaphase.[23] Karyotype has been used for decades in prenatal diagnosis and has the ability to detect changes in the number or structure of chromosomes, including aneuploidy, larger deletions or duplications generally greater than 5 to 10 Mb in size, balanced and unbalanced translocations, inversions, marker chromosomes, chromosomal rings, and mosaicism.[23] The interpretation of karyotype relies on cytogenetic analysis of chromosomal bands and subbands and analysis of the structural locations of genetic material. A karyotype cannot detect small CNVs; sequence variants, such as missense variants; regions of homozygosity; abnormal methylation; or low-level mosaicism. Prenatal karyotype requires a culture of live chorionic villi or amniocytes and thus may take 7 to 14 days to generate a result.

Potential indications for a fetal karyotype include increased risk of an unbalanced translocation because of a known balanced parental translocation, cell-free DNA or other positive screening tests for aneuploidy, or imaging findings suggestive of autosomal trisomy or monosomy X. Furthermore, a karyotype should be performed when gain of an entire chromosome is detected with CMA to distinguish between trisomy and an unbalanced translocation, which could be inherited from a parent with a balanced translocation and thus increase recurrence risk of a future pregnancy.

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) uses fluorescent-tagged probes that bind to specific regions of chromosomes, typically when in interphase, enabling the identification of a region for which the probe was designed.[24] Thus, a specific diagnosis caused by changes in a particular chromosomal region must be suspected for its use. FISH probes have been developed for common deletion and duplication syndromes, which are associated with CNVs too small to be detected by conventional karyotyping. This test can be considered when a specific diagnosis is suspected, such as trisomy 21, for which fluorescent probes bound to 3 copies of chromosome 21 would indicate this diagnosis. Although FISH can be performed more quickly than karyotype, FISH is now used less commonly as karyotype is still necessary to confirm the findings and demonstrate the genomic location of abnormal chromosomal material.

Chromosomal microarray

CMA is recommended as the first-line test in cases with fetal structural anomalies and/or stillbirth, and it replaces the need for karyotype in most cases.[2,6] CMA can detect clinically relevant microdeletions or microduplications in approximately 6% of cases with fetal abnormalities and normal karyotype.[1] Among stillbirths, CMA can also yield results more often and provide greater detection of genetic abnormalities than karyotype.[7]

CMA can detect submicroscopic CNVs that are 100 times smaller than those identified by standard karyotyping. Although they are rare on an individual level, there are hundreds of microdeletion and microduplication syndromes that may be detected with CMA. These conditions are not associated with increasing reproductive age, and thus, CMA should be considered for all pregnant individuals undergoing prenatal diagnostic testing regardless of age.[2,6] CMA does not require actively dividing cells, which may lead to shorter turnaround times. Single-nucleotide polymorphism (SNP) microarrays have largely replaced comparative genomic hybridization microarrays in clinical practice; only SNP microarrays can detect triploidy and regions of homozygosity, which may enable the detection of uniparental disomy and consanguinity.[2,6] Unlike karyotype, CMA cannot demonstrate the genomic location of deleted or duplicated chromosomal material, such as Down syndrome resulting from trisomy 21, compared with a Robertsonian translocation. In addition, CMA cannot detect balanced chromosomal rearrangements, such as balanced translocations or inversions, and will not detect low-level mosaicism.

Methylation studies

Methylation abnormalities may include hypermethylation or hypomethylation on specific regions of maternal or paternal chromosomes.[25] Methylation studies are used when particular genetic diseases resulting from abnormal methylation or imprinting are suspected. Examples of diagnoses more frequently considered in the prenatal setting with this mechanism of disease are Beckwith-Wiedemann syndrome (BWS), Russell-Silver syndrome, and Prader-Willi syndrome.

Targeted testing

Targeted testing is used primarily in situations with a family history of genetic disease in which the genetic variant causing that disease is known or when a fetus is determined to be at risk of inheriting a condition identified through carrier screening. This approach focuses on testing only on the presence or absence of specific genetic variants. For example, targeted testing would be used in scenarios where a pregnant individual has an autosomal dominant genetic disease to determine if the fetus inherited the disease-causing variant, or where both individuals in a reproductive couple are found to be carriers of an autosomal recessive genetic disease to determine if the fetus inherited the variants and thus is predicted to have the disease.

Targeted gene panels

Targeted gene panels examine a select set of genes or gene regions with known or suspected associations with a phenotype or disease. Coverage in terms of the number of genes and examination of sequence variants vs deletions and duplications varies by the panel and

company offering the test. Gene panels do not examine any genes beyond those for which the panel was designed, which can limit diagnostic abilities when fetal manifestations of diseases are not well understood. Gene panels are most appropriate in situations where the fetal phenotype is consistent with a disease category that has known or suspected genetic associations, such as skeletal dysplasias or RASopathies.

Exome sequencing

ES evaluates protein-coding regions of the genome, specifically the exome. This amounts to coding regions of more than 20,000 genes, but only 1% to 2% of the entire genome.[26] Most ES platforms will not routinely detect CNVs, although some may detect them. In addition, ES cannot detect aneuploidy, structural rearrangements, or other genomic alterations, such as methylation defects.

Over recent years, prenatal ES has become more available in both clinical and research contexts.[3,4] Based on existing data, ES may be beneficial in situations with a nondiagnostic CMA and imaging findings of a single fetal anomaly or multiple organ system anomalies that suggest a genetic etiology. Furthermore, ES can be considered for cases with no CMA result if the phenotype is strongly suggestive of a single-gene disorder.[8] Additional potential indications for ES are listed in Table 1. The incremental yield of ES vs CMA for establishing a prenatal diagnosis in the setting of fetal anomalies is approximately 8% to 30%, with higher yields often observed among cases with multiple structural anomalies and certain phenotypes, such as skeletal dysplasias and nonimmune hydrops fetalis (NIHF).[3,4,27] The most accurate interpretation of ES results depends on a clear understanding of phenotypic abnormalities, so expansion of fetal phenotypes of genetic diseases will be key for improving the accuracy of fetal ES.[28,29]

Genome sequencing

GS evaluates the entire genome, including sequence variants in both coding and noncoding regions of the genome, small CNVs, and structural rearrangements, such as inversions and many others.[30,31] GS has only recently begun to be used for prenatal diagnosis, primarily through research studies. The incremental yield of prenatal GS vs more standard genetics tests is not yet well understood. Similar to ES, accurate interpretation of GS results depends on a clear understanding of the phenotypic manifestations of diseases.

Importance of clinical history and phenotype

Details of the family and obstetrical history are essential for every case, as they can provide important clues to focus on the differential diagnosis. For example, a family history of learning difficulties and cardiac anomalies might increase suspicion of a RASopathy, such as Noonan syndrome in the setting of a fetus found to have pleural effusions. An obstetrical history notable for multiple pregnancies with NIHF and a low mean corpuscular volume in a pregnant individual should raise suspicion of alpha thalassemia. The testing approaches for these diagnoses are quite different, and testing, such as a gene panel, to evaluate for a RASopathy would not identify alpha thalassemia carrier status. This highlights the necessity

of gathering the unique details for each case and pursuing genetic tests designed to evaluate the diagnoses in question.

In addition, fetal phenotyping is a key component of the evaluation.[32,33] In some cases, multiple anomalies are present and can point more clearly to a particular set of potential diagnoses. When this is the case, more narrow testing may be appropriate, such as a gene panel for skeletal dysplasias. In other cases, the constellation of fetal features may not strongly suggest a set of diagnoses, and broader testing, such as ES, may be beneficial to avoid inappropriately limiting the focus on a set of genes that are unrelated to the phenotype. ES should additionally be considered in scenarios where a gene panel does not yield an explanation for the phenotype.

Another important consideration is that fetal phenotypes of genetic diseases are often incompletely understood. Although fetal features of omphalocele and macroglossia have been well described with BWS, we have only recently begun to understand the spectrum of single-gene disorders that may underlie cystic hygromas.[25,27] The fetal phenotype of a genetic disease may also be distinct from the postnatal phenotype of that disease, such as with Niemann-Pick disease type C (NPC), where the only clue in utero may be fetal ascites; however, after birth, there can be hypotonia, liver dysfunction, seizures, neurodevelopmental regression, and many other findings.[34,35] As ES and GS are used more in the prenatal setting, we will gain a better understanding of the unique fetal phenotypes of genetic diseases and the specific genetic variants that lead to those diseases.

Case examples

Case 1

A 40-year-old G2P1 presented at 16 weeks of gestation with cell-free DNA that was positive for trisomy 18, and ultrasound that identified fetal growth restriction (FGR) and abnormal skull shape. The patient chose to proceed with amniocentesis, and karyotype was performed because of the high level of suspicion for trisomy 18, which confirmed the diagnosis. If CMA had instead been performed, this would have identified 3 copies of chromosome 18 but would have been unable to distinguish between true trisomy 18 and an unbalanced translocation. This highlights the importance of karyotype in this scenario to identify the genomic location of additional chromosome 18 material and counsel accurately about recurrence risk.

Case 2

A 38-year-old G2P0 presented at 30 weeks of gestation after transferring her care. The fetal anatomy at 21 weeks of gestation was reported as normal, and cell-free DNA screening was low risk. Ultrasound at 30 weeks of gestation showed ambiguous genitalia and FGR with all parameters <1% and overall size >4 weeks less than expected based on first-trimester dating. The patient chose amniocentesis, and CMA showed an 11 Mb pathogenic terminal deletion of 4p16.3p15.32, consistent with a diagnosis of Wolf-Hirschhorn syndrome. Postnatal features of this syndrome include dysmorphic facial features; cardiac, skeletal, central nervous, and genitourinary system anomalies; seizures; and developmental delays. Because

of the large size of the deletion in this case, this diagnosis would also have been detected by karyotype but would have been missed if a gene panel or many ES platforms had been pursued.

Case 3

A 35-year-old G1P0 was referred for consultation at 20 weeks of gestation because of fetal omphalocele. The most likely diagnoses under consideration were BWS and aneuploidy. Amniocentesis was performed, and CMA and methylation studies for BWS were performed. CMA results returned normal, and methylation studies revealed hypomethylation at IC2, consistent with BWS. This highlights the importance of understanding the mechanisms of disease, as methylation studies must be separately performed when a disease can be caused by abnormal methylation. If BWS remained high on the differential and methylation studies had been normal, further testing strategies would need to be considered to address the less common etiologies of BWS, such as sequence variants.

Case 4

A 29-year-old G2P0 presented for preconception counseling after a recent pregnancy with unexplained fetal arthrogryposis that resulted in stillbirth. A review of prenatal laboratory tests showed the patient to have 1 copy of *SMN1*, indicating spinal muscular atrophy (SMA) carrier status. Her partner had also been tested, with results showing the expected 2 copies of *SMN1*, reducing the chance of SMA carrier status as most SMA cases result from homozygous *SMN1* deletions.[36] Genetic counseling was performed at the preconception visit, where it was discussed that 2% to 5% of pathogenic *SMN1* variants are sequence variants rather than deletions.[36] As the fetal findings in her previous pregnancy could be consistent with a diagnosis of SMA, the partner underwent sequence analysis, which identified a pathogenic *SMN1* variant (c.796T>C) and the partner to also be a carrier for SMA. The remaining DNA from the previous pregnancy was tested, confirming the fetus to be compound heterozygous for the maternally inherited *SMN1* deletion and the paternally inherited sequence variant. This illustrates the importance of thorough genetic counseling and understanding of disease mechanisms, as carrier status for the partner was missed by standard SMA gene-targeted deletion analysis.

Case 5

A 27-year-old G3P1 with a previously uncomplicated pregnancy had a 28-week ultrasound for clinical suspicion of size greater than dates. Ultrasound identified polyhydramnios, skin edema, fetal arrhythmia, and macrosomia, a constellation of findings that have been reported in association with RASopathies.[37,38] Shortly thereafter, the patient developed NIHF and mirror syndrome and was delivered. Because of suspicion of RASopathy, a gene panel was performed after birth. This showed a missense variant in *HRAS* (c.34G>A) consistent with a type of RASopathy called Costello syndrome. Karyotype and CMA would have missed this diagnosis.

Case 6

A 39-year-oldG2P0 presented for a growth ultrasound at 30 weeks of gestation because of decreased fetal movement. Ultrasound showed NIHF, and the patient chose to proceed with amniocentesis. CMA, viral studies, and other appropriate tests to evaluate NIHF results returned normal. Because of the nonspecific phenotype of NIHF alone and the broad differential diagnosis, the patient chose to proceed with ES. This identified compound heterozygous missense variants in *NPC1*, c.3182T>C, and c.2072C>A, interpreted as pathogenic and likely pathogenic, respectively, leading to a diagnosis of NPC. CMA missed this important diagnosis, and many gene panels that do not include *NPC1* would similarly have missed this diagnosis.

Discussion

Our ability to establish a diagnosis of fetal genetic disease in utero has expanded substantially, particularly with the incorporation of NGStechniques.[1–8] More tests are available, and contributions from advancing fetal imaging enable more accurate, and often earlier, diagnoses of fetal abnormalities. With such rapidly expanding prenatal genetic tests, it is becoming increasingly essential for obstetrical providers to be well versed in the indications, benefits, and limitations of each one.

The selection of appropriate tests relies heavily on key details from fetal imaging, family and obstetrical history, suspected genetic diagnoses and mechanisms of disease, and the detection abilities of each test. No 1 test is perfect or can detect all abnormalities, and providers must understand the potential diagnoses that are missed when selecting tests for each unique case. Occasionally, multiple tests are needed to arrive at a diagnosis, so communication with the laboratory is crucial to ensure that samples of cultured cells, extracted DNA, or others are preserved while the diagnostic evaluation is underway. We recommend that thorough pre- and posttest counseling, by or in conjunction with providers trained in prenatal genetics, be performed for all cases, given that many issues are unique to pregnancy, such as fetal manifestations of genetic diseases, and that gestational age must be considered with the turnaround time of tests.. This counseling should ensure that individuals accurately understand genetic tests, their potential results and limitations, and the clinical implications of their findings; that interpretation of results can change over time or with additional phenotypic information; and that secondary or unexpected findings can be detected with some tests.

By establishing the diagnosis of a fetal genetic disease in utero, we can expand decision-making opportunities for individuals during pregnancy, tailor both prenatal care and surveillance to disease-specific risks, and, in many cases, engage in early planning to optimize neonatal care. However, not all individuals have equal access to the genetic tests discussed in this article, primarily because of limitations in insurance coverage and access to research studies offering advanced testing. There is a crucial need for more equitable access to the array of available genetic tests in the prenatal setting. In addition, further research will be necessary to clarify the optimal order of genetic tests according to fetal phenotype and clinical history, features and timing with which diseases manifest in the fetal setting, and specific genetic variants capable of leading to in utero disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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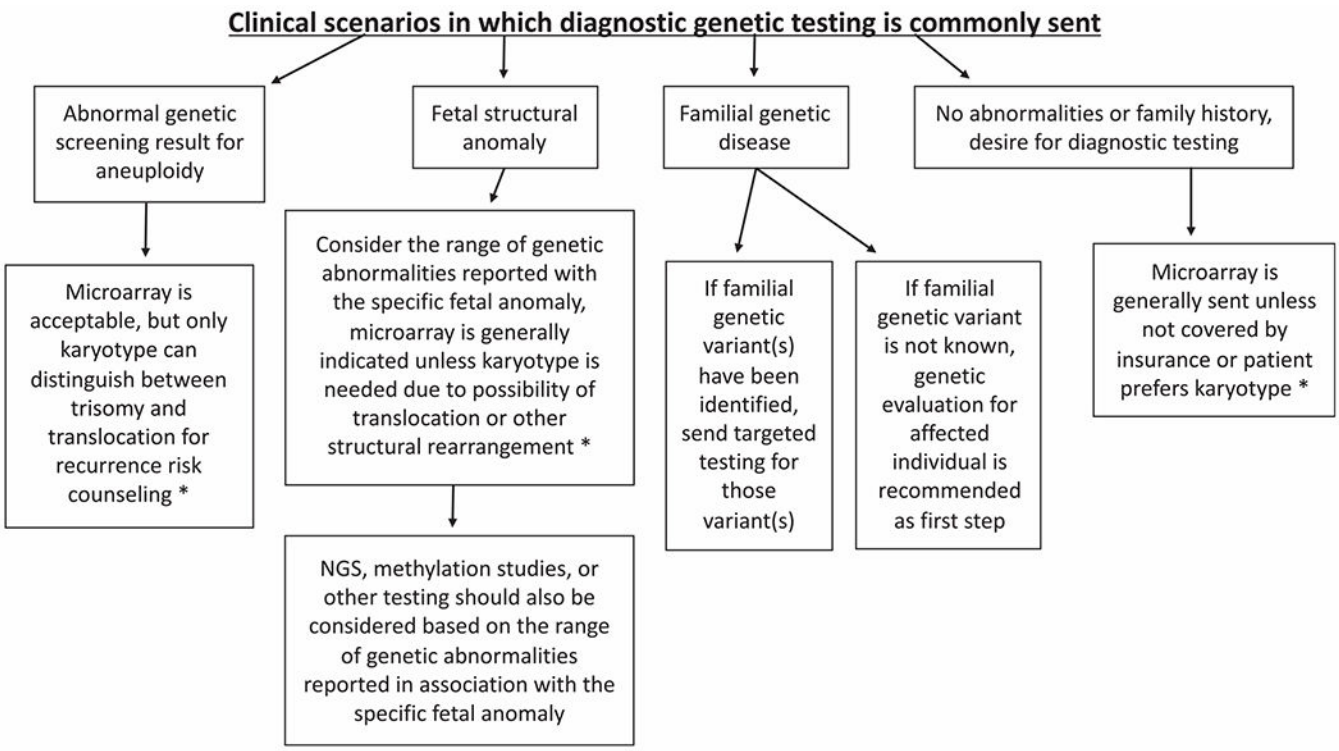

FIGURE. Diagnostic genetic testing strategies based on common clinical scenarios

*Table 2 provides other advantages and disadvantages of each test.

NGS, next-generation sequencing.

TABLE 1

Pre- and posttest counseling suggestions

Counseling	Content suggestions
Pretest	- Explore individual's or couple's goals of genetic screening and/or diagnostic genetic testing - Involve providers trained in prenatal genetics whenever possible[a] - Discuss that no test can detect all diseases; tests can yield positive, uncertain, or negative results; interpretation of test results can change over time or with additional phenotypic information; and secondary or unexpected findings can be detected with some tests - Explore willingness to accept uncertain information - Address risk of psychological distress if a diagnosis is identified - Discuss potential effect of the results on health, life, or disability insurance - Review that results of genetic testing could possibly have health or further testing implications for other family members - Discuss advantages and disadvantages of the genetic screening and/or diagnostic tests under consideration and expected turnaround times for results
Posttest	- Involve providers trained in prenatal genetics whenever possible[a] - Review the findings of genetic testing and their clinical relevance (including possibilities of reduced penetrance or variable expressivity when applicable) - Discuss any further testing that may be indicated for the fetus, pregnant individual, or other family members - Counseling about VUS must be with consideration for the clinical situation, reason for variant uncertainty (ie, gene-level data, variant-level data, or incomplete phenotype), and appropriate caution - Explain that any finding with genetic testing could be reclassified as additional data that could be published over time and that those reclassifications could either increase or decrease the degree of concern that a particular finding can lead to genetic disease - Discuss that autoreanalysis of genetic data may be performed in the future depending on individual laboratory policies and individuals may be contacted by the laboratory with results of those reanalyses - Explore the effects of results on future reproductive decision-making - Address psychological effect of results

VUS, variant of uncertain significance.

[a]Genetic counselors or geneticists.

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TABLE 2

Genetic test indications, advantages, and disadvantages

Test	Indications	Advantages	Disadvantages
Karyotype	- Increased risk of unbalanced translocation because of balanced parental translocation - Cell-free DNA or other positive screening tests for aneuploidy - Imaging findings suggestive of autosomal trisomy or monosomy X, particularly in conjunction with a positive aneuploidy screening test - Gain of an entire chromosome detected on CMA to distinguish between trisomy and an unbalanced translocation, which could be inherited from a parent with a balanced translocation and thus increase recurrence risk for a future pregnancy - Can be offered in cases with no fetal structural abnormality	- Lower risk of VUS - Can detect aneuploidy, larger deletions, or duplications generally greater than 5–10 Mb, balanced and unbalanced translocations, inversions, mosaicism, marker chromosomes, and chromosomal rings - Can demonstrate the genomic location of CNV (such as trisomy 21 vs Robertsonian translocation)	- Generally cannot detect a CNV of less than 5–10 Mb - Cannot detect sequence variants, such as missense variants, regions of homozygosity, or abnormal methylation - Requires live, dividing cells - May not detect low-level mosaicism
FISH	- A specific diagnosis is suspected - Detection of common microdeletion and microduplication syndromes, which are associated with CNVs too small to be detected by conventional karyotyping	- Generally has a faster turnaround time than karyotype and can be useful when a rapid preliminary result is desired - Probes detect the presence of specific chromosomal material	- Karyotype is necessary to confirm findings and demonstrate the structural location of abnormal chromosomal material - Must suspect a specific diagnosis caused by changes of a particular chromosomal region
Microarray	- First-line test for fetal structural anomalies and/or stillbirth - Can be offered in cases with no fetal structural abnormality	- Can detect a CNV of <5 Mb and mosaicism - SNP microarray can detect regions of homozygosity and identify uniparental disomy and consanguinity - Does not require cell culture and so can be performed on nonviable, nondividing cells - Rapid turnaround time	- Cannot demonstrate genomic location of CNV (such as trisomy 21 vs Robertsonian translocation) - Cannot detect balanced translocations or inversions - CGH microarray cannot detect regions of homozygosity or triploidy - May not detect low-level mosaicism
Methylation studies	- Genetic diseases resulting from hypermethylation or hypomethylation are suspected	- Evaluates hypermethylation or hypomethylation on specific regions of maternal or paternal chromosomes	- Does not detect other mechanisms of disease (such as sequence variants that less commonly lead to imprinting disorders)
Targeted testing	- Family history of genetic disease in which the genetic variant causing the disease is known - Fetus is determined to be at risk of inheriting a condition identified through carrier screening	- Identifies presence or absence of familial variants	- Does not evaluate remainder of gene in question or other genes
Targeted gene panels (sequencing and deletion and duplication analyses)	- Cases where fetal phenotype is consistent with a disease category that has known or suspected genetic associations, such as skeletal dysplasias or RASopathies	- Evaluate numerous genes with known or suspected associations with a phenotype or disease	- Evaluation is limited to only a selected subset of genes - Not useful when the differential diagnosis is more broad - Fetal phenotypes of disease often do not match postnatal phenotypes, potentially leading to missed diagnoses
Exome sequencing[8]	- Cases with a nondiagnostic CMA and imaging findings of a single fetal anomaly or multiple organ system anomalies that suggest a genetic etiology - Cases with nondiagnostic CMA and imaging findings of a recurrent single fetal anomaly or multiple organ system anomalies that suggest a genetic etiology and occurred in a similar pattern during a previous pregnancy - Fetus with a pattern of anomalies previously observed in a family with a history of recurrent stillbirths	- Evaluates protein-coding regions of genome (the exome, coding regions of >20,000 genes) - Evaluates genes that may not otherwise be strongly considered, sometimes in situations where fetal phenotypes of disease do not match postnatal phenotypes	- Not designed to routinely detect CNV or structural rearrangements - Turnaround time can be longer than other testing methods

Test	Indications	Advantages	Disadvantages
	- Can be considered for cases with no CMA result if the phenotype is strongly suggestive of a single-gene disorder		
Genome sequencing	- Can be considered for cases that are suspicious for a genetic etiology with nondiagnostic results of more standard and clinically available genetic testing - Currently primarily performed in the setting of a research protocol	- Evaluates entire genome, including sequence variants in both coding and noncoding regions of the genome, small CNVs, and structural rearrangements, such as inversions and many others	- Incremental diagnostic yield for fetal diagnosis not yet clear - Possibility of additional results with uncertain clinical significance

CGH, comparative genomic hybridization; *CMA*, chromosomal microarray; *CNV*, copy number variant; *FISH*, fluorescence in situ hybridization; *Mb*, megabase; *SNP*, single-nucleotide polymorphism; *VUS*, variant of uncertain significance.