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# From Conception to Revelation: Non-Invasive Prenatal Testing Explored

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**Abstract.** Prenatal testing plays a crucial role in addressing the inherent unpredictability of genetic disorders that can affect unborn children. Non-Invasive Prenatal Testing (NIPT) has emerged as a groundbreaking tool in the realm of prenatal care, enabling the early detection of chromosomal abnormalities. By examining fetal DNA present in the mother's bloodstream, NIPT has achieved remarkable accuracy rates, surpassing invasive techniques that carry inherent risks. However, with these advancements come ethical considerations. The results of NIPT can significantly influence a mother's decision regarding the continuation or termination of pregnancy. This decision can be further complicated by religious beliefs, which often play a pivotal role in guiding choices related to pregnancy. Moreover, receiving a positive result from NIPT can subject expectant mothers to profound emotional stress, highlighting the need for comprehensive counseling and support services. In addition to these ethical concerns, practical limitations persist. The cost of NIPT remains a substantial barrier for many families, potentially limiting its accessibility. Furthermore, NIPT's accuracy can be influenced by factors such as gestational age, with tests often yielding inconclusive results when fetal fractions are low. Despite these challenges, technological innovations, including microarrays and Next Generation Sequencing (NGS), have bolstered the capabilities of NIPT. These advancements enable a more comprehensive assessment of fetal DNA, enhancing the overall quality of prenatal care. As NIPT continues to evolve and integrate with broader genomic technologies, society must engage in thoughtful reflection on its implications. The potential normalization of pregnancy terminations based on genetic information underscores the importance of ethical and societal discussions surrounding the use of NIPT, with a focus on preserving informed patient choices and ensuring comprehensive support for expectant parents facing difficult decisions.

**Keywords:** Non-Invasive prenatal sequencing; Next Generation Sequencing; massive parallel sequencing; genetic disorder; cell-free fetal DNA; Microarrays.

## 1. Introduction

### 1.1. Necessity of prenatal testing

A genetic disorder is a disease that results from abnormalities in an individual's DNA. It is caused by a change in the sequence of a part of a gene in DNA. This change in gene sequence is usually caused by a mutation in the gene, which means that there is no direct relation between the behavior of the parents before or during pregnancy. A fetus can develop a genetic disorder even if the mother maintains healthy habits and a balanced diet. One well-known example of a genetic disorder is Trisomy 21, more commonly known as Down syndrome. In this condition, an additional copy of chromosome 21 triggers developmental issues. Down syndrome is the most common genetic disorder in the United States, with an estimated 6,000 babies born with it every year. The ratio of fetuses with Down syndrome to healthy fetuses is approximately 1 to 700, and there are about 200,000 people in the United States diagnosed with Down syndrome [1]. It's important to note that this data does not encompass other genetic disorders besides Down syndrome. Since genetic disorders cannot be

predicted by the behavior of parents before or during pregnancy, and there is no way to completely cure genetic disorders, prenatal diagnosis becomes the only means to prevent such diseases. Non-Invasive Prenatal Testing (NIPT), which examines fragments of fetal DNA in a pregnant woman's blood, has become a breakthrough in identifying potential chromosomal abnormalities in a fetus.

### 1.2. Advances of NITP

Questions often arise about why the NIPT (Non-Invasive Prenatal Testing) should be applied across such a wide range when people are considering whether to take the NIPT test or not. Aneuploidy, with its strong drug resistance and adaptability, has the potential to disrupt the normal function of chromosomes, causing fatal effects [2]. Introduced into clinical practice in 2011, NIPT has become a routine part of antenatal care, including screening for fatal trisomy conditions [3]. NIPT is capable of detecting trisomy 21, 18, and 13, which are the main causes of fatal aneuploidy. The resulting sensitivity rates are impressive: 99.17% for trisomy 21, 98.24% for trisomy 18, and 100% for trisomy 13, while the specificity for NIPT stands at 99.95% for trisomy 21, 99.95% for trisomy 18, and 99.96% for trisomy 13, respectively. Moreover, NIPT has shown excellent performance in a large sample of 72,382 high-risk and 40,287 low-risk pregnancies. This performance is outstanding, especially considering that NIPT was previously considered suitable only for high-risk groups [4]. Furthermore, NIPT is capable of detecting various other disabilities, including Sex Chromosome Abnormalities (SCAs), monogenic diseases, and other rare trisomies or microdeletions [3]. As the Invasive Prenatal Test (IPT) involves the risk of miscarriage, many pregnant women are choosing NIPT over invasive diagnostic methods [5].

### 1.3. Ethical Consideration for NIPT

Undergoing an NIPT test serves as an example of making an informed choice. An informed choice refers to a voluntary decision made by the patient to undergo a diagnostic or therapeutic procedure. Genetic disorders, often incurable, can potentially lead to the termination of pregnancy upon a positive diagnosis. However, such decisions and their outcomes can raise ethical concerns. For instance, women with intermediate and high levels of education tend to make more informed choices, while those with strong religious beliefs may be less inclined to make such decisions. Religious convictions can significantly influence the willingness to undergo NIPT, with some expectant mothers declining the test for religious reasons [6]. Furthermore, receiving a positive diagnosis of a genetic disorder in an unborn fetus during pregnancy can impose substantial emotional distress on expectant mothers. Neither abortion nor long-term medical treatment offers a perfect solution to the challenges posed by genetic disorders. Additionally, the legality of abortion varies across different countries and states, and debates concerning the rights of an unborn fetus continue to pervade public discourse.

## 2. Major introduction of NIPT

### 2.1. Brief history of NIPT

The innovative use of NIPT in diagnosing genetic abnormalities can be traced back to the hypothesis proposed by Lo and his colleagues regarding the presence of cell-free fetal DNA (cffDNA) in maternal plasma. This hypothesis was inspired by the discovery of tumor DNA in the plasma of cancer patients. In 1997, research was conducted to confirm this hypothesis [7], thereby establishing NIPT as a viable technique for prenatal genetic analysis.

### 2.2. Methods of NIPT

During pregnancy, cell-free fetal DNA (cffDNA) originating from the placenta combines with the mother's free DNA, collectively referred to as cell-free DNA (cfDNA), in the maternal bloodstream. This presence in maternal plasma and serum provides an opportunity to identify genes from the unborn fetus through the isolation of fetal DNA from maternal plasma or serum for NIPT. Genetic

disorders can be detected from the cffDNA originating from the unborn fetus. In the context of NIPT, the ratio of cffDNA to the total cfDNA in maternal plasma is termed the fetal fraction (FF). There is a positive correlation between FF and gestational age; a higher FF enhances the accuracy of results [8]. A higher FF indicates an increased concentration of cffDNA in maternal plasma, potentially improving the accuracy of NIPT. In conclusion, the process of using NIPT technology for sequencing and analysis first involves collecting an appropriate amount of blood sample from the maternal body. After a series of further processing steps on the maternal blood sample, it then undergoes a series of centrifugation processes to ultimately extract the sample containing cf-DNA from the maternal plasma [9].

### 2.3. Types of labels and detections

Sequencing and detection in later stages can be achieved using various sequencing technologies, such as massive parallel sequencing (MPS), also known as Next-Generation Sequencing (NGS). MPS technology is classified into several sequencing tools [10] and is one of the most cutting-edge methods for profiling genes in greater detail [11]. MPS simplifies the management and analysis of human genes by converting the dataset into a digital format [11].

Abnormal DNA can be monitored by detecting single-base variations (SNP), which are common types of variations in DNA that can lead to changes in phenotypical features [10], or copy number variations (CNV), which are structural variations involving alterations in the number of repetitions in specific gene regions, representing essential mutation forms in the human genome [12]. The MPS can classify thousands of SNPs based on their genotypes in a single experiment, even when dealing with numerous different samples [13]. These two types of markers play a significant role in human genome sequencing for understanding diseases and inheritance, often serving as the primary factors for determining the presence of abnormal data.

As the analysis heavily relies on CNV and SNP detection through NGS, NIPT techniques become more accessible for predicting diseases associated with changes in chromosome numbers. To make the sequencing more apparent, the polymerase chain reaction (PCR) is employed to amplify the DNA [14]. The resulting products are continuously monitored using quantitative real-time PCR, allowing for the quantification of the initially extracted sample to a specific level of amplification [15].

### 2.4. Applied scenarios

Diseases such as trisomy 21, 13, and 18 [16], as well as other diseases resulting from variations in the number of sex chromosomes [17], can be identified through the NIPT technique. This identification is possible because differences in the number of chromosomes in the extracted sample, particularly abnormalities in the initial sample amplified by PCR, result in a significant variation in the quantity of the final amplification products compared to the normal or standard levels. Diseases sharing similar characteristics of chromosomal number changes can be detected and predicted using the NIPT technique [18].

## 3. Methodology advances

### 3.1. Microarrays

Microarray technology, a groundbreaking technique in genomics, has found significant applications in the field of NIPT. Originating from traditional solid-phase assays like DNA/RNA dot blot assays and Enzyme-Linked Immunosorbent Assays (ELISA) [19], microarrays have evolved to offer a high-throughput and miniaturized version of these traditional assays. The essence of microarray technology lies in its ability to simultaneously probe a sample for hundreds to millions of distinct molecules, making it particularly efficient for genetic analysis in NIPT [19].

In the context of NIPT, microarrays utilize 'capture molecules' or 'probes' affixed to a solid support [19]. These probes are designed to detect the presence of specific target molecules in a sample, such

as fetal DNA fragments present in maternal plasma. The specificity and affinity of these probes are crucial, as they determine the accuracy of the test. Given the versatility of the probes, which can be derived from sources like PCR products, oligonucleotides, or even plasmids, microarrays allow for comprehensive analysis of fetal genomes [19].

A defining feature of microarray technology in NIPT is its capacity for parallel analysis. By immobilizing diverse probes onto a solid support in arrays of spots, with each spot containing multiple copies of a specific capture molecule, microarrays can detect multiple genetic markers simultaneously [19]. This parallel analysis is particularly beneficial in NIPT, where the objective is to detect potential genetic abnormalities in the fetus. The hybridization process, where the sample interacts with the probes, is optimized to ensure maximum sensitivity, especially given that cfDNA from maternal plasma typically contains only about 11% fetal DNA [20].

One of the prominent applications of microarrays in NIPT is array CGH (Comparative Genomic Hybridization) [20]. While array CGH is commonly employed to analyze amniotic samples and preimplantation embryos, its use in NIPT presents challenges due to the dilution of fetal DNA in maternal plasma [20]. However, by integrating targeted DNA analysis with the expansive analysis space provided by arrays, a robust microarray-based NIPT can be developed, offering a comprehensive genetic screening of the fetus in a non-invasive method [19].

### 3.2. NGS Advances

NGS has ushered in transformative changes in the realm of genomics, and its application in NIPT serves as a testament to its potential. Among the various NGS technologies, Ion Torrent stands out due to its unique sequencing approach tailored for NIPT [21].

Ion Torrent technology, a subset of NGS, operates on a distinctive principle. Unlike traditional sequencing methods that rely on fluorescent signals, Ion Torrent detects the release of a hydrogen ion (H+) during the nucleotide extension process [21]. This pH change is captured within the sensor wells of the Ion Torrent chip, facilitating the identification of the incorporated nucleotide, making it particularly suitable for NIPT [21]. The design of the Ion Torrent chip, with its flow compartment and solid-state pH sensor micro-arrayed wells, is optimized to detect subtle pH changes resulting from the release of H+ ions.

For NIPT, the high-throughput capability of NGS offers a more in-depth and comprehensive analysis of fetal DNA fragments present in maternal blood. Ion Torrent technology, with its rapid sequencing time and unique pH-based detection method, ensures accurate results without the need for fluorescent imaging. This not only expedites the NIPT process but also minimizes potential errors commonly associated with imaging-based methods, making it the preferred choice for many clinicians and researchers in the field of prenatal testing [21].

## 4. More to debate

Despite the advances of NIPT, it is worth noting some limitations of this technique. The cost of NIPT is relatively high, ranging from $800 to $2000 in the USA and about $500 to $1500 in other places [22]. Additionally, the principle of NIPT makes it impossible to provide an accurate diagnosis of fetal disorders. Therefore, a definitive diagnosis requires an invasive test. There are also systemic issues that need to be considered during NIPT. The accuracy of NIPT is inherently linked to gestational age. The fetal fraction (FF), which represents the concentration of cff-DNA in the total circulating cf-DNA, significantly affects the test's precision. Commercial NIPT considers a test with FF below 4% as failed [8]. Various ethical issues cannot be neglected as well.

The most prevalent concern relates to the impact of NIPT on a mother's autonomy in deciding whether to terminate the pregnancy. Some believe that the ease of offering NIPT and the lack of appropriate counseling may lead to its routine use, resulting in overuse of this test. This trend is referred to as "normalization," and such a phenomenon may shift people's views on abortion, reducing the incidence

of disorders significantly. The potential consequence of this could be the mistreatment of patients with genetic disorders [5]. Others believe that as NIPT becomes more linked with other genomics technologies, informing patients about NIPT may become more challenging, potentially leading to unconscious acceptance of the test. Patients may not have intended for NIPT to influence their decision about abortion, but approaches that detect other types of abnormalities could alter their autonomy in deciding to terminate the pregnancy [8].

## 5. Conclusion

Non-Invasive Prenatal Testing (NIPT) stands as a monumental advancement in the realm of prenatal genetic screening, offering a safe and efficient means of detecting potential chromosomal abnormalities in fetuses. Its development has been driven by the recognized imperative for prenatal testing, considering the unpredictable nature of genetic disorders, which can manifest irrespective of parental behaviors prior to or during pregnancy. Rooted in technological breakthroughs like microarrays and Next Generation Sequencing (NGS), notably the Ion Torrent technology, NIPT has expanded our understanding of fetal DNA, thereby enhancing the quality of prenatal care.

The significance of NIPT lies in its remarkable sensitivity and specificity, outperforming traditional methods, which often carry potential risks. This revolutionary approach has enabled the early identification of major trisomy diseases, empowering expectant parents with critical information to make informed decisions about their pregnancy.

Nonetheless, NIPT is not without its challenges. The cost of NIPT remains a substantial barrier for many families, limiting its accessibility and potentially exacerbating healthcare disparities. Additionally, NIPT's inherent design necessitates the need for confirmatory invasive tests to establish a definitive diagnosis of fetal disorders, adding to the emotional and financial burdens faced by expectant parents.

Furthermore, the accuracy of NIPT is intricately linked to gestational age. Tests are sometimes deemed inconclusive when the fetal fraction (FF) in maternal blood is low. This presents a limitation in the application of NIPT, particularly in the early stages of pregnancy.

On the ethical front, the emergence and increasing accessibility of NIPT have raised critical concerns. One prominent issue revolves around the potential normalization of the test, which could inadvertently influence societal perspectives on genetic disorders and decisions related to pregnancy termination. Striking a balance between offering a valuable diagnostic tool and preserving the autonomy of expectant parents is paramount.

As NIPT continues to interface with broader genomic technologies, maintaining the clarity and autonomy of informed patient choices will be pivotal. Ensuring that comprehensive counseling and support services are readily available to expectant parents facing challenging decisions is essential to navigating the complexities that accompany this powerful advancement in prenatal care. Addressing the barriers, ethical concerns, and limitations associated with NIPT is crucial to harnessing its full potential for the benefit of all expectant families.

## Authors Contribution

All the authors contributed equally, and their names were listed in alphabetical order.

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