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Review Article **Pharmacy**

Comprehensive Overview of Non-Invasive Prenatal Testing (NIPT) and its Ethical Considerations

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Abstract

This article provides an overview of Non-Invasive Prenatal Testing (NIPT), also known as noninvasive prenatal screening. It is a technique for estimating the likelihood that the fetus will have specific genetic defects at birth for pregnant women. The content is specifically tailored for pregnant women and ncompasses a detailed exploration of topics such as Genomics-based noninvasive prenatal tests, sample details for noninvasive prenatal testing, factors influencing test results, the interpretation of test results, limitations associated with the test, and the content included in the Declaration of Consent Form for Noninvasive Prenatal Testing.

Keywords: Fetal aneuploidies, genetic disorders, trisomy. Circulating cells: free DNA, amniocentesis, Down syndrome, gNIPT.

INTRODUCTION

The NIPS, also known as noninvasive prenatal screening, is a noninvasive test for pregnancies. technique used to assess the likelihood that a fetus would possess from birth specific genetic defects. Little DNA fragments that are present in a pregnant woman's blood are analyzed by this test. These pieces of DNA are flying freely and not most DNA, which is contained within cells, in contrast to the majority of DNA, which is located inside a cell's nucleus. For this reason, they are referred to as DNA free of cells (cfDNA). These tiny pieces, which are typically composed of Less than 200 base pairs of DNA, are produced when cells divide and release their constituent parts into the circulation.

Background

There are 46 chromosomes in humans (23 pairs). Unusual chromosomal counts can result in hereditary illnesses for which there is no treatment. A sex chromosomal abnormality (SCA) is an excess (or less) of a sexual chromosome, whereas a trisomy is an additional chromosome. About one in 1,000 newborns have Down syndrome, the most prevalent trisomy. Slow development, distinctive facial traits, mild to moderate intellectual handicap, and the need for specialized education later in life are all characteristics of children with Down syndrome. Nonetheless, some babies have reasonably normal lives despite the symptoms, which range from mild to severe. Although the degree of dysfunction in the various trisomy or SCA disorders varies, the likelihood of a newborn being impacted is far lower [1]. When it comes to identifying Down syndrome, NIPT has a sensitivity greater than 99%and a false-positive rate of less than 0.1%. NIPT offers a safe substitute for intrusive testing when women show elevated risk determined by the findings of the first-trimester combination test (FCT). It is crucial to keep in mind that invasive testing should be used to confirm a positive NIPT result. NIPT additionally be used as the main screening exam for all expectant mothers because of its because of its remarkable accuracy [2]

Turner syndrome (45,X), Triple X syndrome (47,XXX), Klinefelter syndrome (47,XXY), Edward syndrome (trisomy 18 or T18), Patau syndrome y Among the prevalent fetal aneuploidies are Down syndrome (trisomy 21 or T21), (trisomy 13 or T13), and 47,XYY syndrome (47,XYY).

Although screening for fetal aneuploidies during pregnancy has become routine practice in numerous nations,there are still significant false-positive

and false-negative rates with contemporary ultrasound and biochemical assays. circulating cell-free DNA (ccfDNA) from fetal in mother blood suggests that a more precise genomics-based non-invasive prenatal diagnostic screening technique (gNIPT) may be possible. Two techniques are utilized for massively parallel shotgun sequencing (MPSS) and targeted massively parallel sequencing (TMPS) in gNIPT.

Other Important Information to Consider

The statistical analysis demonstrated that gestational age and counseling day had a significant impact on women's decisions for Contrasting invasive prenatal tests with non-invasive prenatal testing (NIPT) in pregnancies with an elevated a priori risk of fetal aneuploidies [3]. The gNIPT technique seems to work effectively for detecting fetuses that have an unusually high number of chromosomes. However, before making decisions about pregnancy, confirmatory tests (such as amniocentesis or CVS) are still required if a gNIPT reveals an aberrant chromosomal number.

Genomics Based Non-Invasive Pregnancy Tests

The discovery that placental cells constantly produce measurable quantities of fetal ccfDNA in mother blood is the foundation for non-invasive prenatal diagnostics based on genomics. This ccfDNA from fetus is mostly composed of relatively small pieces, less than Three hundred base pairs and comes from the placenta's normal cell death [1]. One crucial factor in accurately detecting the fetal fraction of gNIPT is an aneuploid fetus, or the portion of total ccfDNA of fetal origin in the bloodstream of mothers. Fetal ccfDNA can be found as early as During five weeks of pregnancy, while making up just between 2 and 20% of the total ccfDNA in the mother's blood. Amniocentesis is one invasive operation that might be or might not be linked to a statistically noteworthy rise in ccfDNA in mother blood. This might have an impact on concentration of fetal DNA and gNIPT outcomes. Thus, mother For the gNIPT, blood is often obtained either prior to or following a minimum of twenty-four hours of an intrusive test in a clinical setting investigations. In fact, It's been calculated that the half-life of ccfDNA is less than a day.

With reference to a chromosomal reference set, the goal of The goal of genomics-based non-invasive prenatal testing (gNIPT) is to quantify the quantity of circulating cell-free DNA (ccfDNA) from a pregnant woman that contains DNA fragments from the chromosomes of interest (chromosome 21; see example in figure 1). Top illustration shows DNA fragments flowing in maternal blood within the cases of aneuploid (right) and euploid (left) pregnancies. Although TMPS produces a greater percentage of readings from the target chromosomes (bottom), MPSS generates a substantial total number of reads for each chromosome's sequence. Sequence reads may be utilized in both techniques to detect a little excess of fetal genetic material originating from the target chromosomes.

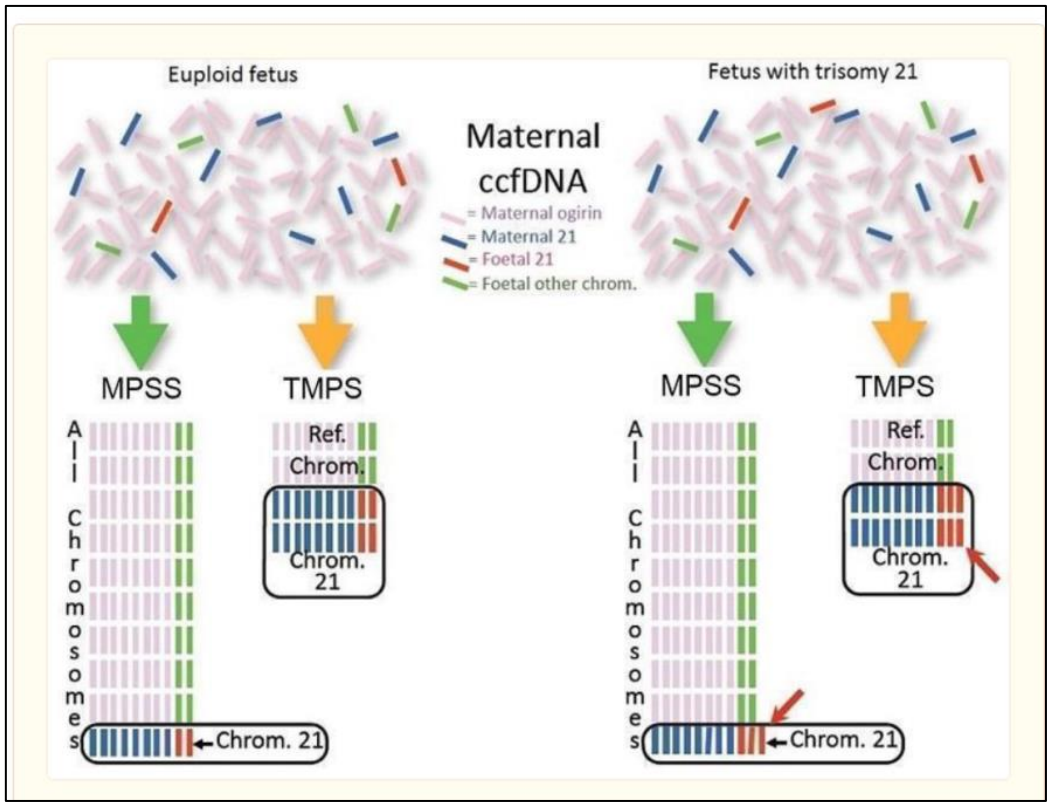

Figure 1: The distinction between targeted massively parallel sequencing (TMPS) and massively parallel shotgun sequencing (MPSS)

During pregnancy genetic screening investigation, NIPT (fetal DNA testing without cells) is frequently used; however, due to its limitations, further invasive tests must be performed for confirmation. On the other hand, a potential non-invasive diagnostic technique that extracts whole fetal cells from the mother's cervix is called trophoblast retrieval and isolation from the cervix, or TRIC. TRIC has other benefits beyond its non-invasive nature, such the possibility of a timely diagnosis as early as five weeks of pregnancy and reliable outcomes that are not influenced by maternal obesity. Additionally, Prior to the onset of clinical signs, TRIC's trophoblast yields can offer important insights into obstetrical issues associated to aberrant placentation [4].

Laboratory Leadership and Quality Management of NIPT Testing

Quality assurance is a crucial aspect of any laboratory testing, including prenatal non-invasive testing (NIPT). Here are a some of the key elements of quality assurance for NIPT:

Laboratory Accreditation: The laboratory performing the The NIPT ought to accredited by a recognized accrediting agency, such as the College of American Pathologists (CAP), International Organization for Standardization (ISO). The Amendments to Clinical Laboratory Improvement (CLIA), or an international equivalent. Accreditation ensures that the laboratory has demonstrated proficiency in conducting the examination andmeets rigorous standards of quality control.

Verification and Validation: The laboratory should have performed validation and verification studies to ensure that the NIPT is accurate and reliable for the conditions it detects.

Control of Quality: A strong quality control procedure need to be implemented in the laboratory to keep an eye on the NIPT's accuracy and precision. This entails involvement in outside quality assurance initiatives additionally routine testing of internal quality control samples and reference samples.

Data Interpretation and Analysis: For data processing and NIPT result interpretation, the lab needs a well-defined and tested bioinformatics workflow. To guarantee correctness and dependability, this pipeline has to be checked and updated on a regular basis.

Reporting of Results

A clear and simple reporting system for NIPT findings should be in place at the laboratory. It should include details on test limits, the necessity of follow-up testing, and the consequences of both favorable and unfavourable results. In addition, individuals who obtain positive or inconclusive results from the laboratory ought to go through genetic counseling services. Continuing instruction and preparation: To stay current with developments in NIPT technology and quality assurance procedures, laboratory personnel should regularly participate in continuing instruction and preparation:

Currently, commercialized gNIPT services for detecting common aneuploidies are available from companies across Europe, Asia, and America, with their respective diagnostic performance data accessible via their websites. Additionally, Numerous clinical and research laboratories have formulated their own in-house gNIPT solutions. As stated by the latest recommendations by the ACOG, or American College of Obstetricians and Gynecologists, gNIPT is not to exist regarded as an alternative to diagnostic testing. ACOG advises that pregnant individuals with favorable gNIPT outcomes should undergo diagnostic procedures before considering any irreversible actions such as terminating the pregnancy. Furthermore, guidelines suggest that pregnant individuals with inconclusive gNIPT results should be provided with thorough ultrasound care and additional genetic counseling evaluations along with diagnostic testing options.

As stated by the clinical route, most upper-middle-class and high-income nations include screening for fetal aneuploidy during pregnancy (usually T21) in their public health initiatives, and they usually make it available to all expectant mothers. Thus far, Aneuploidy screening tests have been performed using blood-based testing placental markers biochemically in conjunction either with or without ultrasonic imaging to determine if additional indicators of aneuploidy in the fetus first trimester, such as thickness of nuchal translucency. The age of the expectant mother is paired with biomarker levels as well as nuchal translucency prediction indicators throughout the first trimester of T21.

NIPT Sample Details:

1. Sample Requirement
   A. 10 ml mother blood sample with unbound DNA in cells(of DNA) collection tube (Streck tube)

2. Sample Rejection Criteria
   - Sample not collected in a ofDNA tube
   - Hemolyzed sample
   - Inadequate blood sample

3. Documents Required
   A. Test request form (TRF) and Form G
   B. Ultrasound report
   C. Maternal serum screening report (If available)

Interpretation of Test Findings:

The analysis software compares the DNA levels of the exemplar with the DNA levels of a bioinformatic baseline, which represents a chromosomally normal

state. The following results can be obtained from this comparison:

No alteration was detected: The amounts of Maternal blood DNA resembles that of the baseline chromosomally normal population. These outcomes are in line with a evaluation for the examined chromosomes that is normal. For the condition(s) under study, the patient is thought to pose little danger.

Alteration Detected: For the designated chromosome, There was one increase in DNA levels found in comparison to the baseline chromosomally normal state. These findings align with an anomalous test for the designated chromosome. With a very high favorable predictive value, the patient is deemed to be in a position where the stated disease.

Suspected Alteration Detected: For the designated chromosome, a statistically significant increase in DNA levels was seen, although a small one, as compared to the chromosomally normal baseline. These findings align with an anomalous test for the designated chromosome. With a low PPV, the patient is deemed to be at high risk for the ailment that has been recorded.

Non-Informative: Due to insufficient derived DNA quality and/or quantity, information on the chromosomal status of the pregnancy cannot be obtained from maternal blood. Additionally, an inadvertent chromosomal finding—that is, the discovery of a change in the DNA levels of a different chromosome from the ones reported by the test—may result in a non-informative result.

Sex of the Fetus (Sexual Chromosomes): When the reported result is XY in a single pregnancy, the fetus is male; when the reported result is XX, it is female. It is impossible to identify the specific sex of each fetus in a twin pregnancy. The Y chromosome's existence or absence is reported in these situations. It is necessary to understand that the presence of the Y chromosome indicates that one or both fetuses are male. It is necessary to conclude that both fetuses are female since one of the Y chromosomes is absent.

Test Description:

This test is a genetic screening procedure that examines DNA without cells that is present in pregnant women's bloodstreams. Next-generation sequencing is used to detect chromosomal number change, or aneuploidy (NGS). This screening test has a very high accuracy rate. There is evidence of clinical connection. Amniocentesis or chorionic villus sample would be required if a conclusive diagnosis was sought. This method uses FF estimate as one of its components, which is paired with other quality measures to establish the degree of confidence in the outcome. Samples are not excluded solely based on the FF estimate.

Limitations:

This assessment has been developed and verified to identify aneuploidies across all 24 chromosomes. It has been validated specifically for singleton pregnancies with a gestational age of at least 10 weeks, estimated from the last menstrual period. A negative outcome from the test does not completely rule out the possibility of chromosomal variations in the assessed chromosomes. There is a minor chance that the test outcomes may not accurately represent the fetal chromosomes but could instead reflect chromosomal alterations in the placenta (confined placental mosaicism) or maternal chromosomal mosaicism. Notably, the test does not detect triploidy. Furthermore, while these findings do not dismiss the potential for other chromosomal or sub chromosomal irregularities, birth defects, or clinical conditions in the pregnancy, they are not designed to identify risks related to open neural tube defects. The precision of these assessments might be compromised in cases of maternal chromosomal aneuploidy, mosaicism (either fetal or confined to the placenta), or if the analysis is conducted before the 10th week of pregnancy. There exists a slight possibility that the test results may not accurately reflect the chromosomal status of the fetus, potentially yielding false positives that instead indicate subchromosomal changes in the placenta or maternal factors like allogeneic blood transfusion, transplant or stem cell therapy, vanishing twin syndrome, and multiple gestations. Additionally, ongoing treatment with low molecular weight heparin may interfere with the analysis.

Factors affecting NIPT results:

When dealing with NIPT, low fetal fraction and high data noise can impact the reliability of the test results which is shown in figure 2.

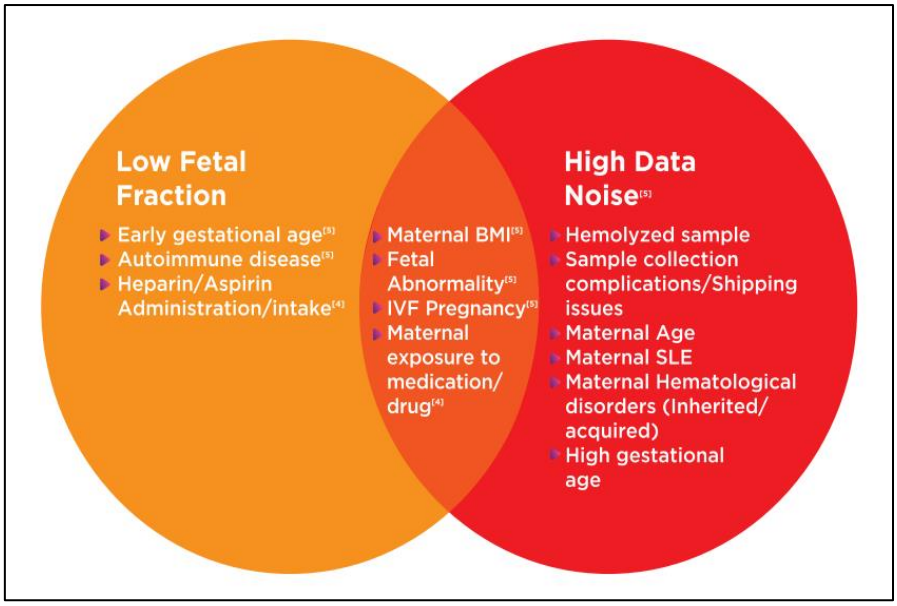

Figure 2: Factors affecting NIPT results

When we consider False-positives and false-negatives in non-invasive prenatal testing (NIPT), false positive rates of different NIPT tests may be relatively accurate, it is probable that reported false-negative rates are too low. The latter is as NIPT-cases with negative results for tested conditions are yet not in detail followed up for cases with other genetic or teratogenic disorders [5].

Non-Invasive Prenatal Testing Consent Form

It is mandatory to ensure that a patient has signed their consent to conduct genetic analyses, and a NIPT declaration form may include information such as the purpose of the test, the risks and benefits of the test, the limitations of the test, the accuracy of the test, the types of conditions that can be detected, the need for confirmatory testing, the potential implications of the test results, and the patient's rights and responsibilities. It is important to read and understand the contents of the NIPT declaration form before signing it and undergoing the test. Debates persist within the medical genetics community regarding the ethics surrounding parental consent and the disclosure of genetic conditions that offer limited therapeutic options [6].

Declaration of Consent Form Content for NIPT Testing

- By signing this declaration of consent, I acknowledge that:
- I have received, read, and understood the preceding written justification for non-invasive prenatal testing and the further explanation contained in the requisition form.

A. I have been provided thorough explanations by my physician regarding the Non-Invasive Prenatal Testing (NIPT), covering various aspects such as its genetic basis, purpose, scope, types, and significance of the intended test(s), the potential results obtainable, the importance of the analyzed genetic traits for my baby's health, and the options for disease prevention or treatment. Additionally, I have been briefed on the risks associated with sample generation required for NIPT and understanding the test results.

All my inquiries have been addressed, and I have been given adequate time for consideration.

B. I've been made aware of the limitations associated with NIPT, including:
C. NIPT's capability to analyze full chromosome aneuploidies of the fetus only after 10 weeks of gestation, focusing on chromosomes 21, 18, 13, and sex chromosomes (XO, XXX, XXY, and XYY) in both singleton and twin pregnancies.
D. NIPT being a statistical procedure for risk assessment rather than a diagnostic test.
E. The inability of NIPT to determine the fetal sex chromosome status in cases of maternal organ transplantation from a male donor.
F. The slight possibility of test findingsreflecting chromosomal changes in the placenta or the mother, instead of thebaby's chromosomes.
G. Inability to analyze triple or higher gestations.
H. The inability to determine the individual fetal gender during twin gestations if only one Y chromosome is detected.
I. Chromosome aneuploidies in twin gestations were found, but they were not able to be linked to individual twin fetuses.
J. When test results are unclear or uncertain, an invasive prenatal diagnosis is required for confirmation.
i. In certain countries—mainly China and India—fetal gender will not be included in the report or divulged, even upon request, due to regulatory constraints.

ii. Negative findings (stated as "No Aneuploidy Detected") do not rule out the possibility that the analyzed chromosomes had chromosomal abnormalities.
iii. A negative outcome does not rule out the chance of more chromosomal abnormalities (microdeletions, for example), genetic disorders, or birth problems throughout the pregnancy. Maternal and/or fetal variables, such as recent blood transfusions, cancer, weight, stem cell therapies, and others, may complicate test findings.
iv. I was informed that in case of a positive aneuploidy finding, invasive testing is recommended.

- I provide my consent for the non-invasive prenatal testing by signing this declaration, by First Genomix, for the subject stated above and which is described in more detail in the preceding written explanation of NIPT and in the requisition form [2], to the collection, processing and use by my physician and First Genomix of my personal data (that may also refer to my health) required to conduct that examination and totransfer my personal data between physician and First Genomix electronically across country borders [3], to the generation of the necessary sample as specified by my physician and above [4], to the storage and usage of the sample for as long as it is required to verify/check the results [5] to add to my record and use for the above purposes; if applicable, personal data on members of my family if these members have consented; and [6] to inform me or my physician or, if First Genomix has been instructed by a laboratory acting on behalf of my physician, this laboratory, about outcomes of the NIPT. I also release First Genomix and its employees from their secrecy obligation as a physician or healthcare professional vis-à-vis service and external data storage providers that administer and maintain systems, databases, and software of First Genomix or provide external data storage to First Genomix.

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