ACCURACY OF NON-INVASIVE PRENATAL TESTING USING MASSIVELY PARALLEL SEQUENCING FOR DETECTING TRISOMY 21: A META-ANALYSIS

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Abstract: Background:*Non-Invasive Prenatal Testing (NIPT) for fetal trisomy 21 aneuploidy has been widely adopted in clinical practice due to its superior accuracy. NIPT has also been developed and validated as an option for early detection of genetic abnormalities prior to invasive diagnostic procedures. The aim of this study was to evaluate the accuracy of NIPT using Massively Parallel Sequencing (MPS) technology for screening Down syndrome in singleton pregnancies across all trimesters.* ***Methods:****This systematic review and meta-analysis analyzed literature on the accuracy of NIPT with MPS technology for Down syndrome screening in singleton pregnancies across all trimesters, following PRISMA guidelines. Eight studies were included, and data extraction was performed independently by three reviewers. Study quality was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool.* ***Results:****Among 485,365 pregnant women screened for major autosomal trisomies confirmed by fetal karyotype or newborn phenotype, 2,092 cases were verified from the total population. The sensitivity of NIPT for detecting trisomy 21 ranged from 0.80 (95% CI: 0.73–0.87) to 1.00 (95% CI: 0.48–1.00) based on the eight identified studies. NIPT showed very high specificity, with most studies reporting a specificity of 1.00 (95% CI: 1.00–1.00).* ***Conclusion:*** *NIPT using MPS technology demonstrated very high sensitivity and specificity in detecting trisomy 21 (Down syndrome) in singleton pregnancies across all trimesters. It is a reliable and effective screening tool due to its high accuracy in detecting true-positive cases and minimizing false-positive results.*

INTRODUCTION

Chromosomal abnormalities may include the absence or addition of entire chromosomes, as well as deletions, duplications, and translocations of varying sizes.[1] Aneuploidy is defined as the presence of an extra or missing chromosome, while

microdeletions and duplications refer to the loss or gain of small chromosomal segments, known as copy number variants.[1]

Trisomy 21 (T21), known as Down syndrome (DS), is the most common chromosomal abnormality in humans.[2] Down syndrome is a chromosomal condition associated with intellectual disability and is characterized by a wide range of additional clinical features.[3] This condition occurs in approximately one out of every 800 live births worldwide.[3] The phenotype of DS involves manifestations that affect multiple body systems, particularly the musculoskeletal, neurological, and cardiovascular systems.[4] Individuals with DS typically present with muscle hypotonia, short stature, reduced neuronal density, atlantoaxial instability, intellectual disability, cerebellar hypoplasia, and congenital heart defects (CHD), particularly atrioventricular septal defects (AVSD).[4] Individuals with DS are also more likely to develop certain health conditions, including autoimmune diseases, hypothyroidism, epilepsy, obstructive sleep apnea, hearing and vision problems, recurrent infections, hematologic disorders (including leukemia), anxiety disorders, and early-onset Alzheimer's disease.[4]

Several factors are associated with an increased likelihood of chromosomal abnormalities, including advanced maternal age, parental translocation or other chromosomal defects, a previous pregnancy affected by a chromosomal abnormality, prenatal ultrasonographic anomalies, or positive screening test results.[1] Although the risk of aneuploidy increases with maternal age, most children with trisomy 21 are born to younger mothers, as a greater proportion of all births occur among younger women.[5]

Currently, there is no cure for Down syndrome; therefore, prenatal detection provides an opportunity for parents to prepare themselves in various ways—such as seeking appropriate support, understanding the potential special needs, or, in some cases, considering the option of pregnancy termination.[6] Traditionally, fetal aneuploidy screening has been performed during the first and second trimesters using maternal serum biochemical markers, including pregnancy-associated plasma protein A (PAPP-A), human chorionic gonadotropin (hCG), alpha-fetoprotein (AFP), unconjugated estriol (uE3), and Inhibin A.[6] These serum biochemical tests (SBT) demonstrate detection rates of 49–76% with a 5% false-positive rate.[7] Pregnancies identified as high-risk by SBT are then referred for Chorionic Villus Sampling (CVS) or amniocentesis (AC), which are considered the gold standards for definitive diagnosis of fetal aneuploidy. However, both invasive procedures are time-consuming and carry a miscarriage risk of up to 1%.[7]

Cell-free DNA (cfDNA) from the fetus has been detected in the plasma of pregnant women and has been successfully used for non-invasive determination of fetal sex and RhD genotype in RhD-negative women.[8] The principle of this test lies in the detection of fetal-specific DNA sequences in maternal plasma. Similar approaches, such as the identification of fetal-specific DNA methylation markers and mRNA in maternal plasma, have been proposed for non-invasive detection of fetal aneuploidy.[8]

Major professional guidelines have supported the use of Non-Invasive Prenatal Testing (NIPT) as a secondary screening tool when conventional Down syndrome screening identifies pregnancies at high risk.[9] An alternative approach for trisomy 21 screening involves analyzing cfDNA in maternal blood.[1,10] NIPT works by detecting and quantifying chromosome 21 sequences in maternal plasma. In pregnancies with trisomy 21, there are three copies of

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chromosome 21 in the fetus, resulting in an increased proportion of chromosome 21 sequences in maternal plasma. However, a major challenge is that fetal DNA constitutes only about 10–20% of the total cfDNA in maternal plasma, meaning that the increased fetal chromosome 21 sequences are diluted by maternal DNA, which complicates detection.[11]

Non-Invasive Prenatal Testing (NIPT) using Massively Parallel Sequencing (MPS) of cfDNA in maternal plasma was introduced into clinical practice in late 2011 in the United States. MPS technology enables simultaneous sequencing of a large number of DNA fragments, allowing highly accurate detection of chromosomal abnormalities such as trisomy 21 from maternal blood samples.[12] Although the technology for non-invasive prenatal detection of Down syndrome continues to be refined, the significant improvements in both safety and accuracy compared with previous screening methods make it an increasingly attractive option. As its name suggests, NIPT is safer and less invasive than current diagnostic tests, which require fetal or placental cell samples obtained through amniocentesis or Chorionic Villus Sampling (CVS).

Therefore, the purpose of this study is to evaluate the accuracy of Non-Invasive Prenatal Testing (NIPT) using Massively Parallel Sequencing (MPS) technology in screening for trisomy 21 (Down syndrome) in singleton pregnancies across all trimesters.

METHODS

Literature Search Strategy

This study is a diagnostic test accuracy review. The literature search followed the Cochrane Guidebook for Systematic Reviews of Diagnostic Test Accuracy to identify studies that evaluated the accuracy of Non-Invasive Prenatal Testing (NIPT) using Massively Parallel Sequencing (MPS) technology for detecting Down syndrome or trisomy 21 abnormalities in pregnant women with singleton pregnancies across all trimesters. Searches were conducted in databases including PubMed, Scopus, and Web of Science. The search terms used were “noninvasive,” “non-invasive,” “prenatal diagnosis,” “cell free fetal DNA,” “cell-free fetal DNA,” “Down syndrome,” and “trisomy 21.” The publication date was limited to studies published between 2014 and 2024. Searches were restricted so that the keywords appeared in the titles of all databases. In addition, manual searches were performed based on the bibliographic references of the selected publications. Data extracted from each study included country, objectives, conditions, study design, participants, tests performed, results, and conclusions.

Study Criteria and Screening

The inclusion criteria applied in this review were as follows: (1) studies with an observational study design; (2) articles published between 2014 and 2024; (3) articles evaluating the accuracy of NIPT for screening Down syndrome; (4) populations consisting of pregnant women with singleton pregnancies in all trimesters; (5) studies utilizing NIPT with Massively Parallel Sequencing (MPS) technology as a screening method for detecting trisomy 21 (Down syndrome); (6) studies employing definitive diagnostic tests as a reference standard, such as amniocentesis or Chorionic Villus Sampling (CVS), as sampling methods for karyotype testing to confirm the NIPT results. The exclusion criteria included: (1) studies with non-observational designs; (2) studies not written in English; (3) non-original articles such as conference papers; (4) studies that did not use NIPT with MPS technology; (5) studies outside the defined population criteria.

Data Extraction

Data extraction was performed independently by three reviewers, with discrepancies resolved through discussion until a consensus was reached. The extracted information included: (1) study characteristics; (2) subject characteristics; (3) diagnostic characteristics; (4) accuracy results of Non-Invasive Prenatal Testing using MPS technology for detecting Down syndrome in singleton pregnancies across all trimesters.

Quality Assessment of Included Studies

The quality of the studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool. The Cochrane QUADAS-2 assessment covers four main domains to evaluate the risk of bias and applicability of primary diagnostic accuracy studies, including patient selection, index test, reference standard, and flow and timing. Responses were categorized as "Yes," "Probably Yes," "Probably No," "No," and "Not Included." These were subsequently summarized and translated into "yes," "no," or "unclear," with the first three domains also assessed for applicability (representativeness), coded as "high," "low," or "unclear."

When a quality item was rated as "unclear," additional information was sought from the original study authors. This systematic evaluation ensured that each study was thoroughly assessed for quality and risk of bias, ensuring that the meta-analysis results reflected reliable and relevant data. The quality assessment of studies was performed independently by three reviewers, and final judgments were reached by consensus. The extracted data were transferred to Review Manager 5.4 (The Nordic Cochrane Centre, Copenhagen, Denmark) for further data analysis.

Statistical Analysis of Test Accuracy Studies

For each study, 2×2 data tables were used to calculate sensitivity and specificity with 95% confidence intervals (CIs). Heterogeneity was explored by assessing the distribution of results in forest plots. Summary measures, including sensitivity, specificity, diagnostic odds ratio, and positive and negative likelihood ratios, along with their 95% CIs, were calculated for each study using the Review Manager software.

RESULTS AND DISCUSSION

Included Studies

Prisma

A total of 746 articles were identified after a comprehensive search of the primary databases: PubMed (n = 460), Scopus (n = 204), and Web of Science (n = 82). The articles were required to be observational studies published in English between 2014 and 2024. A total of 163 duplicate records were removed.

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From the remaining 583 articles screened based on title and abstract, 538 were excluded as they were not relevant to the topic of this systematic review. The remaining 45 articles were retrieved for full-text review. Of these, 37 studies were excluded due to issues related to study design, patient population, or lack of diagnostic accuracy data. This systematic review ultimately included data from eight observational studies. See Figure 1 for the PRISMA flow diagram of the study selection process.

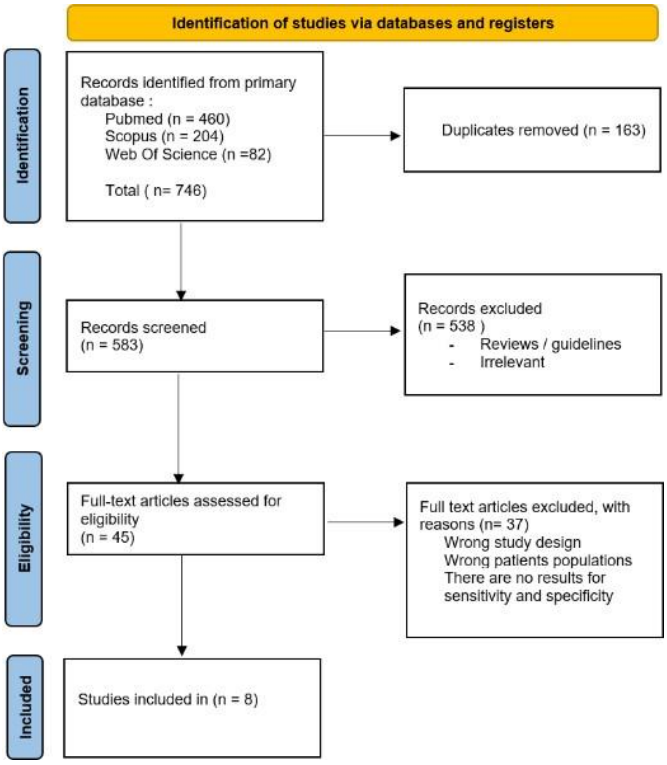

Figure 1. PRISMA Flow Diagram

Study Design and Participants

Eight publications, ranging from 2014 to 2024, reported Non-Invasive Prenatal Testing (NIPT) results for 485,365 pregnant women for the major autosomal trisomies related to fetal karyotype or neonatal phenotype and met the inclusion criteria. A total of 2,092 patients were confirmed among all pregnant participants. Most of the included studies were cohort studies (n = 5)[12-16] with retrospective data collection. One study [17] had a case-control design, and two studies [18,19] had unclear designs. Five studies [12,13,15,16,19] used samples from high-risk pregnant women (such as those with positive standard screening results, abnormal ultrasound findings, advanced maternal age, or personal/family history of aneuploidy) who underwent invasive testing. Three studies [14,17,18] involved pregnant women with mixed risk factors.

Testing Strategy

All studies used karyotyping as the verification standard, in which participants received confirmed results based on karyotype analysis. Karyotyping was performed using Chorionic Villus Sampling (CVS) and amniocentesis as sampling methods.

All studies employed Non-Invasive Prenatal Testing (NIPT) using Massively Parallel Sequencing (MPS) technology as the testing method. MPS is a DNA sequencing technique in which the entire genome is fragmented into small pieces, then sequenced randomly and in parallel. In NIPT, MPS is used to sequence cell-free fetal DNA (cfDNA) obtained from maternal blood samples. Maternal blood sampling for NIPT in all studies was performed prior to invasive testing.

Methodological Quality of Included Studies

The methodological quality of the eight included studies, as assessed by the QUADAS-2 tool, is summarized in Figures 2 and 3. Most studies demonstrated a low to moderate risk of bias, with one of eight studies identified as having a high risk of bias in at least one domain. One study was rated as having an unclear risk of bias across all three domains, while five others were rated as unclear in one or two domains. Two studies were considered to have an overall low risk of bias across all four domains.

Figure 2 shows that patient selection was the domain with the highest risk of bias. Incomplete or unclear reporting was common, particularly in the index test domain, observed in 5 (62.5%) out of 8 studies. Similar issues were also identified in the reference standard and flow and timing domains, where half of the publications (50%) exhibited unclear bias risks. The remaining six studies were judged to have a low risk of bias for the reference standard and flow/timing domains. Overall, two studies were classified as having low risk of bias, while six were considered to have potential bias concerns.

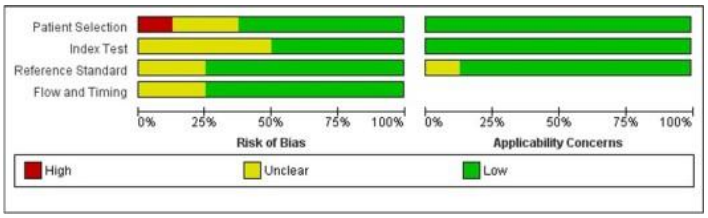

Figure 2. Quality Assessment Summary Based on QUADAS-2

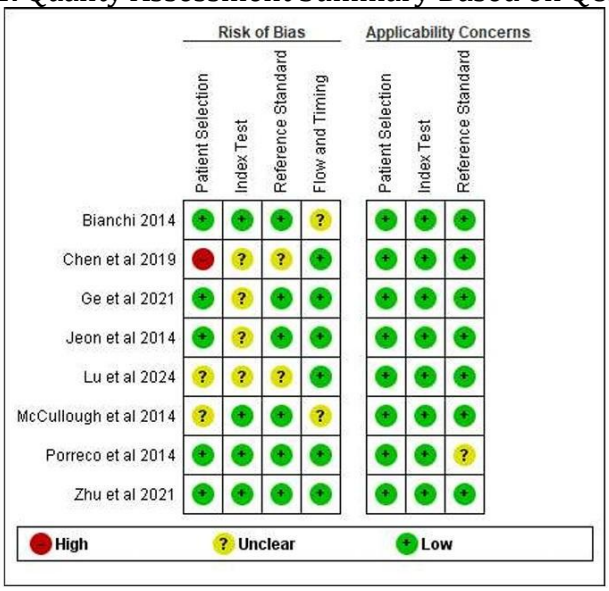

Figure 3. Quality Assessment Graph Based on QUADAS-2

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Meta-Analysis

The meta-analysis of studies evaluating the diagnostic accuracy of Non-Invasive Prenatal Testing (NIPT) demonstrated high sensitivity and specificity, despite variations among the included studies. NIPT sensitivity ranged from 0.80 to 1.00. The study with the lowest sensitivity, Chen et al. [14], reported a value of 0.80 (95% CI: 0.73–0.87), indicating that while NIPT detected most cases of trisomy 21, some cases went undetected, reflecting the occurrence of false negatives. Other studies, Bianchi et al.[10], Ge et al.[13], Porreco et al.[16], Jeon et al.[15], and Lu et al.[17]—reported perfect sensitivity values of 1.00, with their respective confidence intervals including 1.00. This suggests that in these studies, NIPT successfully identified all trisomy 21 cases without missing any, indicating no false-negative results.

Specificity was consistently high across all studies, reaching 1.00 in every one of them. This perfect specificity shows that NIPT almost always correctly identifies individuals without trisomy 21. Bianchi et al.[10], Ge et al.[13], Porreco et al.[16], Jeon et al.[15], Lu et al.[17], McCullough et al.[18], and Zhu et al.[12] all reported specificity values of 1.00, reinforcing NIPT's reliability in avoiding false positives. Overall, the consistently high sensitivity and specificity observed in this meta-analysis strongly support NIPT's use as a highly effective screening tool in clinical settings. Variations in specificity values may be attributed to differences in study populations, testing methodologies, or uncontrolled variables across studies.

Test Failure Rate

The test failure rate can be inferred from the reported sensitivity and specificity values. Sensitivity, which reflects the test's ability to correctly identify positive cases, varied among studies but was generally very high. The study by Chen et al.[14] reported a sensitivity of 0.80 (80%), indicating that the test failed to detect 20% of true positive cases, or in other words, had a 20% detection failure rate. This highlights a non-negligible risk that some positive cases might go undetected in certain contexts. However, most other studies, Bianchi et al.[10], Ge et al.[13], Porreco et al.[16], Jeon et al.[15], and Lu et al.[17], demonstrated perfect sensitivity (1.00 or 100%), meaning that no positive cases were missed.

Overall, the test failure rate of NIPT was very low, with failures primarily observed in a few studies showing slightly reduced sensitivity, such as Chen et al.[14] These failures remain minimal compared to the overall outstanding performance of the test in detecting positive cases and avoiding false positives. Therefore, although there is a small risk of missed positive detections, NIPT can be considered a highly reliable and effective screening tool with a minimal failure rate.

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bianchi 2014	5	6	0	1994	1.00 [0.48, 1.00]	1.00 [0.99, 1.00]
Chen et al 2019	103	27	25	7961	0.80 [0.73, 0.87]	1.00 [1.00, 1.00]
Ge et al 2021	139	14	0	18363	1.00 [0.97, 1.00]	1.00 [1.00, 1.00]
Jeon et al 2014	11	0	0	144	1.00 [0.72, 1.00]	1.00 [0.97, 1.00]
Lu et al 2024	457	54	0	268875	1.00 [0.99, 1.00]	1.00 [1.00, 1.00]
McCullough et al 2014	1129	79	20	23156	0.98 [0.97, 0.99]	1.00 [1.00, 1.00]
Porreco et al 2014	137	2	0	3291	1.00 [0.97, 1.00]	1.00 [1.00, 1.00]
Zhu et al 2021	111	11	1	27489	0.99 [0.95, 1.00]	1.00 [1.00, 1.00]

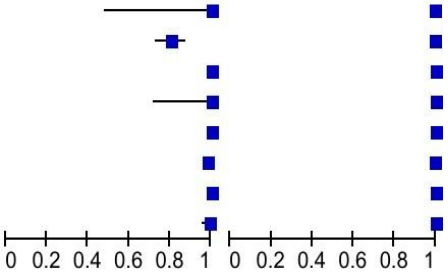

Figure 4. Forest plot of studies evaluating trisomy 21 in singleton pregnancies using Non-Invasive Prenatal Testing (NIPT)

Discussion

Principle and Mechanism of Non-Invasive Prenatal Testing (NIPT)

Non-Invasive Prenatal Testing (NIPT) is a screening method used to detect potential fetal genetic abnormalities from cell-free DNA (cfDNA) circulating in maternal blood.[20] NIPT analyzes the amount of cfDNA circulating in the maternal bloodstream, which consists of both maternal and fetal components.[20] It has now been widely adopted in clinical practice because it poses no risk to the pregnancy compared with traditional invasive methods, which carry a miscarriage risk of approximately 0.1–2%.[21] Moreover, it demonstrates higher accuracy than other non-invasive screening approaches, such as the use of maternal serum biochemical markers combined with fetal ultrasound markers. NIPT has been endorsed by several professional bodies and organizations as a primary screening method, regardless of pregnancy risk status.[1]

Cell-free DNA (cfDNA) refers to short DNA fragments present in plasma or other body fluids, distinct from DNA contained within intact cell nuclei.[22] These cfDNA fragments are released from various organs during cellular processes, including apoptosis, necrosis, and microparticle secretion.[22] In non-pregnant individuals, the proportional representation of each chromosome in plasma cfDNA reflects both chromosome size and the individual's karyotype. In euploid pregnant women, any deviation from the expected chromosomal profile in plasma cfDNA, due to an excess or deficiency of fragments from a specific chromosome, may indicate the presence of fetal trisomy or monosomy.[22]

Fetal Fraction (FF) represents the percentage of fetal cfDNA relative to the total cfDNA circulating in maternal plasma and serves as a key determinant of test sensitivity.[23,24] FF is defined as the amount of fetal cfDNA divided by the total cfDNA present in maternal plasma.[24] It is therefore a function of both maternal and fetal cfDNA concentrations. The so-called "fetal" DNA actually originates from placental tissue. Between 10 and 20 weeks of gestation, the average FF typically ranges from 10% to 15%.[22] Measuring FF is a critical component of sample quality control and statistical confidence, ensuring that sufficient placental cfDNA is available to produce meaningful test results.[22]

The minimum FF threshold required for adequate NIPT performance varies depending on the testing platform but is generally between 2% and 4%. Higher FF levels allow greater statistical separation between aneuploid and euploid pregnancies, thereby increasing diagnostic confidence. For this reason, NIPT should not be performed before 10 weeks of gestation. FF also correlates directly with crown–rump length (CRL), PAPP-A, and free β-hCG MoM levels and tends to be higher among East Asian populations (Chinese and Japanese) and smokers (largely due to decreased maternal cfDNA levels). Conversely, FF decreases with increasing maternal age and body mass index (BMI) (as a result of higher maternal cfDNA), and is also lower in twin pregnancies, women of Afro-Caribbean or South Asian descent, pregnancies conceived via in vitro fertilization (IVF), and those with elevated uterine artery pulsatility index (PI) during the first-trimester scan.[25]

The NIPT approach for detecting trisomy 21 involves the analysis of cfDNA in maternal plasma.[1,10] NIPT works by detecting and quantifying the number of chromosome 21 sequences in maternal plasma. In pregnancies affected by trisomy 21, the fetus carries three copies of chromosome 21, leading to an increased number of chromosome 21 sequences in maternal blood. However, a major challenge is that fetal DNA constitutes only 10–20% of the

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total cfDNA in maternal plasma. This dilution of fetal DNA by maternal cfDNA makes detection more difficult.

To overcome this challenge, NIPT employs cfDNA enrichment, highly sensitive sequencing techniques, and advanced bioinformatics analysis. Bioinformatics algorithms compare the proportion of chromosome 21 sequences to those of reference chromosomes, accounting for natural variation in cfDNA levels to improve detection accuracy. These analytical results are then interpreted to determine whether a significant increase in chromosome 21 sequences indicates trisomy 21. Despite technical challenges, numerous studies and meta-analyses have demonstrated that NIPT achieves a detection rate of approximately 99.7% and a false-positive rate of only 0.04%. In cases of NIPT failure or inconclusive results, options include retesting, invasive testing, or management guided by ultrasound and first-trimester combined screening. Thus, despite the challenges associated with low fetal cfDNA fractions, NIPT provides a highly accurate and non-invasive prenatal screening method for trisomy 21.

Test Reliability

Fetal cfDNA appears to originate primarily from cytotrophoblasts in the chorionic villi. Therefore, placental mosaicism confined to these tissues can cause false-positive results and represents one of the main reasons for discrepancies between NIPT and invasive test outcomes.[24] Other major contributors to false-positive (FP) or false-negative (FN) results include low fetal fraction, maternal chromosomal abnormalities, fetal mosaicism, pathogenic copy number variants, and vanishing twin phenomena.[25]

According to recent meta-analyses, the detection rate (DR) and false-positive rate (FPR) for trisomy 21 in singleton pregnancies are 99.7% and 0.04%, respectively. This demonstrates that NIPT possesses exceptionally high accuracy for detecting trisomy 21, with most true-positive cases accurately identified and only a very small proportion producing false-positive results. These findings reaffirm the reliability of NIPT as an effective and safe prenatal screening tool.[22]

NIPT Using Massively Parallel Sequencing (MPS)

Non-Invasive Prenatal Testing (NIPT) utilizing Massively Parallel Sequencing (MPS) of cfDNA has revolutionized prenatal screening and diagnosis. This technology enables highly precise molecular analysis, allowing accurate and non-invasive detection of chromosomal aneuploidies. MPS simultaneously sequences millions of DNA fragments, generating rich genomic data that enables the detection of trisomy 21 with extremely high sensitivity and specificity. These advantages make MPS-based NIPT superior to conventional screening methods, which typically exhibit higher error rates.[25]

MPS-based NIPT significantly reduces the number of missed aneuploidy cases due to its greater sensitivity and specificity. Consequently, fewer false positives occur, thereby decreasing the need for invasive confirmation procedures such as amniocentesis or chorionic villus sampling (CVS). This reduction in invasive testing minimizes miscarriage risks and associated maternal complications. Therefore, MPS-based NIPT not only offers earlier and more accurate detection but also enhances safety and comfort for pregnant women.[24]

Implementation of NIPT in Clinical Practice

There are two primary strategies for introducing NIPT into clinical practice. The first approach involves performing NIPT for all patients at 10 weeks of gestation, followed by a

first-trimester ultrasound and combined testing at 12 weeks.[20] For patients identified as high-risk, invasive testing may be scheduled during the first trimester. If NIPT fails or yields a negative result, subsequent management can be guided by ultrasound and combined test findings. This strategy is known as universal NIPT screening.[24]

The second approach is the contingent testing strategy, which considers the results of first-trimester ultrasound and combined testing before deciding whether to perform NIPT. This method maintains the key advantages of NIPT—high detection rate and low false-positive rate—while reducing costs compared with universal screening at 10 weeks. However, one limitation is the potential delay of diagnosis from the first to the second trimester in cases of NIPT failure. In such instances, direct access to invasive testing may be proposed for high-risk patients or NIPT may be considered for those with intermediate risk. Contingent testing also utilizes careful ultrasound examination, which is valuable for several reasons, such as accurate dating, exclusion of major anomalies, identification of both aneuploidy-related and non-aneuploidy markers, and early prediction of pregnancy complications like preterm birth or preeclampsia.[25] If NIPT fails, options include repeat sampling, proceeding with invasive testing, or discontinuing further testing.[22] When first-trimester scans reveal potential structural abnormalities, confirmation via invasive testing is warranted. Conversely, if no abnormalities are detected, a repeat NIPT sample may be appropriate. If a second failure occurs, options again include invasive testing or no further testing.[23]

If no ultrasound abnormalities are observed during combined screening—even after a second NIPT failure—and the a priori risk for trisomy 21 is low, it is acceptable to wait for an anomaly scan.[24] However, if the risk for trisomy 21 is high, invasive testing such as amniocentesis is recommended.

The choice between these approaches depends on several factors, including resource availability, cost, patient preference, and local health policies. Both strategies require adequate pre- and post-test counseling to help parents understand the results and implications of testing, ensuring they make informed decisions.

Three main limitations exist in integrating cfDNA testing into clinical practice. The first is its higher cost compared with other screening tests, similar to that of invasive tests involving karyotype analysis.[22] Broader adoption may reduce costs over time, though the rate and extent of such reductions remain uncertain. The second limitation involves test failures, which can complicate clinical management. As explained by Gratacós and Nicolaides,[25] when NIPT was first introduced in clinical settings, another limitation was the turnaround time for results, as few laboratories conducted such analyses. This could delay diagnosis from the first to the second trimester, negating advantages gained from prenatal screening advancements over the past 30 years. However, it is now recognized that for the three major chromosomal abnormalities, average turnaround time is no more than one week.

Social and Ethical Implications

Numerous studies have explored the social implications of NIPT use.[21] Stakeholders generally view NIPT positively due to its clinical benefits—easy accessibility, improved accuracy, and reduction in invasive procedures compared with traditional screening tests. [23] The use of Non-Invasive Prenatal Testing (NIPT) to detect chromosomal abnormalities such as trisomy 21 is becoming increasingly popular among expectant parents, including those

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who previously avoided invasive prenatal tests due to miscarriage risks.[24] Many parents choose NIPT not to decide on pregnancy termination, but rather to prepare for the birth of a child with Down syndrome—emotionally, financially, and medically. However, concerns remain that NIPT may be perceived as a routine blood test, potentially leading to insufficient discussion of its broader implications and creating social pressure on parents to undergo NIPT simply because it is safe and easy to perform. There is also a risk that uninformed decision-making could occur if NIPT is viewed as a standard, risk-free procedure.[25]

To address these concerns, comprehensive and sensitive pre- and post-test counseling by trained healthcare professionals is essential. Thorough counseling helps parents understand potential results and implications, including the options available following test outcomes, while ensuring adherence to professional and regulatory guidelines that promote best practices in NIPT use. Most stakeholders—including healthcare providers, regulatory authorities, and related organizations—believe that these concerns can be mitigated through proper training and effective communication between healthcare professionals and parents to ensure accurate understanding and informed decision-making. Clinical and ethical implications of NIPT use must also be considered, particularly regarding patient autonomy and social pressures. Therefore, raising awareness about the importance of comprehensive counseling, encouraging healthcare providers to follow updated training and regulatory guidance, and offering resources and support to parents who continue their pregnancies after a positive NIPT result are all essential steps.

CONCLUSION

This systematic review and meta-analysis confirm that Non-Invasive Prenatal Testing (NIPT) using Massively Parallel Sequencing (MPS) demonstrates excellent diagnostic performance in detecting trisomy 21 (Down syndrome) among singleton pregnancies across all trimesters. The findings indicate that NIPT provides consistently high sensitivity and specificity, supporting its role as a superior, safe, and non-invasive alternative to traditional prenatal screening methods.

The implications of this study suggest that implementing NIPT in clinical practice could significantly reduce the need for invasive diagnostic procedures such as amniocentesis and chorionic villus sampling, thereby minimizing associated risks to both mother and fetus. Despite the strengths of this review—including comprehensive data analysis and methodological rigor—limitations exist, such as inter-study heterogeneity and restricted generalizability due to varying population characteristics. Further large-scale, multicenter, and longitudinal studies are recommended to confirm these results and evaluate the broader application of NIPT in diverse populations. Overall, NIPT with MPS represents a major advancement in precision prenatal screening and has the potential to improve early detection and reproductive decision-making.

Conflict of Interest

The authors declare no conflicts of interest related to this study. The authors retained full control over all aspects of the research, including study design, data collection, analysis, interpretation, and manuscript preparation, without external influence.

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Declaration of Using AI

The authors affirm that artificial intelligence (AI) tools were used solely for linguistic enhancement, including grammar correction, paraphrasing, and improving clarity. No AI tools were utilized to generate original content, perform data analysis, or interpret research findings. The authors take full responsibility for the integrity and accuracy of the content presented in this manuscript.

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