International Journal of Women's Health

Dovepress
Taylor & Francis Group

Open Access Full Text Article

COMMENTARY

The Art (and Science) of Individualized Selection of Non-Invasive Prenatal Screening (NIPS)

Vicky Hsieh[1], David M Sherer[1,2], Kateryna Davydovych[1], Mila Kheyman[2], Mudar Dalloul[1,2]

[1]The Division of Medical Genetics, The Department of Obstetrics and Gynecology, State University of New York (SUNY), Downstate Health Sciences University, Brooklyn, NY, USA; [2]The Division of Maternal Fetal Medicine, The Department of Obstetrics and Gynecology, State University of New York (SUNY), Downstate Health Sciences University, Brooklyn, NY, USA

Correspondence: David M Sherer, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, State University of New York (SUNY), Downstate Health Sciences University, 450 Clarkson Avenue, Box 24, Brooklyn, NY, 11203, USA, Tel +1 718 270 2081, Fax +1 718 270-4122, Email davidmsherer@gmail.com

Abstract: Non-invasive prenatal screening (NIPS), utilizing cell-free fetal DNA (cffDNA), has revolutionized prenatal care, transitioning from primarily detecting common fetal aneuploidies to encompassing detection of an increasingly broader spectrum of autosomal dominant and recessive conditions. This Commentary delves into the evolution of NIPS, emphasizes the importance of individualized selection of NIPS strategies based on specific clinical scenarios including patient characteristics, and explores its applications beyond aneuploidy screening. The optimal NIPS strategy should be carefully selected based on individual patient factors, including the specific clinical indications, maternal characteristics such as BMI, medical history, medication use, history of previous pregnancies, fetal characteristics such as multiple gestation or suspected anomalies, and the available NIPS technologies. There are also considerations in choosing between MPSS and SNP-based NIPS based cfDNA screening technologies. NIPS is a screening test; hence, diagnostic testing remains crucial for confirmation of any abnormal screening results. Notwithstanding, NIPS has significantly transformed prenatal care, offering valuable insights into fetal health and enabling earlier identification of potential risks. By carefully considering individual patient factors and selecting the most appropriate NIPS strategy, clinicians have the ability to maximize the benefits of this innovative technology while minimizing potential limitations. Continued research and technological advancements will further refine NIPS and expand its applications in the future.

Plain Language Summary: Development and implementation of NIPS has truly revolutionized the field of prenatal screening and prenatal care. This technology has the potential to remove barriers for patient access and provide early and accurate risk assessment for all pregnancies. Through continued research and progress for more methods of utilizing cfDNA technology in a variety of situations, there are now complexities in the selection of the optimal cfDNA screening approach depending on the clinical scenario. Insurance coverage is often also worthy of consideration for patients. It is important to note that insurance (at least in the United States) will often only cover one aneuploidy screening per pregnancy. Therefore, it is important to select the most appropriate test at the forefront. This commentary has outlined common situations where the prenatal care provider may require guidance in selection of the optimal screening NIPS for their patients.

Keywords: cell-free fetal DNA, noninvasive prenatal screening, prenatal care, fetal aneuploidy

Introduction

Non-invasive Prenatal Screening (NIPS, also known as NIPT) refers to a form of prenatal screening for chromosomal aneuploidies that has revolutionized prenatal care by allowing early detection of fetal chromosome anomalies. Trisomy 21, Trisomy 18, Trisomy 13, and sex chromosome aneuploidies have historically been viewed as common chromosomal aneuploidies with the potential to reach viability.[1] Trisomy 16 and Trisomy 22, on the other hand, are considered common chromosome abnormalities often associated with first-trimester pregnancy losses. It is estimated that between 20% and 25% of all first-trimester pregnancy losses are the result of Trisomy 16 or Trisomy 22.[2] Before the development

Received: 1 February 2025
Accepted: 17 April 2025
Published: 7 May 2025

and widespread adaptation of NIPS, maternal serum screening was the main screening method for fetal aneuploidies beginning in the mid-1980s. Maternal serum screening utilizes maternal serum analytes to provide a risk evaluation for fetal aneuploidy. Such testing has a sensitivity ranging from 80% to 90% but low specificity. Of note, maternal serum screening involves a predetermined 5% false positive rate.[3–5] Therefore, before the development of NIPS, invasive diagnostic procedures such as amniocentesis and chorionic villus sampling were widely used in the prenatal testing landscape.

The concept of utilizing fetal cell free-DNA (cfDNA) circulating in maternal blood for prenatal testing was first proposed in the late 1990s by Dennis Lo, a clinical scientist at the University of Hong Kong.[6] It was not until the early 2000s that the development of massively parallel shotgun sequencing (MPSS) technology provided the necessary tools for detecting and analyzing fetal cfDNA in maternal circulation.[7–9] The first successful application of utilizing cfDNA for the detection of aneuploidy was published in 2007.[10] The method of using MPSS on circulating cfDNA of placental origin for the detection of fetal chromosomal abnormalities was replicated across different laboratories and has become the dominant approach for screening for fetal aneuploidies.

Such testing was coined "Non-invasive prenatal testing" or NIPT. This methodology became commercially available by the early 2010s.[11] In 2013, the American College of Medical Genetics and Genomics (ACMG) issued guidelines supporting the use of NIPT for pregnancies at increased risk for fetal aneuploidies.[12] By 2016, NIPT had revolutionized the prenatal care landscape and became a routinely utilized test widely covered by insurance in the United States for high-risk patients. Around this time, the test was renamed as "Non-invasive prenatal Screening or NIPS" to reflect its screening (and not diagnostic) nature to avoid potential confusion among patients and clinicians. NIPS has much higher sensitivity and specificity for the detection of fetal chromosome aneuploidies than traditional maternal serum screening.[13–15] Perhaps trivially, NIPS is also able to correctly identify fetal sex as early as 9–10-week gestation and has been welcomed by many. By 2020, the American College of Obstetrics and Gynecologists (ACOG) issued an updated guideline supporting the use of NIPS for all pregnancies.[16] Notably, following the widespread application of NIPS, invasive diagnostic testing rates have decreased across the US.[17]

Amniocentesis and CVS are two diagnostic procedures that can be performed in pregnancy to obtain cells from the amniotic fluid or placental trophoblasts. In the age of widespread use of NIPS, arguments have been made regarding the utility of CVS in diagnosing chromosomal aneuploidies since NIPS and CVS both obtain cells of placental origin.[18,19] Amniocentesis is considered the gold standard when it comes to the evaluation of fetal chromosomes and mosaicism.[20] Mosaicism has been identified as a limitation of NIPS due to origin of cfDNA. It is important to distinguish here that NIPS is considered screening when it comes to chromosome aneuploidies and diagnostic procedures should be offered in patients who screened high-risk or positive by NIPS.[20] Long wait times and limited availability of additional resources to high-risk obstetric providers may hinder patient care.[21] This is precisely where considerations of different types of NIPS may have a place in patient management. NIPS can be performed as early as 9 weeks' gestation, or around the same time patients would be obtaining a first dating sonogram. Thus, it can be a useful tool in determining risk levels in early pregnancy.

MPSS vs SNP-Based NIPS

There are two primary methods used for NIPS, Massively parallel shotgun sequencing (MPSS) and single nucleotide polymorphism (SNP)-based sequencing. There are benefits and limitations to both methods.

MPSS sequences the entirety of all fragments of cfDNA found in maternal circulation. This includes cfDNA of maternal and placental ("fetal") origin. Maternal cfDNA originates from maternal adipose tissue and fetal cfDNA originates from placental trophoblasts. This method has the potential to provide a comprehensive analysis of copy number variants across the fetal genome and can detect a wider range of chromosomal abnormalities such as micro-deletions and microduplications of varying sizes depending on the analysis platform. It can also be used more narrowly to focus on common chromosome aneuploidies.[7–9,14,16,22,23]

SNP-based sequencing technology emerged as an alternative to MPSS in 2012. This method focuses on specific genetic markers that are known to be different between maternal and fetal DNA and provides a snapshot of the nucleotide at each specific location. Since the main application of NIPS is for the detection of common chromosome aneuploidies, the SNPs used in this method have been concentrated on chromosomes 13, 18, 21, X, and Y. This method may or may

not offer slightly better sensitivity and specificity for common chromosomal abnormalities but has limitations in detecting other copy number variants across other chromosomes. SNP-based technology may be less expensive than MPSS-based technology due to its more targeted approach.[24–30]

Both methods offer high sensitivity and specificity rates when it comes to common chromosome aneuploidies such as Trisomy 21, Trisomy 18, and Trisomy 13. The choice between the two methods often depends on other clinical indications, the desired level of sensitivity, and the available resources for the patient. This commentary will address matters worthy of consideration in different clinical conditions.

Whole Genome Screening

In clinical situations where a non-specific birth defect or growth restriction has been identified and a patient already had low-risk NIPS for aneuploidy, a rare chromosome aneuploidy or a microdeletion/microduplication may be suspected. It should be noted here that diagnostic testing remains the standard and the recommended method for the evaluation of a fetus with congenital anomalies. However, in situations where diagnostic testing is not possible or when a patient declines invasive testing, genome-wide NIPS should be considered.[23]

Genome-wide NIPS refers to the use of MPSS technology to screen for chromosomal gains and losses across the genome.[23,31] Large microdeletions and microduplications of greater than 3-5Mb can be detected using this method, however, only copy number variants larger than 7Mb are reported.This can be a powerful screening tool in the absence of other testing.[23] A copy number variant larger than 7Mb has a high potential of being pathogenic.

Balanced Translocation Carriers

MPSS-based cfDNA screening may also be offered to patients with large balanced translocations where a fetal unbalanced translocation may be detected. Patients should be counseled on the screening nature of NIPS and the limitations of not performing diagnostic testing. In situations where a known balanced translocation exists in the family, genome-wide NIPS may be able to provide the patient with pertinent answers sooner than diagnostic CVS testing.[32,33] Srebniak et al suggest that NIPS more readily detects familial unbalanced translocations compared to de novo aneuploidy.[32] A report of the familial balanced translocation should accompany the test requisition to ensure the technology has the capability of detecting unbalanced translocations of the size and location in question. It is advised to contact the cfDNA screening laboratory prior to sending the sample to confirm their ability to detect an unbalanced translocation of the specific size and location.[32,33]

The 2–3-week difference in obtaining results drastically decreases patient anxiety, especially when the patient has experienced previous recurrent early pregnancy losses. Diagnostic testing is recommended following the cfDNA screening for confirmation.[32,33]

Twins

Twin pregnancies carry increased obstetric and fetal risks compared to singleton pregnancies. Twin pregnancies increase the maternal risk for gestational diabetes, gestational hypertension, preeclampsia, hemorrhage, and early delivery.[1] These risks also vary depending on whether the twins are monochorionic or dichorionic. Monochorionic twin pregnancies are at increased risk for twin-to-twin transfusion syndrome (TTTS), twin anemia-polycythemia syndrome, and twin reversed arterial perfusion.[1,34] These conditions can cause fetal growth restriction, growth discordancy and even fetal death. In general, dichorionic twins have lower risks for obstetrical complications compared with monochorionic twins but the risks remain considerably increased in comparison with singleton pregnancies.[1,35] All dizygotic twins and 25% of monozygotic twins are dichorionic.[34,36]

Due to the differences in risk level when it comes to monochorionic vs dichorionic twin pregnancies, SNP-based NIPS should be considered for twin pregnancies as it is also able to identify the zygosity.[35,37] In prenatal units where early sonograms can identify the chorionicity and amnionicity of twin pregnancies, the importance of using NIPS to identify zygosity may be overlooked. Since sonograms can be operator-dependent in their accuracy and not all patients have access to early sonograms, an SNP-based NIPS can be a powerful tool in determining whether a twin pregnancy could be at risk for TTTS and whether high-risk monitoring protocol should be initiated.[34,38] Furthermore, determining

zygosity may be helpful in providing the risk for suspected genetic disorders, in one or both of the fetuses, in the presence of abnormal ultrasound findings.[34]

Since the fetuses in monozygotic pregnancies have identical DNA, SNP-based NIPS would only see a maternal DNA profile and one fetal profile. Dizygotic twin pregnancies are the product of two eggs being fertilized by two separate sperms, the SNP-based NIPS would see a maternal DNA profile and two fetal profiles. SNP-based NIPS is highly accurate in determining the zygosity of twin pregnancies.[36,37] This method of testing is available to all patients where blood can be drawn and the analysis is not operator-dependent. SNP-based NIPS should be considered in known twin gestations.

Vanishing Twin

Vanishing twin pregnancies occur when one of the fetuses in a multifetal pregnancy ceases to develop and is reabsorbed. This typically occurs early in the first trimester.[39] Depending on the timing of blood draw for cfDNA screening for aneuploidy, there is a potential for residual cfDNA from the vanished twin to still be present in maternal circulation weeks after the fetal demise.[39–41] The presence of cfDNA from the vanished twin can potentially lead to inaccurate results, as fetuses with a chromosome aneuploidy have an increased risk of being non-viable, especially early in the first trimester. This may lead to false positive or inconclusive results.[39,42]

An SNP-based aneuploidy screen has the potential to detect a vanishing twin pregnancy, even in the absence of sonographic data to suggest the occurrence. An SNP-based screen can report on the presence or absence of an additional DNA profile when a singleton pregnancy is reported.[39] However, aneuploidy risks are often not evaluated in these scenarios. These reports often solely flag and report on the additional DNA profile attributing to triploidy or vanishing twin, not the aneuploidy risk.

An MPSS-based approach, on the other hand, will not identify the presence of additional DNA profile but it will provide an aneuploidy risk. Although the report provided can have a higher chance false positive with low positive predictive value or be inconclusive.[39,42–44]

CfDNA screening for aneuploidies is unable to determine whether the vanished or surviving twin has a suspected aneuploidy.[41] Diagnostic testing is strongly recommended in cases of a positive result from MPSS-based screening, although patients should be counseled on the increased chance of a false positive result. Patients are also encouraged to inform providers if an early sonogram suggested a twin gestation so that SNP-based aneuploidy screening can be performed.

Triplet and Higher-Order Multiples

In the United States, multifetal pregnancy rates have increased significantly in recent decades attributed to the introduction of assisted reproductive technology (ART) and older maternal age at conception.[45,46] Higher-order multiple pregnancies are associated with a higher risk of maternal and fetal morbidity and mortality compared to twin and singleton pregnancies.[47]

NIPS has not been validated for triplet or higher order pregnancies due to the limited data available.[46] Few studies have been published assessing the performance of MPSS NIPS on triplet pregnancies. Available studies suggest that the performance of NIPS is comparable between singleton and multifetal gestations if sufficient fetal fraction is present.[46,48] Currently, the MPSS platform is the only platform able to evaluate triplet pregnancies.[49] SNP-based platforms cannot currently accept triplet pregnancy samples.[39,50] Fetal sex determinants will likely only be able to specify whether Y chromosome material is detected and not state how many of the fetuses are expected to be male or female.

Currently, pregnancies with four or more fetuses cannot be evaluated through cfDNA screening.[48] These patients should be counseled specifically about the lack of aneuploidy screening technologies available, as maternal serum screening also has similar limitations. Sonography and diagnostic testing remain their only method for aneuploidy evaluation.

Triploidy

Triploidy is a rare chromosomal condition where a fetus has three sets of chromosomes instead of two sets.[25,51] Instead of 46 chromosomes total, fetuses with triploidy will have 69 chromosomes. The estimated incidence of triploidy is 1–3% and can represent up to 20% of first-trimester pregnancy losses.[51] Triploidy is often caused by a sporadic event where an

error in cell division in the gametes causes the embryo to receive a whole extra set of chromosomes.[25,51] Triploidy pregnancies rarely carry to term. Most triploid fetuses that are alive at birth are mosaic for the condition, have severe malformations, and rarely survive infancy.[52] Paternal triploidy can lead to a large cystic placenta with a risk of malignancy.[39,52] Due to this risk, additional testing is often recommended when triploidy is identified to assess whether it is of paternal or maternal origin. Management may differ depending on the result.

SNP-based NIPS is the only NIPS able to screen for triploidy, with a 5-10% PPV.[13,25,52] Since triploidy causes a balanced and proportional increase across all fetal chromosomes, MPSS-based NIPS is unable to identify the condition.[52] Thus, in cases where triploidy is suspected or if a patient has a history of triploidy, SNP-based NIPS should be considered for the patient for screening. Since triploid pregnancies rarely survive the first trimester, whether screening for triploidy should be performed for all patients as baseline screening remains at the individual provider's discretion.

IVF Pregnancies

In vitro fertilization (IVF) is the most common form of assisted reproductive technology (ART). Individuals can pursue this method of conception for a multitude of reasons, including advanced maternal age, recurrent pregnancy loss, infertility, and increased risk for certain genetic conditions.[53] The first step of IVF is retrieving eggs and sperm. Then, the gametes are combined by insemination or intracytoplasmic sperm injection (ICSI). After fertilization, the embryos are cultured before being transferred into the gestational carrier. Prior to embryo transfer, there is an optional step of pre-implantation genetic testing (PGT) that can be done by blastocyst biopsy at 5–6 days. For an additional cost, the embryo can be tested for aneuploidy (PGT-A); monogenic disorders (PGT-M); structural chromosomal abnormalities such as chromosomal translocations, inversions, deletions, and insertions (PGT-SR); and polygenic conditions (PGT-P). The procedure involves removing 5–10 cells from the trophectoderm (which eventually becomes the placenta). PGT-A is the most commonly performed test, the goal is to select the embryo with the highest likelihood of a successful implantation/pregnancy.[53] It is important to understand that PGT is considered screening. The biopsied cell population might not be representative of the entire embryo since mosaicism is observed more frequently in early embryos. Furthermore, the inner cell mass is untouched; therefore, placental mosaicism is another limitation of this testing. Since PGT is screening, diagnostic testing should still be offered during pregnancy.[53]

While maternal serum screening can be performed for IVF pregnancies, some studies have identified an association with alternate serum marker levels, which may lead to a higher rate of false positive results.[54,55] NIPS can also be performed in this population; however, fetal fraction has been shown to be lower.[56,57] While 95% patients will receive a result on their first attempt, it is important to discuss these factors prior to screening.[56] MPSS NIPS is the preferable option since it is less sensitive to lower fetal fractions.

While some studies have identified an increased incidence of imprinting disorders after IVF and ICSI,[58–60] other studies have disputed this.[61,62] MPSS and SNP NIPS cannot identify methylation defects since they do not result in copy number variants. Genome-wide NIPS should be considered in IVF pregnancies for the most comprehensive screen of overall copy number variants.

SNP-based cfDNA screening offers an effective method for confirming the biological parentage of embryos in IVF pregnancies. By analyzing the SNPs, this approach provides information regarding the maternal parentage of embryos, verifying the correct embryo was transferred. This technology may be valuable in cases of embryo freezing, ensuring that the transferred embryo aligns with the intended genetic material. Notably, incorrect maternal parentage is often implied and not explicitly reported.

Blood Transfusions and Transplant Recipients

Cell-free DNA refers to DNA fragments released into the bloodstream from cell turnover or cellular damage. In the case of a transplant recipient, whether organ transplant, stem cell transplant, or blood transfusion, the recipient would have circulating cfDNA from the donor cell source.[63,64] For this reason, cfDNA has been used in recent years as a promising biomarker for transplant rejection, infection surveillance, graft versus host disease, and even tumor recurrence.[65–71]

In situations where a patient has received a recent blood transfusion or previous organ/stem cell transplant, MPSS-based NIPS should be the only screening method utilized. Since additional donor DNA would be marked in the SNP-based method, it limits the accurate evaluation of fetal chromosome status. MPSS-based NIPS assumes the donor DNA to be balanced. Donor DNA,

maternal DNA, and placental DNA will all be analyzed to determine whether an underlying imbalance is present for detecting aneuploidy and other copy number variants. Fetal sex discrepancy between NIPS and ultrasound can occur if the organ donor is male, due to the presence of donor Y chromosome in the cfDNA.[64,72,73] For blood transfusion recipients, unlike for organ transplantation recipients, fetal sex chromosome discrepancy is only a concern if the transfusion occurred <4 weeks prior to the NIPS blood draw.[74]

Maternal Weight and Fetal Fraction

Placental ("fetal") cell free DNA enters maternal circulation due to blood exchange between maternal circulation and placenta. This cfDNA is quickly cleared from maternal circulation a few hours after delivery.[75] cfDNA is detectable in maternal serum at 7 weeks GA.[43] Most NIPS can be performed as early as 9 weeks GA, but sufficient cfDNA is necessary to provide a clinically significant result. The minimum fetal fraction (FF) varies by laboratory but is generally between 3% and 4%. FF is the percentage of cfDNA of fetal or placental origin compared to maternal cfDNA.[76] It increases at a slow and non-constant rate throughout pregnancy. Between 10 and 20 weeks gestational age, the FF ranges from 10% to 15%.[76,77] Low FF can be caused by high maternal BMI, certain aneuploidy, chronic hypertension, certain maternal conditions, medications, and assisted reproductive technology.[76,78]

Low FF can cause a non-reportable or failed NIPS result.[22,78,79] In theory, since maternal cfDNA is derived from adipocytes, if a woman has more adipose tissue, more of her cfDNA will be present in circulation compared to fetal cfDNA, or possibly adipocytes will turnover at an accelerated rate.[22,77–81] According to Livergood et al, women with a BMI of 25–29.9, 30–34.5, 35–39.9, and >40 had a 2.31, 3.66, 7.52, and 8.55 fold higher risk of a non-reportable result on cfDNA screening, respectively.[78] In women with a high BMI, the median FF is lower and increases at a slower rate compared to women with a lower BMI.[22,78,79] At 21 weeks' gestation GA the no call rate is the same in most weight groups. However, at this GA, follow-up options may be more limited.[78] Failed NIPS results can cause increased maternal anxiety and delay timely pregnancy management. Repeating NIPS has been shown to produce a result in 56% of cases.[22,76]

MPSS-based NIPS has a lower non-reportable threshold when it comes to fetal fraction, while SNP-based NIPS has a higher fail rate.[79] Therefore, MPSS NIPS is preferable in women with a higher BMI. Furthermore, ultrasound at 11–14 weeks and MSS can be performed to help assess for aneuploidy, septated cystic hygroma, structural fetal anomalies and overall obstetric risks.[79]

Maternal Medication Use

Maternal medication use can have an impact on cfDNA screening, particularly by impacting the fetal fraction. Some medications such as anticoagulants have been shown to lower fetal fraction and increase the chance of non-reportable results.[40,41,82–84] In certain situations, lowering the fetal fraction can lead to false negatives on cfDNA screening. It has been proposed that anticoagulants can lower fetal fraction by decreasing trophoblast apoptosis or by increasing maternal cfDNA.[40,82]

When patients are taking these medications, it is advised that they get their blood drawn before taking the medication. Drawing blood immediately before the medication intake will allow for the lowest impact medications can have on fetal fraction. In some circumstances, patients may not be appropriate candidates for cfDNA screening due to medication use. It is not advised to skip medication dosage for cfDNA screening. These patients should be counselled, and diagnostic testing should be offered.

MPSS technologies are generally less sensitive to small reductions in fetal fraction.[79] If the patient has a failed cfDNA screening through SNP-based NIPS, switching to the MPSS-based NIPS can be considered.

Summary

NIPS has significantly transformed prenatal care, offering valuable insights into fetal health and enabling earlier identification of potential risks. By carefully considering individual patient factors and selecting the most appropriate NIPS strategy, clinicians can maximize the benefits of this technology while minimizing potential limitations. Table 1 summarizes the abovementioned clinical scenarios and the recommended NIPS approach for each. It is also important to

Table 1 Comparison of MPSS and SNP-Based NIPS Technologies

Clinical Consideration	MPSS-Based Approach	SNP-Based Approach
Balanced translocation carrier	Genome-Wide screening recommended	
Twin Pregnancies		Only method to determine zygosity
Vanishing Twin Pregnancies	Able to determine aneuploidy risk but will not detect the possibility of vanishing twin	Will report on suspicion of vanishing twin but might not provide aneuploidy risk
Triploidy		Only method to detect triploidy
IVF pregnancies	Consider Genome-wide approach for broader screen	If maternal parentage confirmation is desired
Triplets	Only approach to evaluate aneuploidy risk	
Transfusion/Transplant recipient	Only approach to evaluate aneuploidy risk	
High BMI and low fetal fraction	Lower likelihood for non-reportable result	May be attempted
Medication use	Lower likelihood for non-reportable result	May be attempted

Note: This table summarizes the optimal use of MPSS vs SNP-based NIPS technologies across various clinical considerations, highlighting which technology is most effective in each clinical scenario.

note that not all laboratories performing MPSS-based NIPS will offer analysis in each scenario. It is crucial to speak with the laboratory prior to ordering to ensure proper analysis of the results for your patients.

Technologies Used for Single Gene NIPT

Application of NIPS to single gene conditions was initially reported in 2000, three years after the discovery of cffDNA in maternal circulation.[85,86] Since then, numerous case studies have been published utilizing this technology to detect paternally inherited or de novo autosomal dominant conditions, such as achondroplasia.[87,88] Analysis of autosomal recessive conditions is analytically more challenging due to the high background of the maternal pathogenic variant in the cfDNA. In 2002, to overcome this barrier, only the paternal pathogenic variant was assessed for exclusion in the cfDNA.[89] Despite single gene NIPT (sgNIPT) becoming technologically available around the same time as aneuploidy NIPS, it had not become commercially available until 2018 and 2019 for autosomal dominant and recessive conditions, respectively. The slower adaptation of this testing was likely due to a combination of low commercial interest, high cost, insufficient volume of samples for validation, and low prevalence of individual conditions.[90–92]

Autosomal dominant conditions targeted for cfDNA analysis (AD sgNIPT) typically include severe gestational or neonatal phenotypes with high de novo rates. While sgNIPT for autosomal recessive conditions (AR sgNIPT) assess for inherited disorders. AD sgNIPT compares maternal and fetal DNA to find differences in genes of interest, while AR sgNIPT compares the quantities of pathogenic to wild type alleles in the relevant genes.

Multiple studies have referred to sgNIPT as non-invasive prenatal diagnosis (NIPD) because unlike aneuploidy, placental mosaicism is not a limiting factor.[90–92] While the sensitivity and specificity are at times comparable to diagnostic testing, sgNIPT can still produce false results due to vanishing twin.[90] Furthermore, many monogenic conditions currently present on the available screening panels have variable penetrance and high clinical variability and thus phenotype cannot always be predicted from genotype. It is important to keep these factors in mind when interpreting results.

Autosomal Dominant Conditions

Currently, a commercially available panel for dominant single gene NIPT includes 25 conditions across 30 genes that have severe phenotypes and a collective incidence of 1 in 600. The conditions include Noonan spectrum disorders, skeletal disorders, craniosynostosis syndromes, Cornelia de Lange syndrome, Alagille syndrome, tuberous sclerosis,

epileptic encephalopathy, SYNGAP1 related intellectual disability, CHARGE syndrome, Sotos syndromes, and Rett syndrome.[93,94]

Indications for this testing can include (1) advanced paternal age (>40 years old),[95] (2) paternal family history of the aforementioned conditions, (3) ultrasound anomalies such as cardiac defects, lymphatic anomalies (cystic hygroma, increased nuchal translucency, pleural effusion, polyhydramnios, fetal hydrops, hydrocephalus), skeletal abnormalities (skull, facial, long bone, and digit malformations), and/or growth restriction.[93,94]

Single gene NIPT for autosomal dominant conditions compares the cfDNA to the maternal genomic DNA (gDNA). If a mutation is identified in the former and not the latter, the variant is assumed to originate from the fetal cfDNA and is either de novo or paternally inherited. Differentiating between mutations in the cfDNA and sequencing errors is a challenge with this technology. To overcome this, unique sequences are attached to each fragment of DNA. The sample is enriched and sequenced with next generation sequencing (NGS). Since errors can arise during this process, the fragments with the same unique sequences are compared and deduplicated, a call is made when two-thirds of the reads match. The results are then compared to the maternal gDNA (in the past a paternal DNA sample was required, but recent NIPS developments have negated the need for a paternal sample). Pathogenic or likely pathogenic variants identified in the cfDNA are confirmed by repeat testing with deeper sequencing. Since this testing technology is assessing for novel variants the sensitivity (>99%) and specificity (>99%) are high. According to the laboratory's 2018 validation study and 2022 clinical experience study, no false negatives nor false positives were reported; however, the cohorts were small, and no call rates were high.[93,94,96]

It is important to note that sgNIPT is still considered screening and, hence, irreversible pregnancy decisions should not be determined on these results alone. The most common genes associated with the aforementioned conditions are on this panel, not all associated genes are included. Furthermore, only sequence variants are assessed, deletions, duplications, structural rearrangements, and deep intronic variants are not evaluated.[94] Lastly, this methodology cannot assess fetal risk if the pregnant patient has a pathogenic variant in any of the 30 genes, the fetus would be at a 50% risk of inheriting the mutation and diagnostic testing would be the only way to accurately assess this risk.[93,94]

Autosomal Recessive Conditions

Screening for autosomal recessive and X-linked recessive conditions is recommended by ACOG and ACMG prior to pregnancy; however, most screening does not occur until pregnancy begins and less than half of partners complete carrier screening.[97–99] These barriers can result in uptake of diagnostic testing that a patient might have otherwise declined, limit the time for reproductive decision-making, and increase maternal anxiety. Currently, ACOG recommends screening for hemoglobinopathies, cystic fibrosis, and spinal muscular atrophy in all pregnant patients.[100] They recommend additional screening in the presence of a positive family history or specific ethnic background.[101] ACMG recommends screening for 113 conditions that have a worldwide prevalence of ≥1/200.[102] Generally, the carrier screening turnaround time is 3–4 weeks and diagnostic testing results are reported in 2–3 weeks.

Single gene NIPT for autosomal recessive conditions can overcome some of the barriers posed by the traditional testing workflow. Currently, this testing is available for cystic fibrosis (*CFTR)*, spinal muscular atrophy (*SMN1)*, and hemoglobinopathies (*HBA1, HBA2, and HBB)*.[103] The list of screened conditions using this platform is likely to expand in the future. If the pregnant patient is found to be a carrier for the aforementioned conditions, the testing is reflexed to the fetal cfDNA without the need for an additional blood sample. A paternal sample is not required to produce a result. The goal of sgNIPT for AR conditions is to evaluate the chance that the fetus has two homozygous or compound heterozygous pathogenic variants in the conditions tested, and therefore, be affected.[104] There are a number of different possible test results. If the calculated risk is less than the a priori risk, then a low risk (<1/500) or decreased risk result is reported. If the calculated risk is higher than the a priori risk, then high risk (>1/4) or increased risk result is reported. Furthermore, the high risk category is split into four risk estimates, 1/4, 1/2, 2/3, and 9/10, that correspond to the PPV to assist in patient counseling.[103]

Single gene NIPT for AR conditions is a powerful tool that is especially important in situations where the pregnant patient wants carrier screening but (1) she is late to care, (2) her partner is unavailable, uninsured, or unwilling to undergo testing, (3)if there is a time constraint for reproductive decision-making, (4) if paternity is undetermined, or (5) if the couple is determined to be at 25% risk of having an affected child, yet are reluctant to pursue diagnostic testing. The potential for this tecnology to serve as a

primary carrier screening modality raises compelling questions that deserve dedicated investigations. Single gene NIPT is not diagnostic and should not be used to make irreversible pregnancy decisions.

Fetal Antigen Typing

RhD positive or negative status refers to the presence or absence of the RhD antigen on the surface of red blood cells, respectively.[105] RhD negative individuals are homozygous for variants on the *RHD* gene, compound heterozygous for one variant and one deletion, or most commonly, homozygous for deletions of the *RHD* gene. These individuals are at risk of creating antibodies against the RhD antigen (alloimmunization) after exposure events. During pregnancy, sensitization events can include vaginal bleeding, miscarriage, trauma, placental abruption, diagnostic testing (amniocentesis or chorionic villus sampling), and labor. Non-pregnancy events can include situations where RhD positive blood is introduced into circulation, such as blood transfusions.[105] Alloimmunization can result in maternal antibodies crossing the placenta and destroying fetal red blood cells, also known as, hemolytic disease of the fetus and newborn (HDFN). To prevent HDFN, the FDA approved use of prophylactic Rho(D) immune globulin (RhIg) in RhD negative non-alloimmunized women in the late 1960's. In the United States, RhIg has been given to all RhD negative women since then, and RhD associated HDFN has decreased from 16% to 1%.[106] The presence of other red blood cell (RBC) antigens or minor antigens can also cause HDFN, although they are much less common. Antibodies towards non-RhD antigens are identified in 1.5–2.5% of pregnant women.[105] RhIG is not applicable for these pregnancies.

If the patient is RhD negative or is identified to have antibodies against any erythrocyte antigen associated with HDFN, the traditional next step involves testing the partner for antigen status.[105] If negative, then the fetus is presumed to not be at risk.If positive or if reproductive partner is unavailable for testing, the pregnancy may need additional surveillance and monitoring. Maternal antibody titers and Doppler measurements of peak systolic velocity (PSV) in the fetal middle cerebral artery (MCA) can be used to monitor the pregnancy. If necessary, the benefits and risks of intrauterine blood transfusion and early delivery should be weighed.[105]

Beginning in March 2024, in the United States, there was a nationwide shortage of RhIg, which prompted the integration of fetal RhD testing to prioritize RhIg for RhD negative women with RhD positive fetuses.[107] Non-invasive fetal RhD testing has been the standard of care throughout many countries in Europe for quite some time.[108–113] One likely reason that this practice has not been adopted in the United States is the high rate of inconclusive or false results in the presence of non-deletion *RHD* variants.[106,114] Qualitative PCR technology (that is used in European countries) cannot determine if the maternal sequence variant is present or absent in the fetal cfDNA; thus, the fetal antigen profile could not be predicted, causing inconclusive or false results.[106] Non-deletion *RHD* variants are more common in non-European ancestries.

Recently, two tests have been validated in the United States to assess fetal antigens with high sensitivity, specificity, and low inconclusive rate. In 2020, one lab validated their methodology for detection of fetal RhD, C, c, E, Kell, and Fya.[106] They were able to achieve 100% sensitivity (95% CI 99–100%) and 100% specificity (95% CI 99–100%) because they developed a method to accurately quantify sequence variants, which allowed them to predict whether the fetus inherited the maternal variant. In 2024, a different laboratory used SNP-based NIPS to successfully detect the fetal *RHD* genotype, with 100% sensitivity (95% CI 98.9–100%) and 99.3% specificity (95% CI 97.6–99.8%).[115]

Due to high sensitivity and specificity of fetal RBC antigen NIPT, it is reasonable to use this methodology to evaluate the risk of HDFN when a patient declines diagnostic testing. NIPT can be informative about fetal status when (1) the patient is RhD negative, (2) the patient developed antibodies towards other RBC antigens known to be associated with HDFN, (3) the father is unavailable/unwilling to test or paternity is unknown, and/or (4) the father tests positive for the antigen in question. As of March 2024, ACOG supports the use of NIPT to detect fetal RhD status in the context of an RhIg shortage, but has not taken a stance on this testing for minor antigens[116].

Ethical Considerations

Widespread use of NIPS in pregnancies to screen for chromosomal abnormalities in fetuses raises ethical questions related to genetic privacy, reproductive decision-making, and the potential for unintended consequences. Globally, the birth rate of individuals with Down syndrome has drastically decreased since the mid-2010s and has been attributed to

cfDNA screening. As cfDNA testing and screening aims to detect more and more conditions, the question of unintended consequences should remain at the forefront.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Committee on Practice Bulletins—Obstetrics; Society for Maternal–Fetal Medicine. Practice bulletin no. 169: multifetal gestations: twin, triplet, and higher-order multifetal pregnancies. *Obstet Gynecol*. 2016;128(4):e131–46. doi:10.1097/aog.0000000000001709
2. Wang BT, Chong TP, Boyar FZ, et al. Abnormalities in spontaneous abortions detected by G-banding and chromosomal microarray analysis (CMA) at a national reference laboratory. *Mol Cytogenet*. 2014;7:33. doi:10.1186/1755-8166-7-33
3. Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol*. 1999;13 (4):231–237. doi:10.1046/j.1469-0705.1999.13040231.x
4. Wald NJ, Rodeck C, Hackshaw AK, Rudnicka A. SURUSS in perspective. *BJOG*. 2004;111(6):521–531. doi:10.1111/j.1471-0528.2004.00193.x
5. Malone FD, Canick JA, Ball RH, et al. First-trimester or second-trimester screening, or both, for down's syndrome. *N Engl J Med*. 2005;353 (19):2001–2011. doi:10.1056/NEJMoa043693
6. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. *Lancet*. 1997;350(9076):485–487. doi:10.1016/s0140-6736(97)02174-0
7. Chiu RW, Chan KC, Gao Y, et al. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. *Proc Natl Acad Sci U S A*. 2008;105(51):20458–20463. doi:10.1073/pnas.0810641105
8. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *Proc Natl Acad Sci U S A*. 2008;105(42):16266–16271. doi:10.1073/pnas.0808319105
9. Lo YM. Non-invasive prenatal diagnosis by massively parallel sequencing of maternal plasma DNA. *Open Biol*. 2012;2(6):120086. doi:10.1098/rsob.120086
10. Lo YM, Lun FM, Chan KC, et al. Digital PCR for the molecular detection of fetal chromosomal aneuploidy. *Proc Natl Acad Sci U S A*. 2007;104(32):13116–13121. doi:10.1073/pnas.0705765104
11. Palomaki GE, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to detect down syndrome: an international clinical validation study. *Genet Med*. 2011;13(11):913–920. doi:10.1097/GIM.0b013e3182368a0e
12. Gregg AR, Gross SJ, Best RG, et al. ACMG statement on noninvasive prenatal screening for fetal aneuploidy. *Genet Med*. 2013;15(5):395–398. doi:10.1038/gim.2013.29
13. Bianchi DW, Parker RL, Wentworth J, et al. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med*. 2014;370 (9):799–808. doi:10.1056/NEJMoa1311037
14. Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy. *N Engl J Med*. 2015;372 (17):1589–1597. doi:10.1056/NEJMoa1407349
15. Zhang H, Gao Y, Jiang F, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146 958 pregnancies. *Ultrasound Obstet Gynecol*. 2015;45(5):530–538. doi:10.1002/uog.14792
16. American College of Obstetricians and Gynecologists, Society for Maternal-Fetal Medicine. Screening for fetal chromosomal abnormalities: ACOG practice bulletin, number 226. *Obstet Gynecol*. 2020;136(4):e48–e69. doi:10.1097/aog.0000000000004084
17. Rose NC, Barrie ES, Malinowski J, et al. Systematic evidence-based review: the application of noninvasive prenatal screening using cell-free DNA in general-risk pregnancies. *Genet Med*. 2022;24(7):1379–1391. doi:10.1016/j.gim.2022.03.019
18. Van Opstal D, Eggenhuizen GM, Joosten M, et al. Noninvasive prenatal testing as compared to chorionic villus sampling is more sensitive for the detection of confined placental mosaicism involving the cytotrophoblast. *Prenat Diagn*. 2020;40(10):1338–1342. doi:10.1002/pd.5766
19. Okoror CEM, Arora S. Prenatal diagnosis after high chance non-invasive prenatal testing for trisomies 21, 18 and 13, chorionic villus sampling or amniocentesis? - Experience at a district general hospital in the United Kingdom. *Eur J Obstet Gynecol Reprod Biol X*. 2023;19:100211. doi:10.1016/j.eurox.2023.100211
20. Cherry AM, Akkari YM, Barr KM, et al. Diagnostic cytogenetic testing following positive noninvasive prenatal screening results: a clinical laboratory practice resource of the American College of MEDICAL GENETICS AND GENomics (ACMG). *Genet Med*. 2017;19(8):845–850. doi:10.1038/gim.2017.91
21. Warsof SL, Larion S, Abuhamad AZ. Overview of the impact of noninvasive prenatal testing on diagnostic procedures. *Prenat Diagn*. 2015;35 (10):972–979. doi:10.1002/pd.4601
22. Kinnings SL, Geis JA, Almasri E, et al. Factors affecting levels of circulating cell-free fetal DNA in maternal plasma and their implications for noninvasive prenatal testing. *Prenat Diagn*. 2015;35(8):816–822. doi:10.1002/pd.4625
23. Guseh S, Wilkins-Haug L, Kaimal A, et al. Utility of noninvasive genome-wide screening: a prospective cohort of obstetric patients undergoing diagnostic testing. *Genet Med*. 2021;23(7):1341–1348. doi:10.1038/s41436-021-01147-4
24. Zimmermann B, Hill M, Gemelos G, et al. Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. *Prenat Diagn*. 2012;32(13):1233–1241. doi:10.1002/pd.3993
25. Nicolaides KH, Syngelaki A, Gil M, Atanasova V, Markova D. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. *Prenat Diagn*. 2013;33(6):575–579. doi:10.1002/pd.4103
26. Samango-Sprouse C, Banjevic M, Ryan A, et al. SNP-based non-invasive prenatal testing detects sex chromosome aneuploidies with high accuracy. *Prenat Diagn*. 2013;33(7):643–649. doi:10.1002/pd.4159
27. Dar P, Curnow KJ, Gross SJ, et al. Clinical experience and follow-up with large scale single-nucleotide polymorphism-based noninvasive prenatal aneuploidy testing. *Am J Obstet Gynecol*. 2014;211(5):527.e1–527.e17. doi:10.1016/j.ajog.2014.08.006

28. Hall MP, Hill M, Zimmermann B, et al. Non-invasive prenatal detection of trisomy 13 using a single nucleotide polymorphism- and informatics-based approach. *PLoS One*. 2014;9(5):e96677. doi:10.1371/journal.pone.0096677
29. Pergament E, Cuckle H, Zimmermann B, et al. Single-nucleotide polymorphism-based noninvasive prenatal screening in a high-risk and low-risk cohort. *Obstet Gynecol*. 2014;124(2 Pt 1):210–218. doi:10.1097/aog.0000000000000363
30. Wapner RJ, Babiarz JE, Levy B, et al. Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. *Am J Obstet Gynecol*. 2015;212(3):332.e1–9. doi:10.1016/j.ajog.2014.11.041
31. Lefkowitz RB, Tynan JA, Liu T, et al. Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants. *Am J Obstet Gynecol*. 2016;215(2):227.e1–227.e16. doi:10.1016/j.ajog.2016.02.030
32. Srebniak MI, Vogel I, Van Opstal D. Is carriership of a balanced translocation or inversion an indication for non-invasive prenatal testing? *Expert Rev Mol Diagn*. 2018;18(6):477–479. doi:10.1080/14737159.2018.1468255
33. Srebniak MI, Jehee FS, Joosten M, et al. Non-invasive prenatal diagnosis for translocation carriers—YES please or NO go? *Acta Obstetricia et Gynecologica Scandinavica*. 2021;100(11):2036–2043. doi:10.1111/aogs.14256
34. Benn P, Rebarber A. Non-invasive prenatal testing in the management of twin pregnancies. *Prenat Diagn*. 2021;41(10):1233–1240. doi:10.1002/pd.5989
35. Norwitz ER, McNeill G, Kalyan A, et al. Validation of a single-nucleotide polymorphism-based non-invasive prenatal test in twin gestations: determination of zygosity, individual fetal sex, and fetal aneuploidy. *J Clin Med*. 2019;8(7):937. doi:10.3390/jcm8070937
36. Wang Y, Qiu X, Chen S, et al. Noninvasive evaluation of fetal zygosity in twin pregnancies involving a binary analysis of single-nucleotide polymorphisms. *J Mol Diagn*. 2023;25(9):682–691. doi:10.1016/j.jmoldx.2023.06.003
37. Hedriana H, Martin K, Saltzman D, Billings P, Demko Z, Benn P. Cell-free DNA fetal fraction in twin gestations in single-nucleotide polymorphism-based noninvasive prenatal screening. *Prenat Diagn*. 2020;40(2):179–184. doi:10.1002/pd.5609
38. Kantor V, Mo L, DiNonno W, et al. Positive predictive value of a single nucleotide polymorphism (SNP)-based NIPT for aneuploidy in twins: experience from clinical practice. *Prenat Diagn*. 2022;42(13):1587–1593. doi:10.1002/pd.6262
39. Curnow KJ, Wilkins-Haug L, Ryan A, et al. Detection of triploid, molar, and vanishing twin pregnancies by a single-nucleotide polymorphism-based noninvasive prenatal test. *Am J Obstet Gynecol*. 2015;212(1):79.e1–9. doi:10.1016/j.ajog.2014.10.012
40. Deng C, Liu S. Factors affecting the fetal fraction in noninvasive prenatal screening: a review. *Front Pediatr*. 2022;10:812781. doi:10.3389/fped.2022.812781
41. Grömminger S, Erkan S, Schöck U, et al. The influence of low molecular weight heparin medication on plasma DNA in pregnant women. *Prenat Diagn*. 2015;35(11):1155–1157. doi:10.1002/pd.4668
42. van Eekhout JCA, Bekker MN, Bax CJ, Galjaard R-JH. Non-invasive prenatal testing (NIPT) in twin pregnancies affected by early single fetal demise: a systematic review of NIPT and vanishing twins. *Prenat Diagn*. 2023;43(7):829–837. doi:10.1002/pd.6388
43. Grace MR, Hardisty E, Dotters-Katz SK, Vora NL, Kuller JA. Cell-free DNA screening: complexities and challenges of clinical implementation. *Obstet Gynecol Surv*. 2016;71(8):477–487. doi:10.1097/ogx.0000000000000342
44. van Riel M, Brison N, Baetens M, et al. Performance and diagnostic value of genome-wide noninvasive prenatal testing in multiple gestations. *Obstet Gynecol*. 2021;137(6):1102–1108. doi:10.1097/aog.0000000000004385
45. Bernal Claverol M, Ruiz Minaya M, Aracil Moreno I, et al. Maternal, perinatal and neonatal outcomes of triplet pregnancies according to chorionicity: a systematic review of the literature and meta-analysis. *J Clin Med*. 2022;11(7):1871. doi:10.3390/jcm11071871
46. Zakaria H, Kleinfinger P, Lohmann L, et al. Performance of cell-free DNA testing for common fetal trisomies in triplet pregnancies. *Prenat Diagn*. 2024;44(5):555–561. doi:10.1002/pd.6548
47. Stone J, Kohari KS. Higher-order multiples. *Clin Obstet Gynecol*. 2015;58(3):668–675. doi:10.1097/grf.0000000000000121
48. Chen M, Jiang F, Guo Y, et al. Validation of fetal DNA fraction estimation and its application in noninvasive prenatal testing for aneuploidy detection in multiple pregnancies. *Prenat Diagn*. 2019;39(13):1273–1282. doi:10.1002/pd.5597
49. Dyr B, Boomer T, Almasri EA, et al. A new era in aneuploidy screening: cfDNA testing in >30,000 multifetal gestations: experience at one clinical laboratory. *PLoS One*. 2019;14(8):e0220979. doi:10.1371/journal.pone.0220979
50. Ryan A, Hunkapiller N, Banjevic M, et al. Validation of an enhanced version of a single-nucleotide polymorphism-based noninvasive prenatal test for detection of fetal aneuploidies. *Fetal Diagn Ther*. 2016;40(3):219–223. doi:10.1159/000442931
51. Kolarski M, Ahmetovic B, Beres M, et al. Genetic counseling and prenatal diagnosis of triploidy during the second trimester of pregnancy. *Med Arch*. 2017;71(2):144–147. doi:10.5455/medarh.2017.71.144-147
52. Kantor V, Jelsema R, Xu W, et al. Non-invasive prenatal screening for fetal triploidy using single nucleotide polymorphism-based testing: differential diagnosis and clinical management in cases showing an extra haplotype. *Prenat Diagn*. 2022;42(8):994–999. doi:10.1002/pd.6169
53. Kimelman D, Pavone ME. Non-invasive prenatal testing in the context of IVF and PGT-A. *Best Pract Res Clin Obstet Gynaecol*. 2021;70:51–62. doi:10.1016/j.bpobgyn.2020.07.004
54. Amor DJ, Xu JX, Halliday JL, et al. Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for down syndrome. *Hum Reprod*. 2009;24(6):1330–1338. doi:10.1093/humrep/dep046
55. Gjerris AC, Loft A, Pinborg A, Christiansen M, Tabor A. First-trimester screening markers are altered in pregnancies conceived after IVF/ICSI. *Ultrasound Obstet Gynecol*. 2009;33(1):8–17. doi:10.1002/uog.6254
56. Lee TJ, Rolnik DL, Menezes MA, McLennan AC, da Silva Costa F. Cell-free fetal DNA testing in singleton IVF conceptions. *Hum Reprod*. 2018;33(4):572–578. doi:10.1093/humrep/dey033
57. Revello R, Sarno L, Ispas A, Akolekar R, Nicolaides KH. Screening for trisomies by cell-free DNA testing of maternal blood: consequences of a failed result. *Ultrasound Obstet Gynecol*. 2016;47(6):698–704. doi:10.1002/uog.15851
58. Li L, Wang L, Le F, et al. Evaluation of DNA methylation status at differentially methylated regions in IVF-conceived newborn twins. *Fertil Steril*. 2011;95(6):1975–1979. doi:10.1016/j.fertnstert.2011.01.173
59. Loke YJ, Galati JC, Saffery R, Craig JM. Association of in vitro fertilization with global and IGF2/H19 methylation variation in newborn twins. *J Dev Orig Health Dis*. 2015;6(2):115–124. doi:10.1017/s2040174415000161

60. Nelissen EC, Dumoulin JC, Daunay A, Evers JL, Tost J, van Montfoort AP. Placentas from pregnancies conceived by IVF/ICSI have a reduced DNA methylation level at the H19 and MEST differentially methylated regions. *Hum Reprod*. 2013;28(4):1117–1126. doi:10.1093/humrep/des459
61. Oliver VF, Miles HL, Cutfield WS, Hofman PL, Ludgate JL, Morison IM. Defects in imprinting and genome-wide DNA methylation are not common in the in vitro fertilization population. *Fertil Steril*. 2012;97(1):147–53.e7. doi:10.1016/j.fertnstert.2011.10.027
62. Tierling S, Souren NY, Gries J, et al. Assisted reproductive technologies do not enhance the variability of DNA methylation imprints in human. *J Med Genet*. 2010;47(6):371–376. doi:10.1136/jmg.2009.073189
63. Bianchi DW, Chiu RWK. Sequencing of circulating cell-free DNA during pregnancy. *N Engl J Med*. 2018;379(5):464–473. doi:10.1056/NEJMra1705345
64. Liang D, Lin Y, Luo C, Li H, Hu P, Xu Z. Noninvasive prenatal screening in a pregnant woman with a history of stem cell transplant from a male donor: a case report and literature review. *Mol Genet Genomic Med*. 2024;12(6):e2479. doi:10.1002/mgg3.2479
65. Yan Y-Y, Guo Q-R, Wang F-H, et al. Cell-free DNA: hope and potential application in cancer. Review. *Front Cell Dev Biol*. 2021;9. doi:10.3389/fcell.2021.639233
66. Snyder TM, Khush KK, Valantine HA, Quake SR. Universal noninvasive detection of solid organ transplant rejection. *Proc Natl Acad Sci U S A*. 2011;108(15):6229–6234. doi:10.1073/pnas.1013924108
67. Aubert O, Ursule-Dufait C, Brousse R, et al. Cell-free DNA for the detection of kidney allograft rejection. *Nature Medicine*. 2024;30(8):2320–2327. doi:10.1038/s41591-024-03087-3
68. Oellerich M, Budde K, Osmanodja B, et al. Donor-derived cell-free DNA as a diagnostic tool in transplantation. *Front Genet*. 2022;13:1031894. doi:10.3389/fgene.2022.1031894
69. Hu Y, Zhao Y, Zhang Y, Chen W, Zhang H, Jin X. Cell-free DNA: a promising biomarker in infectious diseases. *Trends Microbiol*. 2024. doi:10.1016/j.tim.2024.06.005
70. Avni B, Neiman D, Shaked E, et al. Chronic graft-versus-host disease detected by tissue-specific cell-free DNA methylation biomarkers. *J Clin Invest*. 2024;134(2). doi:10.1172/jci163541
71. Nader-Marta G, Monteforte M, Agostinetto E, et al. Circulating tumor DNA for predicting recurrence in patients with operable breast cancer: a systematic review and meta-analysis. *ESMO Open*. 2024;9(3):102390. doi:10.1016/j.esmoop.2024.102390
72. Bianchi DW, Parsa S, Bhatt S, et al. Fetal Sex chromosome testing by maternal plasma DNA sequencing: clinical laboratory experience and biology. *Obstet Gynecol*. 2015;125(2):375–382. doi:10.1097/aog.0000000000000637
73. Neufeld-Kaiser WA, Cheng EY, Liu YJ. Positive predictive value of non-invasive prenatal screening for fetal chromosome disorders using cell-free DNA in maternal serum: independent clinical experience of a tertiary referral center. *BMC Med*. 2015;13(1):129. doi:10.1186/s12916-015-0374-8
74. Gregg AR, Skotko BG, Benkendorf JL, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. *Genet Med*. 2016;18(10):1056–1065. doi:10.1038/gim.2016.97
75. Lo YMD, Zhang J, Leung TN, Lau TK, Chang AMZ, Hjelm NM. Rapid Clearance of Fetal DNA from Maternal Plasma. *Am J Hum Genet*. 1999;64(1):218–224. doi:10.1086/302205
76. Hui L, Bianchi DW. Fetal fraction and noninvasive prenatal testing: what clinicians need to know. *Prenat Diagn*. 2020;40(2):155–163. doi:10.1002/pd.5620
77. Canick JA, Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE. The impact of maternal plasma DNA fetal fraction on next generation sequencing tests for common fetal aneuploidies. *Prenat Diagn*. 2013;33(7):667–674. doi:10.1002/pd.4126
78. Livergood MC, LeChien KA, Trudell AS. Obesity and cell-free DNA "no calls": is there an optimal gestational age at time of sampling? *Am J Obstet Gynecol*. 2017;216(4):413.e1–413.e9. doi:10.1016/j.ajog.2017.01.011
79. Rolnik DL, Yong Y, Lee TJ, Tse C, McLennan AC, da Silva Costa F. Influence of body mass index on fetal fraction increase with gestation and cell-free DNA test failure. *Obstet Gynecol*. 2018;132(2):436–443. doi:10.1097/aog.0000000000002752
80. Ashoor G, Syngelaki A, Poon LCY, Rezende JC, Nicolaides KH. Fetal fraction in maternal plasma cell-free DNA at 11–13 weeks' gestation: relation to maternal and fetal characteristics. *Ultrasound Obstet Gynecol*. 2013;41(1):26–32. doi:10.1002/uog.12331
81. Hestand MS, Bessem M, van Rijn P, et al. Fetal fraction evaluation in non-invasive prenatal screening (NIPS). *Eur J Hum Genet*. 2019;27(2):198–202. doi:10.1038/s41431-018-0271-7
82. Burns W, Koelper N, Barberio A, et al. The association between anticoagulation therapy, maternal characteristics, and a failed cfDNA test due to a low fetal fraction. *Prenat Diagn*. 2017;37(11):1125–1129. doi:10.1002/pd.5152
83. Ma G-C, Wu W-J, Lee M-H, Lin Y-S, Chen M. Low-molecular-weight heparin associated with reduced fetal fraction and subsequent false-negative cell-free DNA test result for trisomy 21. *Ultrasound Obstet Gynecol*. 2018;51(2):276–277. doi:10.1002/uog.17473
84. Shree R, MacKinnon HJ, Hannan J, Kolarova TR, Reichel J, Lockwood CM. Anticoagulation use is associated with lower fetal fraction and more indeterminate results. *Am J Obstet Gynecol*. 2024;230(1):95.e1–95.e10. doi:10.1016/j.ajog.2023.07.005
85. Saito H, Sekizawa A, Morimoto T, Suzuki M, Yanaihara T. Prenatal DNA diagnosis of a single-gene disorder from maternal plasma. *Lancet*. 2000;356(9236):1170. doi:10.1016/S0140-6736(00)02767-7
86. Amicucci P, Gennarelli M, Novelli G, Dallapiccola B. Prenatal diagnosis of myotonic dystrophy using fetal DNA obtained from maternal plasma. *Clin Chem*. 2000;46(2):301–302. doi:10.1093/clinchem/46.2.301
87. Chitty LS, Griffin DR, Meaney C, et al. New aids for the non-invasive prenatal diagnosis of achondroplasia: dysmorphic features, charts of fetal size and molecular confirmation using cell-free fetal DNA in maternal plasma. *Ultrasound Obstet Gynecol*. 2011;37(3):283–289. doi:10.1002/uog.8893
88. Li Y, Holzgreve W, Page-Christiaens GCML, Gille JJP, Hahn S. Improved prenatal detection of a fetal point mutation for achondroplasia by the use of size-fractionated circulatory DNA in maternal plasma—case report. *Prenat Diagn*. 2004;24(11):896–898. doi:10.1002/pd.1030
89. Chiu RW, Lau TK, Leung TN, Chow KC, Chui DH, Lo YM. Prenatal exclusion of beta thalassaemia major by examination of maternal plasma. *Lancet*. 2002;360(9338):998–1000. doi:10.1016/s0140-6736(02)11086-5
90. Scotchman E, Chandler NJ, Mellis R, Chitty LS. Noninvasive prenatal diagnosis of single-gene diseases: the next frontier. *Clin Chem*. 2020;66(1):53–60. doi:10.1373/clinchem.2019.304238

91. Jenkins LA, Deans ZC, Lewis C, Allen S. Delivering an accredited non-invasive prenatal diagnosis service for monogenic disorders and recommendations for best practice. *Prenat Diagn*. 2018;38(1):44–51. doi:10.1002/pd.5197
92. Chitty LS. Advances in the prenatal diagnosis of monogenic disorders. *Prenat Diagn*. 2018;38(1):3–5. doi:10.1002/pd.5208
93. Zhang J, Li J, Saucier JB, et al. Non-invasive prenatal sequencing for multiple Mendelian monogenic disorders using circulating cell-free fetal DNA. *Nat Med*. 2019;25(3):439–447. doi:10.1038/s41591-018-0334-x
94. Mohan P, Lemoine J, Trotter C, et al. Clinical experience with non-invasive prenatal screening for single-gene disorders. *Ultrasound Obstet Gynecol*. 2022;59(1):33–39. doi:10.1002/uog.23756
95. Toriello HV, Meck JM. Statement on guidance for genetic counseling in advanced paternal age. *Genet Med*. 2008;10(6):457–460. doi:10.1097/GIM.0b013e318176fabb
96. Adams S, Llorin H, Maher O, et al. Single gene non-invasive prenatal screening for autosomal dominant conditions in a high-risk cohort. *Prenat Diagn*. 2023;43(9):1110–1119. doi:10.1002/pd.6351
97. Giles Choates M, Stevens BK, Wagner C, Murphy L, Singletary CN, Wittman AT. It takes two: uptake of carrier screening among male reproductive partners. *Prenat Diagn*. 2020;40(3):311–316. doi:10.1002/pd.5588
98. Hull LE, Cheng D, Hallman MH, Rieu-Werden ML, Haas JS. Association of patient and site-of-care characteristics with reproductive carrier screening timing in a large integrated health system. *JAMA Network Open*. 2022;5(11):e2240829–e2240829. doi:10.1001/jamanetworkopen.2022.40829
99. Strauss TS, Schneider E, Boniferro E, et al. Barriers to completion of expanded carrier screening in an inner city population. *Genet Med*. 2023;25(7):100858. doi:10.1016/j.gim.2023.100858
100. Rink B, Romero S, Biggio JR, Saller DN, Jr, Giardine R. Committee opinion no. 691: Carrier Screening for Genetic Conditions. *Obstet Gynecol*. 2017;129(3):e41–e55. doi:10.1097/aog.0000000000001952
101. Committee on Genetics. Committee opinion no. 690: carrier screening in the age of genomic medicine. *Obstet Gynecol*. 2017;129(3):e35–e40. doi:10.1097/aog.0000000000001951
102. Gregg AR, Aarabi M, Klugman S, et al. Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2021;23(10):1793–1806. doi:10.1038/s41436-021-01203-z
103. Wynn J, Hoskovec J, Carter RD, Ross MJ, Perni SC. Performance of single-gene noninvasive prenatal testing for autosomal recessive conditions in a general population setting. *Prenat Diagn*. 2023;43(10):1344–1354. doi:10.1002/pd.6427
104. Tsao DS, Silas S, Landry BP, et al. A novel high-throughput molecular counting method with single base-pair resolution enables accurate single-gene NIPT. *Sci Rep*. 2019;9(1):14382. doi:10.1038/s41598-019-50378-8
105. Holmgren C, Flint Porter T. ACOG Practice Bulletin no. 192: management of Alloimmunization During Pregnancy. *Obstet Gynecol*. 2018;131(3):e82–e90. doi:10.1097/aog.0000000000002528
106. Alford B, Landry BP, Hou S, et al. Validation of a non-invasive prenatal test for fetal RhD, C, c, E, K and Fy(a) antigens. *Sci Rep*. 2023;13(1):12786. doi:10.1038/s41598-023-39283-3
107. ACOG Practice advisory: rho(D) Immune Globulin Shortages. *Obstet Gynecol*. Available from: https://www.acog.org/clinical/clinical-guidance/practice-advisory/articles/2024/03/rhod-immune-globulin-shortages. Accessed April 22, 2025.
108. Chitty LS, Finning K, Wade A, et al. Diagnostic accuracy of routine antenatal determination of fetal RHD status across gestation: population based cohort study. *BMJ*. 2014;349:g5243. doi:10.1136/bmj.g5243
109. Clausen FB, Steffensen R, Christiansen M, et al. Routine noninvasive prenatal screening for fetal RHD in plasma of RhD-negative pregnant women-2 years of screening experience from Denmark. *Prenat Diagn*. 2014;34(10):1000–1005. doi:10.1002/pd.4419
110. Daniels G. Variants of RhD--current testing and clinical consequences. *Br J Haematol*. 2013;161(4):461–470. doi:10.1111/bjh.12275
111. Finning K, Martin P, Summers J, Daniels G. Fetal genotyping for the K (Kell) and Rh C, c, and E blood groups on cell-free fetal DNA in maternal plasma. *Transfusion*. 2007;47(11):2126–2133. doi:10.1111/j.1537-2995.2007.01437.x
112. Grande M, Ordoñez E, Cirigliano V, et al. Clinical application of midtrimester non-invasive fetal RHD genotyping and identification of RHD variants in a mixed-ethnic population. *Prenat Diagn*. 2013;33(2):173–178. doi:10.1002/pd.4035
113. Gutensohn K, Müller SP, Thomann K, et al. Diagnostic accuracy of noninvasive polymerase chain reaction testing for the determination of fetal rhesus C, c and E status in early pregnancy. *BJOG*. 2010;117(6):722–729. doi:10.1111/j.1471-0528.2010.02518.x
114. Singleton BK, Green CA, Avent ND, et al. The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype. *Blood*. 2000;95(1):12–18. doi:10.1182/blood.V95.1.12
115. Gilstrop Thompson M, Xu W, Moore B, et al. Clinical validation of a prenatal cell-free DNA screening test for fetal RHD in a large U.S. *Cohort Obstet Gynecol*. 2025;145(2):211–216. doi:10.1097/aog.0000000000005794
116. Gandi, M, Shields, A Rho(D) Immune Globulin Shortages Practice Advisory *American College of Obstetricians and Gynecologists* 2024