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COMMENTARY

PRENATAL DIAGNOSIS WILEY

Position statement from the International Society for Prenatal Diagnosis on the use of non-invasive prenatal testing for the detection of fetal chromosomal conditions in singleton pregnancies

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Key points

What is already known about this topic?

- In 2015, the International Society for Prenatal Diagnosis (ISPD) published its first position statement on the use of non-invasive prenatal testing (NIPT) to screen for aneuploidy. Widespread uptake across the globe and subsequent published research has shed new light on test performance and implementation issues.

What does this study add?

- This new position statement replaces the 2015 statement with updated information on the current technologies, clinical experience, and implementation practices.
- As an international organization, ISPD recognizes that there are important population-specific considerations in the organization of prenatal screening and diagnosis. These

opinions are designed to apply to high income settings where prenatal screening for aneuploidy is an established part of antenatal care.
- This position statement is not a clinical practice guideline but represents the consensus opinion of the current ISPD Board based on the current state of knowledge and clinical practice.

1 | INTRODUCTION

Every pregnancy has a chance of being affected by a clinically significant chromosome condition. For this reason, prenatal screening for the most common serious chromosome conditions (trisomies 21, 18 and 13) has been a routine part of prenatal care in high resource settings for over 50 years. Prenatal screening has evolved through maternal age-based screening, second trimester serum screening, and first trimester combined screening to the current era of maternal plasma cell-free DNA (cfDNA) based screening.

The genomic sequencing technology that facilitates cfDNA based screening brings both benefits and challenges to the field of prenatal care.[1] Non-invasive prenatal testing (NIPT) based on sequencing of cfDNA in maternal blood has been rapidly adopted in high resource settings.[2] In addition to its superior accuracy over traditional forms of screening, NIPT has the capacity to detect genome-wide chromosome imbalances, segmental imbalances, and microdeletion/microduplications as well as to provide incidental information on the maternal genome. The complexity of the issues raised for clinicians and patients by this disruptive technology has been well documented in the literature.[3,4]

The first ISPD position statement on NIPT was written in 2015, when the place of NIPT in routine antenatal care was still evolving.[5] At that time, there were many unanswered questions about its comparative performance with traditional forms of screening, confusion about false positive results (FPR), and concerns about missed opportunities to detect 'atypical' chromosome conditions. Ethical issues and cost-effectiveness were also significant areas of debate. The body of published evidence has since exploded rapidly.[6] Many health care systems now embrace NIPT in their national screening programs (Belgium, the Netherlands, Denmark, the UK), state or private health insurance schemes (United States), while in other countries NIPT remains entirely patient-funded.[7,8] Numerous professional societies, including ISPD, have issued guidance on the implementation of NIPT.[9,10] In some countries, even genome-wide NIPT has become commonplace,[11] while in other countries, NIPT for the common autosomal aneuploidies is not widely accessible.[12]

2 | SCOPE AND PURPOSE

This new ISPD position statement addresses the evolving clinical and research questions that have emerged since the 2015 statement. This document is written for health care professionals and laboratory scientists providing prenatal screening and diagnostic services. The writing group was drawn from current ISPD Board members and membership: maternal fetal medicine specialists (LH, NV, LS, MC,RR), laboratory (JV, MP) and clinical geneticists (LC, DM), and genetic counselors (KE) with broad geographical representation (UK, Europe, China, USA, Mexico, Singapore, Australia). This statement has been approved for publication by the ISPD Board. The ISPD Board gratefully acknowledges consumer representative Jane Fisher, Director of Antenatal Results and Choices, for her review of this statement.

In this statement, we cover the role of NIPT for routine screening for chromosome conditions in unselected populations of people with singleton pregnancies. Non-invasive prenatal testing for twin pregnancies was recently addressed in a separate ISPD position statement and will not be covered here.[13]

As an international organization, ISPD recognizes that there are important population-specific considerations in the organization of prenatal screening and diagnosis. The opinions stated here are designed to apply to high income settings where prenatal screening for aneuploidy is considered an established part of antenatal care. This position statement is not a clinical practice guideline but represents the consensus opinion of the current ISPD Board based on the current state of knowledge and clinical practice.

3 | BACKGROUND ON THE BIOLOGY AND TECHNOLOGY OF NON-INVASIVE PRENATAL TESTING FOR ANEUPLOIDY SCREENING

Cell-free DNA of fetoplacental origin was first demonstrated in maternal plasma in 1997,[14] and since then has been translated rapidly into clinical practice for the detection of fetal chromosomal conditions. CfDNA is released from cells as a by-product of cell-turnover and can be extracted from plasma or other body fluids. Circulating cfDNA arising from the placenta is derived from the outer cytotrophoblast,[15] is detectable as early as 5 weeks of gestation,[16] increases with gestation, and reaches 10%–12% of the total maternal plasma cfDNA pool by the end of the first trimester.[17]

The proportion of cfDNA arising from each chromosome is proportional to the size of the chromosome and the number of chromosomes in an individual's karyotype. Next generation sequencing technologies have facilitated the development of NIPT by allowing these fragments of plasma cfDNA to be sequenced, their chromosome of origin identified by mapping to a reference genome

TABLE 1 Main technological platforms for noninvasive prenatal testing.

Technique	Principle	Clinical relevance
Massively parallel sequencing, random whole genome sequencing[18]	All cfDNA fragments in maternal plasma are sequenced (both maternal and fetal origin)	• Able to detect imbalance in whole chromosomes and segments of chromosomes down to approximately 7Mb in size and so may detect microdeletion/microduplications • employed in genome-wide NIPT • may detect maternal chromosomal imbalances • may detect cfDNA plasma profiles suggestive of maternal malignancy
Chromosome-selective NIPT using amplification of selected regions and microarray hybridization[115]	Chromosome-specific regions on target chromosomes are enriched in a PCR-type reaction and this optimized sample is hybridized to a microarray	• Will not detect aberrations in most 'off target' chromosomes, or report on subchromosomal changes, except 22q11.2 deletion for some platforms • statistical method includes the person's prior chance of aneuploidy
SNP-based chromosome-selective NIPT[116]	Separately analyses DNA from maternal white blood cells and from the plasma; targeted amplification and analysis of unique SNPs on selected chromosomes/regions of interest;	• Use of SNPs allows additional biological information to be obtained using the allelic ratio • able to detect triploidy, • zygosity of twin pregnancies • may detect selected recurrent MMS

Abbreviations: MMS, microdeletion microduplication syndromes; NIPT: noninvasive prenatal testing; SNPs; single nucleotide polymorphisms.

and quantified.[18] This so-called 'counting method' of sequencing can identify more- or less-than-expected quantities of plasma cfDNA from individual chromosomes and thus detect pregnancies with suspected trisomy or monosomy, respectively. Although most of the maternal plasma cfDNA is derived from maternal cells, sequencing for NIPT is performed at a sufficient depth to detect small changes in the plasma profile due to aneuploidy in the placenta.

Several different technologies exist to perform NIPT for trisomies 21, 18, 13 and monosomy X. An overview of the three main approaches is provided in Table 1. Very few direct comparisons of different platforms have been published: one head-to-head comparison of two different sequencing platforms showed equivalent clinical validity.[19]

4 | PERFORMANCE CHARACTERISTICS

Trisomy 21.

Multiple systematic reviews have demonstrated that NIPT is a highly accurate screening test for trisomy 21, 18 and 13.[6,20-22] A recent evidence-based review from the American College of Medical Genetics and Genomics produced summary statistics of NIPT for general unselected populations.[6] The pooled performance characteristics for trisomy 21 screening based on 28 included studies showed a sensitivity of 98.80% (95% CI 97.81–99.34) and a specificity of 99.96% (95% CI 99.92–99.98) (Table 2).

Trisomy 18 and trisomy 13.

Non-invasive prenatal testing also has high performance metrics for trisomy 18 and trisomy 13 with sensitivities of 98.83% (95% CI 95.45–99.71) and 100% (95% CI 0.0–100.00) and specificities of 99.93% (95% CI 99.83–99.97) and 99.96%, (95% CI 99.92–99.98) respectively.[6] (Table 2)

Positive predictive values.

The chance of an affected fetus after a high chance NIPT (positive predictive value (PPV) is influenced by specificity and false-positive rates (1- specificity) as well as the background prevalence of the specific condition. The PPV of NIPT is highest for trisomy 21 (91.78%) because of biological and epidemiological factors (Table 2.) Due to the lower natural prevalence of trisomy 18 and trisomy 13 and the increased incidence of confined placental mosaicism (CPM), their PPVs are lower (65.77% and 37.23% respectively) and the associated confidence intervals are wider.

Despite the high performance of NIPT for the common trisomies, it does not achieve the accuracy of a prenatal diagnostic test. False positive results occur because of biological, technical or statistical reasons, and given the serious implications of a false positive result in the prenatal period, diagnostic testing is strongly recommended if patients are considering termination of pregnancy. Some patients may decline diagnostic testing if they elect to have NIPT for information only and may prefer to continue pregnancy with a presumptive diagnosis of aneuploidy.

Confined placental mosaicism occurs in 1%–2% of pregnancies and can be a cause of false positive NIPT results. Some chromosome abnormalities such as trisomy 13 and monosomy X have higher risks of it and amniocentesis may be preferred over chorionic villus sampling (CVS) if no fetal ultrasound abnormalities are present.[23,24] The modeled rate of a mosaic CVS result after a high chance NIPT result varies by the type of suspected aneuploidy and ranges from 2% to 4% for trisomy 21 or trisomy 18%, to 22% for trisomy 13% and 59% of monosomy X.[25] However, patient preferences and needs should be taken into account as some of them may not want to wait for amniocentesis especially in the setting of an fetal structural anomaly and/or because of legal limits on the termination of pregnancy. Thus, shared decision-making and counseling that amniocentesis may be

TABLE 2 The performance of non-invasive prenatal testing (NIPT) in a general unselected population for trisomy 21, 18 and 13 (adapted from Rose et al[6]).

Condition	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PPV (%)	95% CI
Trisomy 21	98.80	97.81–99.34	99.96	99.92–99.98	91.78	88.43–94.23
Trisomy 18	98.83	95.45–99.71	99.93	99.83–99.97	65.77	45.29–81.68
Trisomy 13	100	0–100	99.96	99.92–99.98	37.23	26.08–49.93

recommended after a CVS if CPM is suspected is important in making this decision.

4.1 | Consensus statements

1. NIPT is the most accurate screening test for the common autosomal aneuploidies (trisomies 21, 13 and 18) in unselected singleton populations, and those at known increased probability.
2. FPR occur with NIPT. Therefore, ISPD strongly recommends that all pregnant individuals with a high chance NIPT result have genetic counseling and diagnostic testing if they are considering termination of pregnancy.

5 | FETAL FRACTION, TEST FAILURES AND INCIDENTAL DETECTION OF MATERNAL NEOPLASIA

Non-invasive prenatal testing represents a major departure from previous aneuploidy screening tests, not just in its superior accuracy but also in the biology and technology that underpin its performance. As a test based on analyzing cfDNA fragments in maternal plasma, it analyses genomic sequences of both maternal and placental origin. The so-called "fetal fraction" refers to the proportion of cfDNA fragments in maternal plasma that arise from the placenta as a percentage of the total cfDNA. The fetal fraction varies between individuals, and by other factors such as gestational age, maternal weight, maternal race, fetal karyotype, and maternal medical conditions.[26,27]

Fetal fraction is an important quality control metric as it has a significant influence on NIPT test performance,[28] however there is no clear consensus regarding the benefits of measuring and reporting fetal fractions.[29,30] Very low fetal fractions are associated with less accurate NIPT results; however, fetal fraction measurement is imprecise and there is substantial variation between laboratories in the fetal fraction threshold below which a result cannot be issued.[31] This threshold depends on the technical platform and bioinformatic algorithms employed by each laboratory, as well as individual sample characteristics.

At very low fetal fractions, a "no call" or 'failed" NIPT result may be issued, creating a clinical dilemma as a 'no call' result is associated with an increased chance of fetal aneuploidy or other potential adverse outcomes.[32–34] Suggested management pathways after a 'no call' result include a repeat blood draw for NIPT analysis, diagnostic testing, or an alternative form of aneuploidy screening such as the first trimester-combined test.[35] Further analysis of the fetal fraction relative to the affected fraction of cfDNA can also provide information on the likely causes of an abnormal NIPT result, such as placental mosaicism, maternal copy number variants (CNVs), or discordant aneuploidy in twins.[36–38]

It is also well-recognised that in about one in 10,000 samples, an occult maternal malignancy may be detected through genome-wide abnormalities in the plasma profile caused by circulating tumor DNA.[39,40] Depending on the NIPT platform used, this may result in an unreportable result, a result indicating an unusual aneuploidy (e.g. simultaneous monosomy and trisomy), or subchromosomal gains and losses on multiple chromosomes. This creates dilemmas for pre-test and post-test counseling, as suspicions of malignancy are often not confirmed, depending on the platform used. There may also be benign sources such as fibroids.[41] Suggested disclosure and investigative approaches in the case of suspected malignancy are described in detail elsewhere.[42,43]

5.1 | Consensus statements

3. Fetal fraction is an important quality control metric, but substantial variation exists between laboratories and test methodologies. Laboratories should perform their own internal validation of their limit of detection and threshold for 'no call' results.
4. Providers (laboratory and clinicians) should have established clinical pathways for the management of patients with a "no call" result. This may include detailed ultrasound, offer of repeat NIPT, alternative screening test, and/or diagnostic testing.
5. If technically relevant, protocols for the identification and disclosure of suspected malignancy should be developed by laboratories.

6 | IMPLEMENTATION MODELS OF NON-INVASIVE PRENATAL TESTING

The screening performance of NIPT for trisomy 21,18 and 13 has been validated in both general populations,[44–47] and high prevalence populations.[6,48–50] The earliest implementation model of NIPT was for patients identified by a prior screening as having a high chance of aneuploidy. In this 'contingent' screening model, pregnant people

with an elevated chance of fetal aneuploidy (e.g. advanced maternal age, previous pregnancy with trisomy, or intermediate/high-chance results from conventional screening) are offered NIPT rather than immediate diagnostic testing.[50] The benefit of this strategy is that it dispels many of the FPR identified via conventional prenatal screening, thus reducing invasive diagnostic tests with their associated risks of miscarriage.[5,51] This strategy also balances the higher costs of NIPT, which make it unaffordable in many countries as a universal first-line screening test.

As more reassuring data on the performance of NIPT in general populations emerged, it was increasingly adopted as a primary screen both in national screening programs[52] and as a self-funded choice.[53] In recent years, several professional society statements have affirmed that NIPT could be offered to pregnant people as a primary screen, with various caveats around funding arrangements and other local access factors.[9,54–56] The majority of pregnant people prefer NIPT to other options due to its superior screening performance, earlier testing, lower risk of requiring an invasive diagnostic test, and fewer anxieties.[57,58] However, NIPT as a primary screen is a more costly strategy than the contingent model.[51,59] Furthermore, if NIPT replaces the nuchal translucency (NT) ultrasound at 11–13 weeks, then its implementation as a primary screen will also reduce opportunities for early ultrasound detection of fetal structural anomalies.[60]

Countries with public health care systems have adopted various approaches, including contingent NIPT (Denmark, UK), primary screening with NIPT (the Netherlands and Belgium), a "case-by-case" approach in Germany,[61] and a self-funded consumer choice approach (Australia).[53] Choosing an appropriate implementation model for a public health system aiming for equitable access is complex and influenced by many factors. These include public funding, cost-effective analysis, the structure of the health care system, and different socio-cultural, ethical, and legal contexts.[62]

6.1 | Consensus statements

6. NIPT for the common autosomal aneuploidies performs sufficiently well to be offered in primary or contingent screening models.
7. The ISPD Board acknowledges that context-specific considerations in health policy influence decisions and implementation models.

7 | EXPANDED NON-INVASIVE PRENATAL TESTING

The common autosomal trisomies comprise only 71% of all prenatally detected chromosome abnormalities.[63] The new capabilities provided by cfDNA-based screening have resulted in laboratories offering detection for conditions that were not previously the subject of prenatal screening, such as the sex chromosome aneuploidies. This trend has created new clinical and ethical questions for our field and is currently one of the most debated areas of clinical practice.[64]

There are significant challenges in synthesizing the evidence base in expanded NIPT due to major differences in technological platforms. Published studies also vary in population characteristics that influence the performance of NIPT, including average gestational age at testing, referral indications, frequency of fetal structural anomalies, and other risk factors. The rarity of some conditions detected with expanded NIPT also makes proper validation in clinical cohort studies unfeasible. Furthermore, the variable clinical phenotype of some conditions assessed in expanded NIPT makes the ascertainment of false negative NIPT results in newborns challenging without universal genetic assessment. The main groups of conditions included in expanded NIPT (sex chromosome aneuploidies, rare autosomal trisomies, and microdeletion/microduplication syndromes) are discussed in separate sections below.

8 | SEX CHROMOSOME ANEUPLOIDY AND FETAL SEX DETERMINATION

Sex chromosome aneuploidies (SCA) are the most common chromosomal conditions as a group, and affect up to one in 400 newborns.[65] The sex chromosomes have a distinctive process of replication, pairing, and unique gene content that makes them more likely to have errors in replication and resultant aneuploidy or mosaicism.[66,67] Many people with SCA are clinically undiagnosed as these have a less distinct phenotype as a group than those with autosomal aneuploidies. Studies comparing newborn screening prevalence to registry studies suggest clinical diagnostic rates of 70% for 45,X, 23% for 47,XXY, 7% for 47,XXX, and 9% for 47,XYY.[65]

An American College of Medical Genetics and Genomics (ACMG) meta-analysis reported PPVs for SCAs that were substantially lower than those for trisomy 21.[9] PPVs ranged from 30% (45,X) to 74% (47,XXY; 47,XYY). The number of studies contributing to these analyses was generally small, with sensitivity ranging from 0% to 100% for 47,XXX and 47,XYY (Table 3).

While the SCA PPVs are lower than those seen in the common autosomal trisomies, ultrasound is an important adjunct to determining the chance of 45,X, which is the only SCA with a consistent prenatal phenotype (including increased NT and/or cystic hygroma). With an abnormal ultrasound and a high chance NIPT result for monosomy X, the PPV may be over 85% (14/16).[68] Expert pretest counseling and consent is recommended when offering NIPT for SCA, with expert post-test counseling if a high chance result is returned.[69]

The ethical issues around screening for the SCA and determining fetal sex for nonmedical reasons are covered in detail by Johnston et al.[70] While many of these ethical issues are not unique to the sex chromosomes, the prenatal detection of SCAs is more controversial than the autosomal trisomies because of their wide phenotypic spectrum. Due to the lower PPV of NIPT and the ethical debate on offering SCA screening, there is more variation in public policy and patient choice surrounding NIPT for SCA, with several countries, states, and territories choosing not to offer SCA screening at all.[8]

TABLE 3 The performance of non-invasive prenatal testing (NIPT) in a general unselected population for sex chromosome aneuploidy (SCA) (adapted from Rose et al[6]).

Condition	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PPV (%)	95% CI
Monosomy X	97.68	84.25–99.70	99.84	99.67–99.92	29.52	22.72–37.36
47,XXX	100	0.0–100	99.97	99.96–99.98	53.95	40.58–66.77
47,XXY	99.25	78.13–99.98	99.99	99.98–99.99	74.05	59.47–84.73
47,XYY	100	0.0–100	99.99	99.99–100)	74.45	58.40–85.81
Overall SCAs	99.63	94.83–99.98	99.80	99.69–99.88	43.13	37.92–48.50

8.1 | Consensus statements

8. NIPT for SCA is sufficiently accurate to be offered alongside autosomal aneuploidy screening with specific pretest counseling and consent.
9. However, other societal, economic, cultural and ethical factors may need to be considered in health policy decisions regarding population-based screening for the SCA. Further studies to evaluate the downstream impacts of offering NIPT for sex chromosome conditions should be considered where such screening is offered.

9 | RARE AUTOSOMAL TRISOMIES

Massively parallel sequencing techniques that perform genome-wide sequencing enable the detection of whole chromosome imbalances in any chromosome. Trisomies in the autosomes other than chromosomes 21, 13 and 18 have become known as the "rare autosomal trisomies" (RATs). Full trisomies of these autosomes are rarely observed in live fetuses after the first trimester; indeed, 97% of all RATs detected in CVS appear to be confined to the placenta and not present in the fetus.[71] CPM is a well-recognized biological cause of false positive NIPT results. This high rate of CPM and the rarity of true fetal RAT is reflected in low PPVs seen with NIPT. A recent systematic review and meta analysis of NIPT for RATs calculated a pooled PPV of 11.46% (95% CI 7.80–15.65).[72] This meta analysis could not calculate the sensitivity and specificity rates of NIPT for RATs due to insufficient data.

Given the high rate of CPM (especially in the presence of a normal ultrasound), amniocentesis is the preferred test for diagnostic assessment of the fetus after a high chance result for a RAT on NIPT. However, if a euploid fetus is confirmed on amniocentesis, the risk of Uniparental disomy (UPD) must then be considered if the RAT involved one of the imprinted chromosomes. The autosomes 6, 7, 11, 14, 15, and 20 contain imprinted regions which cause parent-of-origin differential gene expression.[73] Uniparental disomy is the condition where both homologues of a whole chromosome, or a chromosomal segment, are inherited from the same parent without a contribution from the other parent. A trisomic conception can be rescued by the mitotic loss of one of the three chromosomes but results in UPD. Based on CVS and amniocentesis data, the overall risk of UPD in a pregnancy with a CPM for an imprinted chromosome is 2.1%, though the specific risk varies among the imprinted chromosomes.[73]

It is also evident that CPM may confer an increased risk of adverse obstetric outcomes even after a euploid fetal karyotype has been confirmed.[36,39] Trisomy 16 is associated with early onset fetal growth restriction and fetal surveillance is warranted for people who received a high chance NIPT result for trisomy 16. Further details on management approaches that integrate ultrasound findings and high chance NIPT results for the SCA and RATS are provided by Mardy and colleagues.[24] However, the overall cost-effectiveness of identifying CPM through expanded NIPT is still widely debated. The Netherlands, which conducted a national study of genome-wide NIPT, recently recommended that RATs no longer be reported due to the low PPV.[74] However, their experience may not be generalisable to other settings or providers due to the substantial variation in laboratory techniques and reporting standards in genome-wide screening.

9.1 | Consensus statements

10. There is insufficient data to assess the performance and clinical utility of routine NIPT for RATs. NIPT for RATs is therefore not recommended for the routine care of unselected populations.
11. Where screening for RATs is performed, management after a high chance result requires expert post-test counselling and specialist management.
12. Due to the high likelihood of CPM underlying a positive NIPT result for a RAT, amniocentesis is the single most informative test for fetal karyotype.
13. If NIPT returns a high-chance result for a trisomy involving an imprinted chromosome, amniocentesis with appropriate studies to detect UPD is recommended.
14. Further prospective studies are required to evaluate all aspects of NIPT for RATs.

10 | SUBCHROMOSOMAL IMBALANCES

As well as RATs, genome-wide sequencing can also detect subchromosomal imbalances such as duplications, deletions and

unbalanced translocations (variously referred in the literature as segmental imbalances, structural aberrations, and CNVs). However, the ability of genome-wide NIPT to detect these complex changes depends on the size of the imbalance, the fetal fraction, the sequencing depth, and for SNP-based approaches, the targets included in the individual platform. Two countries (Belgium and the Netherlands) have implemented population-based screening with genome-wide NIPT with analysis for segmental imbalances.[11,75] The frequencies of screen-positive NIPT results in the Belgian and Dutch studies were 0.07% and 0.16%, with PPVs of 47% and 32% respectively. These PPVs are similar to the NIPT screening performance for trisomy 13, although sensitivity data are not available because of incomplete follow-up in screen-negative cases. Based on these results, the Netherlands has recommended routine reporting of suspected subchromosomal imbalances on NIPT.[74] However, as stated above, their experience,[11] and that of Belgium,[75] may not be generalisable to other settings because of variation in laboratory techniques and reporting standards in CNV screening.

It is important that clinicians recognize that genome-wide NIPT for subchromosomal imbalances is not a comprehensive screen for all pathogenic CNVs. More than half of the pathogenic CNVs in the prenatal diagnosis population are <7 Mb in size and thus below the resolution of many current genome-wide NIPT platforms.[76] The use of NIPT for sub chromosomal imbalances continues to be debated due to the risks of false positives, increasing parental anxiety, and potentially increasing diagnostic procedures.[39,77] In selected circumstances, however, it may be of clinical utility, for example, for carriers of balanced reciprocal translocations.[78]

10.1 | Consensus statements

15. There is insufficient data to assess the performance and clinical utility of routine NIPT for subchromosomal imbalances. Large scale population-based evaluations of routine screening for subchromosomal imbalances are being undertaken in several countries and data continue to emerge. Until such time as the outcome data are clear and shown to be reproducible in other settings, NIPT for subchromosomal imbalances is not recommended for the routine care of unselected populations.
16. Where screening for subchromosomal imbalances is performed, management after a high chance result requires expert post-test counselling and specialist management.
17. Genome-wide NIPT that includes subchromosomal imbalances should not be considered a comprehensive screen for all pathogenic CNVs as many pathogenic CNVs are below the limits of resolution of genome-wide NIPT.
18. Further prospective studies are required to evaluate all aspects of NIPT for subchromosomal imbalances.

11 | MICRODELETIONS AND MICRODUPLICATIONS

Microdeletion and microduplication syndromes (MMS) are caused by small subchromosomal CNVs, typically <5 Mb. Due to their small size, NIPT for MMS involves a different technical approach to the detection of larger CNVs discussed in the above section on subchromosomal imbalances; therefore, they are discussed separately here.

The three most common microdeletion syndromes in the prenatal diagnosis population are 22q11.2 deletion syndrome (DiGeorge syndrome), 4p16.3 deletion (Wolf-Hirschhorn syndrome) and 5p15.33 deletion syndrome (Cri-du-Chat syndrome).[76] MMS are not detectable with traditional aneuploidy screening tests and are not related to maternal age. Some current MMS NIPT assays offer either 22q11.2 deletion syndrome screening (in addition to common autosomal trisomy +/− SCA screening) or a panel of five common recurrent MMS including DiGeorge (22q11.2 del), Cri-du-Chat (5p), Prader–Willi/Angelman (15q del), 1p36 deletion, and Wolf–Hirschhorn (4p del) syndromes.

Because of the rarity of these conditions, published data on the technical and clinical validity of microdeletion syndromes are scarce. In a systematic review of NIPT for MMS, Familiari et al found that none of the seven included studies performed genetic confirmation in cases that did not undergo prenatal diagnostic testing or in those with a low chance NIPT result. They were therefore unable to calculate either the sensitivity or specificity of MMS screening.[79] Only one prospective study of 22q11.2 deletion syndrome screening has been published with confirmatory postnatal testing of all included cases, including those with a low chance result.[80] This study with a genetic outcome in 18,289 pregnancies (87.6% of the enrolled cohort) reported a 22q11.2 deletion frequency of 1 in 1524, a screen-positive rate of 0.2%, sensitivity of 75% (95% CI 42.8–94.5), specificity of 99.84% (95% CI, v99.77–99.89), and a PPV of 23.7% (95%CI 11.4%–40.2%). The wide confidence intervals reported in this study reflect the low number of affected cases ($n = 12$). Furthermore, the relatively high frequency of 22q11.2 deletion in this study cohort (1 in 1524) may not reflect the prevalence in the general population, and likely represents the best-case scenario for PPV.

Despite the individual rarity of MMS (aside from 22q11.2 deletion syndrome), a United States cost-benefit study found that additional screening for the five classical microdeletions would improve effectiveness and decrease costs compared to offering NIPT for aneuploidy alone.[81] In 2022, based on these two studies alone,[80,81] the ACMG has suggested that all patients be offered NIPT to screen 22q11.2 deletion syndrome.[9] However, the cost-benefit analysis would not necessarily translate to other health care systems outside the United States. Other considerations, such as increased anxiety and invasive testing rates, need to be considered in more depth. It is also important to be aware that technological platforms used in MMS screening vary, including single-nucleotide polymorphism analysis,[80] random massively parallel sequencing,[82] and microarray hybridization.[83] It is therefore not possible to generalize performance characteristics from one assay to another.

11.1 | Consensus statements

19. 22q11.2 deletion syndrome is the most common microdeletion syndrome. Only one study has evaluated cfDNA-based screening for 22q11.2 deletion syndrome in a clinical cohort with genetic confirmation of all included participants. There is insufficient data to assess the performance and clinical utility of routine NIPT for MMS. NIPT for MMS is therefore not recommended for the routine care of unselected populations.
20. Where screening for MMS is performed, management after a high chance result requires expert post-test counselling and specialist management.
21. Further prospective studies are required to evaluate all aspects of MMS screening with cfDNA.

12 | ROLE OF ULTRASOUND IN NON-INVASIVE PRENATAL TESTING

Multiple studies have shown the clinical importance of an ultrasound prior to blood draw for NIPT to date pregnancies and detect major abnormalities. In one study conducted in a high-prevalence population, 16.1% of pregnant patients had an ultrasound finding at the time of NIPT blood draw that would have altered the provider's counseling, such as a fetal structural anomaly, incorrect dating, multiple gestation, or nonviable pregnancy.[84] Other societies have previously addressed the question of ultrasound prior to cfDNA screening and similarly recommend an early first trimester ultrasound for dating, diagnosis of multiple pregnancy, and confirmation of viability before NIPT.[85,86]

The 11–13 weeks ultrasound, which was originally implemented as a component of the first trimester-combined screen for trisomies 21, 13, and 18, is now regarded as having independent value for the early identification of fetal structural anomalies, regardless of a person's choice of primary screening test. In the Netherlands, where NIPT has replaced the first trimester-combined screen on a national scale, most fetal anomalies are now not diagnosed until the second or third trimester.[60] The detection rate of some major malformations, such as anencephaly and exomphalos, would be expected to approach 100% at 11–13 weeks. However, more advanced first trimester anatomy assessments require expertise that is not uniformly available outside specialist centers.[87]

All patients diagnosed with a fetal anomaly should also be offered diagnostic testing with chromosomal microarray, irrespective of a prior low chance NIPT result.[85,86,88] The residual risk of a clinically significant CNV in the presence of a fetal structural abnormality varies with gestational age and ultrasound finding.[89]

A specific clinical scenario that has arisen in the NIPT era is the management of an isolated increased NT measurement in a pregnant person with a low chance NIPT result.[90] A NT ≥ 3.5 mm is associated with a variety of conditions that are not detectable on NIPT, including atypical chromosome abnormalities, single gene conditions, and structural malformations. Diagnostic testing should therefore be recommended to pregnant patients who have a NT ≥ 3.5 mm at 11–13 weeks gestation, regardless of prior or intended NIPT.[91] Lower NT thresholds for offering diagnostic testing have been proposed, but there is little consensus in the published literature on what standards should be adopted.[92–94] Similarly, the management of nuchal edema before 11 weeks 0 days is still unclear.[95,96]

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22. At least one early first trimester scan for dating, diagnosis of multiple pregnancy and confirmation of fetal viability should be offered before performing NIPT.
23. People who have NIPT as a primary screen should still be offered an 11–13 weeks ultrasound where local resources permit.
24. Fetus with ultrasound abnormalities, including NT measurement ≥3.5 mm, should be offered diagnostic testing and evaluation with chromosomal microarray regardless of the prior NIPT result. There is no consensus on the use of alternative NT thresholds (such as 3.0 mm or 99th centile) for defining a population that should be offered diagnostic testing.

13 | PRE- AND POST-TEST COUNSELING CHALLENGES

International society for prenatal diagnosis supports the principles of autonomy, equity and voluntary informed choice in all prenatal testing. Clinicians should ensure that pre-test counseling promotes patient autonomy whilst providing a supportive environment for shared discussion and decision making in line with the patient's values.[97] (Table 4)

Post-test counseling for those with low chance results should include a caveat that NIPT does not exclude all genetic conditions and that false-negative results may occur. Individuals with a high chance result should also be informed that FPR may occur, and that diagnostic testing is recommended prior to management decisions, particularly if termination of pregnancy is being considered. When a high chance result is discussed with a patient, clinicians should ensure they present an informed description of the suspected condition, including discussion about the range of phenotypic variation. Further, they should provide educational materials that are developed in conjunction with advocacy organizations and consider offering a referral to a health care professional with expertise on that condition. Where NIPT has uncovered incidental findings relevant to maternal health, referral to a genetics specialist is recommended to ensure appropriate management of that parent and any other relevant family members.

The increasing complexity of prenatal screening and the associated genetic counseling workload has been observed in many settings, particularly where expanded NIPT options are available. Further research into alternative genetic counseling delivery techniques such as telehealth, web-based educational videos, and

TABLE 4 Pre-test counseling points for non-invasive prenatal testing (NIPT) (adapted from Sachs et al 2015[97] and Kater-Kuipers et al 2020[107]).

Phase of informed consent process	Content	Rationale
Exploration of personal values, followed by acceptance or rejection of prenatal screening	Aim of prenatal screening	To provide pregnant individuals/expectant couples with more information about their unborn baby's health; to promote reproductive autonomy
	Testing is optional	To assess the pregnant person's preferences and values, ascertain acceptance or rejection of screening; provide alternative screening methods if relevant
Information provision, tailored to individual's information needs; followed by choice regarding participation in screening and range of conditions included (e.g., fetal sex)	Define screening	To distinguish from diagnostic test in accuracy and range and pregnancy risks. Advise need for diagnostic confirmation if high chance result
	Clinical features and variability of conditions	Consent for SCA and expanded NIPT should be discussed separately to consent for the common autosomal trisomies, due to differences in clinical implications and NIPT performance. Information should be tailored to information needs and the health literacy of each person. If MMS are being screened this should be discussed
	Technology	To explain that both maternal and fetal DNA are analyzed, which can lead to maternal incidental findings such as chromosome conditions or neoplasia; tailored to individuals information needs
	Sensitivity	Detection rate
	False positive rate and confirmation of abnormal results	Possibility of a 'false alarm' exists for any screening test; hence need to have confirmatory diagnostic testing before management decisions
	Positive and negative predictive values	Chance of affected pregnancy after a low or high chance result
	Limitations	Does not screen for every chromosomal or genetic condition
		May result in a 'no call' result
	Incidental findings	Maternal malignancy (1 in 10,000), maternal chromosome conditions, confined placental mosaicism
Practical logistics	Timing, description of required blood draw	From 10 weeks (typically)
	Complementary role of ultrasound	Role of first trimester ultrasound to ensure accurate dates, detection of multiple pregnancy, live pregnancy prior to NIPT early detection of structural anomalies
	Costs	Financial disclosure
	Reporting format	When and how their results will be provided
Post-test counseling: Information provision, decision making about follow up and ongoing management	High or low 'chance' result; negative and positive predictive values	Reassuring if low chance result Pathways/next steps if high chance result, including need for invasive test to validate result

BOX 1 Summary of consensus position from the 2023 ISPD board

- NIPT is the most accurate screening test for the common autosomal aneuploidies (trisomies 21, 13 and 18) in unselected singleton populations, and those at known increased probability. It can be offered in primary or contingent screening models with context-specific considerations in local health policy influencing decisions and implementation models.
- False-positive results occur with NIPT. Therefore, ISPD strongly recommends that all patients with a high chance of NIPT result have genetic counseling and are offered diagnostic testing, particularly if the termination of pregnancy is being considered.
- Fetal fraction is an important quality control metric, but substantial variation exists between laboratories and test methodologies. Laboratories should perform their own internal validation of their limit of detection and threshold for 'no call' results.
- Providers (laboratory and clinicians) should have established clinical pathways for the management of patients with a "no call" result. This may include detailed ultrasound, offer of repeat NIPT, alternative screening test, and/or diagnostic testing.
- If technically relevant, protocols for the identification and disclosure of suspected malignancy should be developed by laboratories.
- NIPT for sex chromosome aneuploidy is sufficiently accurate to be offered alongside autosomal aneuploidy screening with specific pretest counseling and consent. However, other societal, economic, cultural and ethical factors may need to be considered in health policy decisions regarding population-based screening for the sex chromosomes.
- There is insufficient data to assess the performance and clinical utility of routine NIPT for rare autosomal trisomies, sub-chromosomal imbalances and microdeletion/duplication syndromes. Further research is required to evaluate these applications of NIPT, but if offered as part of local practice there should be protocols in place to manage high-risk results and detailed platform-specific counseling available both pre- and post-testing.
- At least one early first trimester scan for dating, diagnosis of multiple pregnancy and confirmation of fetal viability should be offered before performing NIPT.
- Fetuses with ultrasound abnormalities, including NT measurement ≥3.5 mm, should be offered diagnostic testing and evaluation with chromosomal microarray regardless of the prior NIPT result.
- The ethical implementation of NIPT requires attention to provision of quality pre-testing counseling, equity of access, and access to appropriate downstream clinical services.
- All stakeholders, including healthcare consumers, should be involved in determining local implementation models and future directions for NIPT.

computerized decision aids is needed to help meet the growing demand for prenatal genetic counseling services.[98–101]

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25. Principles of informed choice should be maintained in the face of 'routinization' of prenatal screening and the expanded scope of some NIPT assays.
26. All patients should have access to pre- and post-test genetic counseling. Those with a high chance NIPT result should be offered diagnostic testing for confirmation.
27. Research should evaluate new ways of providing genetic counseling services that can address the growing demand while maintaining the principles of informed consent.

14 | ETHICAL ISSUES

There are many ethical issues associated with NIPT for aneuploidy; however, detailed discussion of the ethical issues is beyond the scope of this paper. Some are common to prenatal screening generally, such the erosion of informed choice and the potential to increase stigmatization of individuals with chromosome conditions.[102] Other ethical issues are more specific to NIPT, such as the widening scope of aneuploidy screening, inequity of access due to the relatively high cost, increasing complexity of pre-test counseling with limited resources, and in some settings, influences from the competitive commercial environment.[103–106] An ethical framework developed in the Netherlands proposed four limits to the scope of NIPT: "NIPT should generate only test outcomes that are relevant to reproductive decision-making, informed choice should be (made) possible through adequate pre-test counseling, the rights of future children should be respected, and equal access should be guaranteed."[107] Responsible implementation of NIPT requires that all services are available to support patient decision-making and clinical care before and after following the offer of prenatal screening.

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28. The ethical implementation of NIPT requires attention to provision of quality pre- and post-test counseling, equity of NIPT access, and access to appropriate downstream clinical services.

29. All stakeholders, including health care consumers, should be involved in determining local implementation models and future directions for NIPT.

15 | FUTURE OF CELL-FREE DNA SCREENING

Our understanding of circulating cell-free nucleic acids is still in its infancy. Emerging knowledge about plasma cfDNA, cell-free RNA, epigenetics, tissue-specific molecular signatures, and 'fragmentomics' reveals the complexity of this new biology.[108,109] Prenatal screening has proven to be the 'poster child' for clinical translation this field and will likely continue to be at the forefront of new applications.[110] Some of the exciting new directions include analysis of plasma cfRNA to monitor placental function[111,112] and the maternal plasma viral DNA to screen for perinatal infections, such as cytomegalovirus.[113,114]

16 | CONCLUSIONS

This position statement represents the consensus opinion of the current ISPD Board based on the current state of knowledge and clinical practice. (See Box 1 for summary and Supplemental file 1 for the complied list of the 25 consensus statements). Non-invasive prenatal testing is a highly sensitive and specific screening test for trisomies 13, 18, and 21, which can be implemented as a first line screening test for all pregnant people or as a contingent test in people with a higher chance of fetal aneuploidy on prior screening. A high chance result should prompt discussion of an invasive test for confirmation, as NIPT for aneuploidy is not diagnostic. All patients diagnosed with a fetal anomaly should also be offered diagnostic testing with chromosomal microarray, irrespective of a prior low chance NIPT result. Expert pre- and post-test counseling is required. This is resource intensive and new methods for delivering pretest information should be researched as well as greater investment in genetic education and workforce. Pretest information should be tailored to the platform being used and healthcare professionals should be aware of what is being offered through the differing technologies. The clinical utility of NIPT for the detection of subchromosomal rearrangements, including microdeletion and duplication syndromes, RATS, and other adverse pregnancy outcomes is still emerging, and further research is required prior to widespread clinical implementation.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

DISCLAIMER

This is an ISPD Position Statement that has not undergone peer review by this journal. Prenatal Diagnosis is the official society journal of the ISPD and publishes this statement as a courtesy to the ISPD Board.

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REFERENCES

1. Hui L, Bianchi DW. Noninvasive prenatal DNA testing: the vanguard of genomic medicine. *Annu Rev Med*. 2017;68(1):459-472. https://doi.org/10.1146/annurev-med-072115-033220
2. Chandrasekharan S, Minear MA, Hung A, Allyse M. Noninvasive prenatal testing goes global. *Sci Transl Med*. 2014;6(231):231fs15. https://doi.org/10.1126/scitranslmed.3008704
3. Dondorp W, de Wert G, Bombard Y, et al. Bet al; European Society of Human Genetics; American Society of Human Genetics. Noninvasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. *Eur J Hum Genet*. 2015;23(11):1438-1450. https://doi.org/10.1038/ejhg.2015.57
4. Vanstone M, Cernat A, Majid U, Trivedi F, De Freitas C. Perspectives of pregnant people and clinicians on noninvasive prenatal testing: a systematic review and qualitative meta-synthesis. *Ont Health Technol Assess Ser*. 2019;19(5):1-38.
5. Benn P, Borrell A, Chiu RW, et al. Position statement from the chromosome abnormality screening committee on behalf of the

board of the international society for prenatal diagnosis. *Prenat Diagn*. 2015;35(8):725-734. https://doi.org/10.1002/pd.4608
6. 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(9):1992. https://doi.org/10.1016/j.gim.2022.07.002
7. Gadsbøll K, Petersen OB, Gatinois V, et al. Current use of noninvasive prenatal testing in Europe, Australia and the USA: a graphical presentation. *Acta Obstet Gynecol Scand*. 2020;99(6):722-730. https://doi.org/10.1111/aogs.13841
8. Steffensen EH, Skakkebaek A, Gadsbøll K, et al. Inclusion of sex chromosomes in noninvasive prenatal testing in Asia, Australia, Europe and the USA: a survey study. *Prenat Diagn*. 2023;43(2):144-155. https://doi.org/10.1002/pd.6322
9. Dungan JS, Klugman S, Darilek S, et al. Noninvasive prenatal screening (NIPS) for fetal chromosome abnormalities in a general-risk population: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2022;25(2):100336. S1098-3600(22)01004-8. https://doi.org/10.1016/j.gim.2022.11.004
10. Jones R, White K, Batey A, Kostenko E. Cell-free DNA testing for prenatal aneuploidy assessment: analysis of professional society statements. *Ultrasound Obstet Gynecol*. 2021;57(5):840-841. https://doi.org/10.1002/uog.22120
11. van der Meij KRM, Sistermans EA, Macville MVE, et al. TRIDENT-2: national implementation of genome-wide non-invasive prenatal testing as a first-tier screening test in the Netherlands. *Am J Hum Genet*. 2019;105(6):1091-1101. https://doi.org/10.1016/j.ajhg.2019.10.005
12. Takahashi M, Linh LK, M Sayed A, et al. Non-invasive prenatal testing (NIPT) implementation in Japan: a comparison with the United Kingdom, Germany, Italy, Sweden, and Taiwan. *Int J Environ Res Publ Health*. 2022;19(24):16404. https://doi.org/10.3390/ijerph192416404
13. Palomaki GE, Chiu RWK, Pertile MD, et al. International Society for Prenatal Diagnosis Position Statement: cell free (cf)DNA screening for Down syndrome in multiple pregnancies. *Prenat Diagn*. 2021;41(10):1222-1232. https://doi.org/10.1002/pd.5832
14. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. *Lancet*. 1997;350(9076):485-487. https://doi.org/10.1016/s0140-6736(97)02174-0
15. Faas BH, de Ligt J, Janssen I, et al. Non-invasive prenatal diagnosis of fetal aneuploidies using massively parallel sequencing-by-ligation and evidence that cell-free fetal DNA in the maternal plasma originates from cytotrophoblastic cells. *Expert Opin Biol Ther*. 2012;12(Suppl 1):S19e26.
16. Guibert J, Benachi A, Grebille AG, et al. Kinetics of SRY gene appearance in maternal serum: detection by real time PCR in early pregnancy after assisted reproductive technique. *Hum Reprod*. 2003;18(8):1733e6.
17. Wang E, Batey A, Struble C, Musci T, Song K, Oliphant A. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. *Prenat Diagn*. 2013;33(7):662e6-666. https://doi.org/10.1002/pd.4119
18. 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. https://doi.org/10.1073/pnas.0810641105
19. Rousseau F, Langlois S, Johnson JA, et al. Prospective head-to-head comparison of accuracy of two sequencing platforms for screening for fetal aneuploidy by cell-free DNA: the PEGASUS study. *Eur J Hum Genet*. 2019;27(11):1701-1715. https://doi.org/10.1038/s41431-019-0443-0
20. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol*. 2017;50(3):302-314. https://doi.org/10.1002/uog.17484
21. Badeau M, Lindsay C, Blais J, et al. Genomics-based non-invasive prenatal testing for detection of fetal chromosomal aneuploidy in pregnant women. *Cochrane Database Syst Rev*. 2017;11(11):CD011767. https://doi.org/10.1002/14651858.cd011767.pub2
22. Mackie FL, Hemming K, Allen S, Morris RK, Kilby MD. The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. *BJOG*. 2017;124(1):32-46. https://doi.org/10.1111/1471-0528.14050
23. Grati FR, Bestetti I, De Siero D, et al. An Italian multicentric Cytogenetic and cytogenomic Audit of diagnOstic testing (ICARO study). *Prenat Diagn*. 2022;42(13):1575-1586. https://doi.org/10.1002/pd.6271
24. Mardy AH, Norton ME. Diagnostic testing after positive results on cell free DNA screening: CVS or Amnio? *Prenat Diagn*. 2021;41(10):1249-1254. https://doi.org/10.1002/pd.6021
25. Grati FR, Bajaj K, Malvestiti F, et al. The type of feto-placental aneuploidy detected by cfDNA testing may influence the choice of confirmatory diagnostic procedure. *Prenat Diagn*. 2015;35(10):994-998. https://doi.org/10.1002/pd.4659
26. 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. https://doi.org/10.1002/pd.4625
27. Poon LC, Musci T, Song K, Syngelaki A, Nicolaides KH. Maternal plasma cell-free fetal and maternal DNA at 11-13 weeks' gestation: relation to fetal and maternal characteristics and pregnancy outcomes. *Fetal Diagn Ther*. 2013;33(4):215-223. https://doi.org/10.1159/000346806
28. 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. https://doi.org/10.1002/pd.4126
29. Deans ZC, Allen S, Jenkins L, et al. Recommended practice for laboratory reporting of non-invasive prenatal testing of tri-somies 13, 18 and 21: a consensus opinion. *Prenat Diagn*. 2017;37(7):699-704. https://doi.org/10.1002/pd.5068
30. Persson F, Cuckle HS. Consequences of imprecision in fetal fraction estimation on performance of cell-free DNA screening for Down syndrome. *Prenat Diagn*. 2022;42(4):512-517. https://doi.org/10.1002/pd.6126
31. Becking EC, Linthorst J, Patton S, et al. Variability in fetal fraction estimation: comparing fetal fractions reported by noninvasive prenatal testing providers globally. *Clin Chem*. 2022;69(2):hvac207-167. https://doi.org/10.1093/clinchem/hvac207
32. 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. https://doi.org/10.1016/j.ajog.2014.08.006
33. Palomaki GE, Kloza EM, Lambert-Messerlian GM, et al. Circulating cell free DNA testing: are some test failures informative? *Prenat Diagn*. 2015;35(3):289-293. https://doi.org/10.1002/pd.4541
34. Scheffer PG, Wirjosoekarto SAM, Becking EC, et al. Association between low fetal fraction in cell-free DNA testing and adverse pregnancy outcome: a systematic review. *Prenat Diagn*. 2021;41(10):1287-1295. https://doi.org/10.1002/pd.6028
35. Hui L, Bianchi DW. Fetal fraction and noninvasive prenatal testing: what clinicians need to know. *Prenat Diagn*. 2020;40(2):155-163. https://doi.org/10.1002/pd.5620

36. Pertile MD, Halks-Miller M, Flowers N, et al. Rare autosomal trisomies, revealed by maternal plasma DNA sequencing, suggest increased risk of feto-placental disease. *Sci Transl Med*. 2017;9(405):eaan1240. https://doi.org/10.1126/scitranslmed.aan1240
37. Brison N, Neofytou M, Dehaspe L, et al. Predicting fetoplacental chromosomal mosaicism during noninvasive prenatal testing. *Prenat Diagn*. 2018;38(4):258-266. https://doi.org/10.1002/pd.5223
38. Grömminger S, Yagmur E, Erkan S, et al. Fetal aneuploidy detection by cell-free DNA sequencing for multiple pregnancies and quality issues with vanishing twins. *J Clin Med*. 2014;3(3):679-692. https://doi.org/10.3390/jcm3030679
39. van Prooyen Schuurman L, Sistermans EA, Van Opstal D, et al. Dutch NIPT consortium. Clinical impact of additional findings detected by genome-wide non-invasive prenatal testing: follow-up results of the TRIDENT-2 study. *Am J Hum Genet*. 2022;109(6):1140-1152. https://doi.org/10.1016/j.ajhg.2022.04.018
40. Bianchi DW, Chudova D, Sehnert AJ, et al. Noninvasive prenatal testing and incidental detection of occult maternal malignancies. *JAMA*. 2015;314(2):162-169. https://doi.org/10.1001/jama.2015.7120
41. Scott F, Menezes M, Smet ME, et al. Influence of fibroids on cell-free DNA screening accuracy. *Ultrasound Obstet Gynecol*. 2022;59(1):114-119. https://doi.org/10.1002/uog.23763
42. Lannoo L, Lenaerts L, Van Den Bogaert K, et al. Non-invasive prenatal testing suggesting a maternal malignancy: what do we tell the prospective parents in Belgium? *Prenat Diagn*. 2021;41(10):1264-1272. https://doi.org/10.1002/pd.6031
43. Dow E, Freimund A, Smith K, et al. Cancer diagnoses following abnormal noninvasive prenatal testing: a case series, literature review, and proposed management model. *JCO Precis Oncol*. 2021(5):1001-1012. https://doi.org/10.1200/po.20.00429
44. Dan S, Wang W, Ren J, et al. Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11,105 pregnancies with mixed risk factors. *Prenat Diagn*. 2012;32(13):1225-1232. https://doi.org/10.1002/pd.4002
45. Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. *Am J Obstet Gynecol*. 2012;207(5):374.e1-6. https://doi.org/10.1016/j.ajog.2012.08.033
46. Bianchi DW, Parker RL, Wentworth J, et al. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med*. 2014;370(9):799-808. https://doi.org/10.1056/nejmoa1311037
47. 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. https://doi.org/10.1097/01.ogx.0000470657.58577.f2
48. Chiu RW, Akolekar R, Zheng YW, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. *BMJ*. 2011;342(jan11 1):c7401. https://doi.org/10.1136/bmj.c7401
49. 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. https://doi.org/10.1097/aog.0000000000000363
50. Chitty LS, Wright D, Hill M, et al. Vet al. Uptake, outcomes, and costs of implementing non-invasive prenatal testing for Down's syndrome into NHS maternity care: prospective cohort study in eight diverse maternity units. *BMJ*. 2016;354:i3426. https://doi.org/10.1136/bmj.i3426
51. Nshimyumukiza L, Beaumont JA, Duplantie J, et al. Cell-free DNA-based non-invasive prenatal screening for common aneuploidies in a Canadian Province: a cost-effectiveness analysis. *J Obstet Gynaecol Can*. 2018;40(1):48-60. https://doi.org/10.1016/j.jogc.2017.05.015
52. Bilardo CM. The implementation of non-invasive prenatal testing (NIPT) in the Netherlands. *J Perinat Med*. 2021;49(8):941-944. https://doi.org/10.1515/jpm-2021-0290
53. Lindquist A, Hui L, Poulton A, et al. State-wide utilization and performance of traditional and cell-free DNA-based prenatal testing pathways: the Victorian Perinatal Record Linkage (PeRL) study. *Ultrasound Obstet Gynecol*. 2020;56(2):215-224. https://doi.org/10.1002/uog.21899
54. American College of Obstetricians and Gynecologists, Committee on Genetics, Society for Maternal-Fetal Medicine. Screening for fetal chromosomal abnormalities: ACOG practice bulletin, number 226. *Obstet Gynecol*. 2020;136(4):e48-e69. https://doi.org/10.1097/aog.0000000000004084
55. Royal Australian and New Zealand College of Obstetricians and Gynaecologists Statement on Prenatal Screening and Diagnostic Testing for Fetal Chromosomal and Genetic Conditions. C-Obs 59. 2018 Accessed on 16 Feb 2023 at.https://ranzcog.edu.au/wp-content/uploads/2022/05/Prenatal-Screening-and-Diagnostic-Testing-for-Fetal-Chromosomal-and-Genetic-Conditions.pdf
56. Audibert F, De Bie I, Johnson JA, et al. No. 348-Joint SOGC-CCMG guideline: update on prenatal screening for fetal aneuploidy, fetal anomalies, and adverse pregnancy outcomes. *J Obstet Gynaecol Can*. 2017;39(9):805-817. https://doi.org/10.1016/j.jogc.2017.01.032
57. Lewis C, Hill M, Chitty LS. Women's experiences and preferences for service delivery of non-invasive prenatal testing for aneuploidy in a public health setting: a mixed methods study. *PLoS One*. 2016;11(4):e0153147. https://doi.org/10.1371/journal.pone.0153147
58. Lannoo L, van der Meij KRM, Bekker MN, et al. A cross-country comparison of pregnant women's decision-making and perspectives when opting for non-invasive prenatal testing in the Netherlands and Belgium. *Prenat Diagn*. 2023;43(3):294-303. Epub ahead of print. https://doi.org/10.1002/pd.6329
59. Zhang W, Mohammadi T, Sou J, Anis AH. Cost-effectiveness of prenatal screening and diagnostic strategies for Down syndrome: a microsimulation modeling analysis. *PLoS One*. 2019;14(12):e0225281. https://doi.org/10.1371/journal.pone.0225281
60. Bardi F, Beekhuis AM, Bakker MK, Elvan-Taşpınar A, Bilardo CM. Timing of diagnosis of fetal structural abnormalities after the introduction of universal cell-free DNA in the absence of first-trimester anatomical screening. *Prenat Diagn*. 2022;42(10):1242-1252. https://doi.org/10.1002/pd.6224
61. Bowman-Smart H, Wiesemann C, Horn R. Non-invasive prenatal testing in Germany: a unique ethical and policy landscape. *Eur J Hum Genet*. 2022. https://doi.org/10.1038/s41431-022-01256-x
62. Ravitsky V, Roy MC, Haidar H, et al. The emergence and global spread of noninvasive prenatal testing. *Annu Rev Genom Hum Genet*. 2021;22(1):309-338. https://doi.org/10.1146/annurev-genom-083118-015053
63. Wellesley D, Dolk H, Boyd PA, et al. Rare chromosome abnormalities, prevalence and prenatal diagnosis rates from population-based congenital anomaly registers in Europe. *Eur J Hum Genet*. 2012;20(5):521-526. https://doi.org/10.1038/ejhg.2011.246
64. Christiaens L, Chitty LS, Langlois S. Current controversies in prenatal diagnosis: expanded NIPT that includes conditions other than trisomies 13, 18, and 21 should be offered. *Prenat Diagn*. 2021;41(10):1316-1323. https://doi.org/10.1002/pd.5943
65. Bereeglund A, Stochholm K, Gravholt CH. The epidemiology of sex chromosome abnormalities. *Am J Med Genet C Semin Med Genet*. 2020;184(2):202-215. https://doi.org/10.1002/ajmg.c.31805
66. Lu L.-Y, Xiong Y, Kuang H, Korakavi G, Yu X. Regulation of the DNA damage response on male meiotic sex chromosomes. *Nat Commun*. 2013;4(1):2105. https://doi.org/10.1038/ncomms3105

67. Held KR, Kerber S, Kaminsky E, et al. Mosaicism in 45,X Turner syndrome: does survival in early pregnancy depend on the presence of two sex chromosomes? *Hum Genet*. 1992;88(3):288-294. https://doi.org/10.1007/bf00197261
68. Sandow R, Scott FP, Schluter PJ, et al. Increasing maternal age is not a significant cause of false-positive results for monosomy X in non-invasive prenatal testing. *Prenat Diagn*. 2020;40(11):1466-1473. https://doi.org/10.1002/pd.5790. PMID: 32673423.
69. Lewit-Mendes MF, Robson H, Kelley J, et al. Experiences of receiving an increased chance of sex chromosome aneuploidy result from non-invasive prenatal testing in Australia: "A more complicated scenario than what I had ever realized. *J Genet Couns*. 2023;32(1):213-223. https://doi.org/10.1002/jgc4.1635
70. Johnston M, Warton C, Pertile MD, et al. Ethical issues associated with prenatal screening using non-invasive prenatal testing for sex chromosome aneuploidy. *Prenat Diagn*. 2023;43(2):226-234. https://doi.org/10.1002/pd.6217
71. Malvestiti F, Agrati C, Grimi B, et al. Pet al. Interpreting mosaicism in chorionic villi: results of a monocentric series of 1001 mosaics in chorionic villi with follow-up amniocentesis. *Prenat Diagn*. 2015;35(11):1117-1127. https://doi.org/10.1002/pd.4656
72. Acreman ML, Bussolaro S, Raymond YC, Fantasia I, Rolnik DL, Da Silva Costa F. The predictive value of prenatal cell-free DNA testing for rare autosomal trisomies: a systematic review and meta-analysis. *Am J Obstet Gynecol*. 2022;S0002-9378(22):00676-00677. https://doi.org/10.1016/j.ajog.2022.08.034
73. Benn P. Uniparental disomy: origin, frequency, and clinical significance. *Prenat Diagn*. 2021;41(5):564-572. https://doi.org/10.1002/pd.5837
74. Dutch Health Council advice to the Minister of Health, Welfare and Sport. The Non-invasive Prenatal Test (NIPT) as Population Screening. No. 2023/03, the Hague, 2023. Accessed on 3 March 2023 at.https://www.gezondheidsraad.nl/?utm_source=gr.nl&utm_medium=redirect&utm_campaign=redirect
75. Van Den Bogaert K, Lannoo L, Brison N, et al. Outcome of publicly funded nationwide first-tier noninvasive prenatal screening. *Genet Med*. 2021;23(6):1137-1142. https://doi.org/10.1038/s41436-021-01101-4
76. Pynaker C, Norris F, Hui L, Halliday J. Perinatal outcomes and genomic characteristics of fetal copy number variants: an individual record linkage study of 713 pregnancies. *Prenat Diagn*. 2023;43(4):516-526. Epub ahead of print. https://doi.org/10.1002/pd.6305
77. Lo KK, Karampetsou E, Boustred C, et al. Limited clinical utility of non-invasive prenatal testing for subchromosomal abnormalities. *Am J Hum Genet*. 2016;98(1):34-44. https://doi.org/10.1097/01.ogx.0000481796.11218.bd
78. Flowers NJ, Burgess T, Giouzeppos O, et al. Genome-wide noninvasive prenatal screening for carriers of balanced reciprocal translocations. *Genet Med*. 2020;22(12):1944-1955. https://doi.org/10.1038/s41436-020-0930-2
79. Familiari A, Boito S, Rembouskos G, et al. Cell-free DNA analysis of maternal blood in prenatal screening for chromosomal microdeletions and microduplications: a systematic review. *Prenat Diagn*. 2021;41(10):1324-1331. https://doi.org/10.1002/pd.5928
80. Dar P, Jacobsson B, Clifton R, et al. Cell-free DNA screening for prenatal detection of 22q11.2 deletion syndrome. *Am J Obstet Gynecol*. 2022;227(1):79.e71-79.e11. https://doi.org/10.1016/j.ajog.2022.01.002
81. Avram CM, Shaffer BL, Sparks TN, Allen AJ, Caughey AB. Cell-free fetal DNA screening for detection of microdeletion syndromes: a cost-effectiveness analysis. *J Matern Fetal Neonatal Med*. 2021;34(11):1732-1740. https://doi.org/10.1080/14767058.2019.1647161
82. Helgeson J, Wardrop J, Boomer T, et al. Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. *Prenat Diagn*. 2015;35(10):999-1004. https://doi.org/10.1002/pd.4640
83. Schmid M, Wang E, Bogard PE, et al. Prenatal screening for 22q11.2 deletion using a targeted microarray-based cell-free DNA test. *Fetal Diagn Ther*. 2018;44(4):299-304. https://doi.org/10.1159/000484317
84. Vora NL, Robinson S, Hardisty EE, Stamilio DM. Utility of ultrasound examination at 10-14 weeks prior to cell-free DNA screening for fetal aneuploidy. *Ultrasound Obstet Gynecol*. 2017;49(4):465-469. https://doi.org/10.1002/uog.15995
85. Society for Maternal-Fetal Medicine (SMFM)Norton ME, Biggio JR, Kuller JA, Blackwell SC. The role of ultrasound in women who undergo cell-free DNA screening. *Am J Obstet Gynecol*. 2017;216(3):B2-B7. https://doi.org/10.1016/j.ajog.2017.01.005
86. Salomon LJ, Alfirevic Z, Audibert F, et al. ISUOG Clinical Standards Committee. ISUOG consensus statement on the impact of non-invasive prenatal testing (NIPT) on prenatal ultrasound practice. *Ultrasound Obstet Gynecol*. 2014;44(1):122-123. https://doi.org/10.1002/uog.13393
87. Hui L, Johnson JA, Norton ME. ISPD 2022 debate—when offering a first trimester ultrasound at 11 + 0 to 13 + 6 weeks, a detailed review of fetal anatomy should be included. *Prenat Diagn*. 2023;43(4):421-427. ePub ahead of print. https://doi.org/10.1002/pd.6251
88. Wapner RJ, Martin CL, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med*. 2012;367(23):2175-2184. https://doi.org/10.1056/nejmoa1203382
89. Reimers RM, Mason-Suares H, Little SE, et al. When ultrasound anomalies are present: an estimation of the frequency of chromosome abnormalities not detected by cell-free DNA aneuploidy screens. *Prenat Diagn*. 2018;38(4):250-257. https://doi.org/10.1002/pd.5233
90. Kelley J, McGillivray G, Meagher S, Hui L. Increased nuchal translucency after low-risk noninvasive prenatal testing: what should we tell prospective parents? *Prenat Diagn*. 2021;41(10):1305-1315. https://doi.org/10.1002/pd.6024
91. Grande M, Jansen FA, Blumenfeld YJ, et al. Genomic microarray in fetuses with increased nuchal translucency and normal karyotype: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2015;46(6):650-658. https://doi.org/10.1002/uog.14880
92. American College of Obstetricians and Gynecologists. Screening for Fetal Chromosome Abnormalities 2020. Practice Bulletin No. 226 5 September 2020. https://www.smfm.org/publications/328-practice-bulletin-226-screening-fo
93. Hui L, Pynaker C, Bonacquisto L, et al. Re-examining the optimal nuchal translucency cutoff for diagnostic testing in the cell-free DNA and microarray era: results from the Victorian Perinatal Record Linkage study. *Am J Obstet Gynecol*. 2021;3(5):527.e1-527.e12. S0002-9378(21)00537-8. https://doi.org/10.1016/j.ajog.2021.03.050
94. Bardi F, Bosschieter P, Verheij J, et al. Is there still a role for nuchal translucency measurement in the changing paradigm of first trimester screening? *Prenat Diagn*. 2020;40(2):197-205. https://doi.org/10.1002/pd.5590
95. Lugthart MA, Bet BB, Elsman F, et al. Increased nuchal translucency before 11 weeks of gestation: reason for referral? *Prenat Diagn*. 2021;41(13):1685-1693. https://doi.org/10.1002/pd.6054
96. Ramkrishna J, Menezes M, Humnabadkar K, et al. Outcomes following the detection of fetal edema in early pregnancy prior to non-invasive prenatal testing. *Prenat Diagn*. 2021;41(2):241-247. https://doi.org/10.1002/pd.5847
97. Sachs A, Blanchard L, Buchanan A, Norwitz E, Bianchi DW. Recommended pre-test counseling points for noninvasive prenatal

testing using cell-free DNA: a 2015 perspective. *Prenat Diagn*. 2015;35(10):968-971. https://doi.org/10.1002/pd.4666
98. Green S, Hartzfeld D, Terry AB, et al. An evidence-based practice guideline of the National Society of Genetic Counselors for telehealth genetic counseling. *J Genet Couns*. 2023;32(1):4-17. https://doi.org/10.1002/jgc4.1627
99. Battese Ellis K. Effectiveness of alternative prenatal genetic counselling methods in a cohort of pregnant women from a low socio-economic population. *Aust N Z J Obstet Gynaecol*. 2022;62(5):674-680. https://doi.org/10.1111/ajo.13522
100. Carlson LM, Harris S, Hardisty EE, et al. Use of a novel computerized decision aid for aneuploidy screening: a randomized controlled trial. *Genet Med*. 2019;21(4):923-929. https://doi.org/10.1038/s41436-018-0283-2
101. Xian Lim KM, Lewis C, Wong HC, Chong GSL, Gosavi A, Choolani MA. Does an educational video for aneuploidy screening improve informed choice among pregnant women? A randomised controlled trial. *Prenat Diagn*. 2022;43(1):42-50. Epub ahead of print. https://doi.org/10.1002/pd.6279
102. Horn R. NIPT and the concerns regarding 'routinisation. *Eur J Hum Genet*. 2022;30(6):637-638. https://doi.org/10.1038/s41431-022-01053-6
103. Villegas C, Haga SB. Access to genetic counselors in the southern United States. *J Pers Med*. 2019;9(3):33. https://doi.org/10.3390/jpm9030033
104. Kater-Kuipers A, de Beaufort ID, Galjaard RH, Bunnik EM. Rethinking counselling in prenatal screening: an ethical analysis of informed consent in the context of non-invasive prenatal testing (NIPT). *Bioethics*. 2020;34(7):671-678. https://doi.org/10.1111/bioe.12760
105. Bowman-Smart H, Savulescu J, Gyngell C, Mand C, Delatycki M. Sex selection and non-invasive prenatal testing: a review of current practices, evidence and ethical issues. *Prenat Diagn*. 2020;40(4):398-407. https://doi.org/10.1002/pd.5555
106. Gammon BL, Kraft SA, Michie M, Allyse M. I think we've got too many tests!": prenatal providers' reflections on ethical and clinical challenges in the practice integration of cell-free DNA screening. *Ethics Med Public Health*. 2016;2(3):334-342. https://doi.org/10.1016/j.jemep.2016.07.006
107. Kater-Kuipers A, Bunnik EM, de Beaufort ID, Galjaard RJH. Limits to the scope of non-invasive prenatal testing (NIPT): an analysis of the international ethical framework for prenatal screening and an interview study with Dutch professionals. *BMC Pregnancy Childbirth*. 2018;18(1):409. https://doi.org/10.1186/s12884-018-2050-4
108. Chiu RWK, Heitzer E, Lo YMD, Mouliere F, Tsui DWY. Cell-free DNA fragmentomics: the new "omics" on the block. *Clin Chem*. 2020;66(12):1480-1484. https://doi.org/10.1093/clinchem/hvaa258
109. Lo YMD, Han DSC, Jiang P, Chiu RWK. Epigenetics, fragmentomics and topology of cell-free DNA in liquid biopsies. *Science*. 2021;372(6538). eaaw3616. https://doi.org/10.1126/science.aaw3616
110. Lo YMD. Noninvasive prenatal testing: advancing through a virtuous circle of science, technology and clinical applications. *Prenat Diagn*. 2021;41(10):1190-1192. https://doi.org/10.1002/pd.5978
111. Tsang JCH, Vong JSL, Ji L, et al. Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics. *Proc Natl Acad Sci U S A*. 2017;114(37):E7786-E7795. https://doi.org/10.1073/pnas.1710470114
112. Pique-Regi R, Romero R, Tarca AL, et al. Single cell transcriptional signatures of the human placenta in term and preterm parturition. *Elife*. 2019;8:e52004. https://doi.org/10.7554/elife.52004
113. Liu S, Huang S, Chen F, et al. Genomic analyses from non-invasive prenatal testing reveal genetic associations, patterns of viral infections, and Chinese population history. *Cell*. 2018;175(2):347-359.e14. https://doi.org/10.1016/j.cell.2018.08.016
114. Linthorst J, Baksi MMM, Welkers MRA, Sistermans EA. The cell-free DNA virome of 108,349 Dutch pregnant women. *Prenat Diagn*. 2022;43(4):448-456. https://doi.org/10.1002/pd.6143
115. Juneau K, Bogard PE, Huang S, et al. Microarray-based cell-free DNA analysis improves noninvasive prenatal testing. *Fetal Diagn Ther*. 2014;36(4):282-286. https://doi.org/10.1159/000367626
116. 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. https://doi.org/10.1002/pd.3993

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