PRENATALGENOME

Preclinical validation data and performance parameters

Summary

Building on its legacy of genetic innovation, GENOMICA proudly introduces **PrenatalGenome**, the first non-invasive prenatal test (NIPT) that simultaneously screens for **chromosomal abnormalities**, **inherited** and ***de novo* single-gene disorders** (SGD).

While current NIPT methods screen for common and rare aneuploidies, segmental chromosomal imbalances, and syndromes associated with microdeletions or microduplications, **PrenatalGenome** takes a significant step further. It also analyzes circulating cell-free fetal DNA (cfDNA) in maternal blood for pathogenic and likely pathogenic mutations linked to single-gene disorders through **deep exome sequencing**, offering karyotype-level insights and more comprehensive risk assessment. **PrenatalGenome** represents a paradigm shift in prenatal screening, providing a fuller picture of the genetic risks that may affect pregnancy outcomes.

This white paper presents the **analytical performance** of **PrenatalGenome** test, based on a retrospective analysis of 250 frozen plasma samples from pregnant women who underwent traditional NIPT or non-invasive prenatal screening for de novo and/or inherited gene mutations. These results were verified through invasive prenatal diagnostic procedures, including amniocentesis and chorionic villus sampling.

The study, integral to the development of the **PrenatalGenome** laboratory process, demonstrates the test's ability to detect fetal aneuploidy, structural chromosomal abnormalities, and single-gene disorders from cfDNA in maternal blood with remarkable accuracy. Analytical **sensitivity** and **specificity exceeded 99%**, significantly reducing false positive and false negative results compared to traditional NIPT for fetal chromosomal abnormalities and NIPT for single-gene disorders (NIPT-SGD).

In conclusion, the **PrenatalGenome** test reliably identifies fetal chromosomal anomalies, microdeletion/microduplication syndromes, and genetic variants, including de novo mutations—primary contributors to severe pediatric developmental disorders. This innovative method has the potential to redefine non-invasive prenatal testing, enabling the detection of genetic conditions that current technologies cannot identify.

Introduction

Fetal genetic diagnosis plays a critical role in prenatal care, and recent advancements in prenatal exome sequencing have demonstrated significant diagnostic improvements.[1-2] However, due to the invasive nature of fetal sampling, its application remains limited to cases involving identifiable structural anomalies. This limitation leaves many monogenic disorders undetected, as these conditions often do not manifest during the prenatal period. Consequently, a substantial number of neonates are born with severe or fatal genetic conditions.[3] Notably, approximately 60% of severe postnatal monogenic diseases are dominant disorders, with the majority caused by *de novo* mutations.[4]

The development of noninvasive prenatal testing (NIPT) using cell-free fetal DNA (cfDNA) extracted from maternal blood has revolutionized prenatal screening,[5] by enabling detection of fetal aneuploidy and structural chromosomal abnormalities.[6,16]

However, its application has mainly been limited to chromosomal disorders owing to the low resolution available with the existing screens, generally insufficient for identifying mutations causing single-gene disorders (SGDs). Given that SGDs contribute significantly to birth defects, affecting around 1% of births,[7-8] it is fundamental to enhance current prenatal screening methods to include these conditions.

There exists a need for the development of a comprehensive next-generation NIPT assay capable of simultaneously detecting chromosomal abnormalities and single-gene disorder, from circulating cfDNA in maternal blood.

Existing NIPT methods for SGDs (NIPT-SGDs) primarily focus on detection of *de novo* or paternally inherited mutations associated with common dominant monogenic disorders, which occur in approximately 1 in 600 pregnancies.[9] However, these approaches are restricted to specific gene regions, limiting their ability

to detect the wide range of sporadic mutations present in cfDNA.

Recently, to address these challenges, a proof-of-concept approach that leverage deep exome sequencing, enabling the non-invasive detection of fetal *de novo* variants with high accuracy has been recently proposed[10-11].

We developed a high-resolution, non-invasive prenatal screening approach utilizing **ultra-deep exome sequencing**. This technique examines the **whole fetal exome**—the portion of the genome encoding proteins—via cfDNA from maternal blood samples. Since exonic variants account for ~85% of disease-causing mutations in Mendelian disorders, this method provides a comprehensive tool for fetal genetic screening. Additionally, invasive prenatal diagnostics currently recommend exome sequencing for pregnancies involving fetal structural anomalies due to the significant diagnostic yield of approximately 30%.[12]

Our non-invasive fetal exome screening (niFES) test employs **ultra-deep sequencing** to deliver high-resolution results with increased detection rate and reduced false positive rates. By focusing on clinically relevant genes, it minimizes the identification of copy number variations (CNVs) with uncertain significance. This comprehensive approach enables **simultaneous** detection of chromosomal abnormalities, including aneuploidies, segmental imbalances, and microdeletions/duplications, as well as fetal ***de novo* variants**—the leading cause of severe early-onset genetic disorders, such as intellectual disability and developmental disorders.[13]

This represents a paradigm shift in prenatal screening. Our technology identifies novel mutations that standard carrier screenings often overlook, as these mutations are not inherited from either parent. Additionally, our deep exome sequencing approach can detect disorders typically unassociated with abnormal ultrasound findings during the first trimester, which may only become apparent in later stages of pregnancy or after birth. This allows for earlier, more precise risk assessment and intervention, ultimately improving outcomes for affected pregnancies.

MATERIALS AND METHODS

Study design

This study retrospectively analyzed 250 frozen plasma samples collected from pregnant women undergoing traditional NIPT and/or non-invasive prenatal screening for *de novo* and inherited single gene disorders (NIPT-SGD). The test results were confirmed using invasive prenatal diagnostic methods, such as amniocentesis or chorionic villus sampling. Samples were collected between 10 and 22 weeks of gestation.

Traditional NIPT was performed using the VeriSeq NIPT Solution v2 kit and VeriSeq NIPT Assay Software v2 (Illumina, San Diego, CA, U.S.), following the manufacturer's instructions. This method provided an average sequencing depth of 9.6 million reads per sample. The NIPT-SGD approach targeted 61 genes associated with frequent monogenic disorders linked to severe health outcomes.

The primary objective of the study was to assess the performance of the non-invasive prenatal screening test based on deep fetal exome sequencing. The results obtained using this novel method were compared with traditional NIPT, targeted NIPT-SGD, and invasive diagnostic tests. The study cohort also supported the laboratory's development process for the **PrenatalGenome** test.

Cell-free DNA extraction and sequencing library preparation

Plasma was isolated from maternal blood collected in Streck tubes and processed promptly. Blood samples underwent initial centrifugation at 1600 × g for 10 minutes at 4°C to separate plasma from peripheral blood cells. The plasma fraction was transferred to polypropylene tubes and centrifuged again at 16,000 × g for 10 minutes at 4°C to remove residual cells. The isolated plasma was stored at -80°C until further analysis.

Cell-free fetal DNA (cfDNA) was extracted from 1 mL of maternal plasma using the QIAamp DSP Circulating NA Kit (Qiagen) following the manufacturer's instructions.

Sequencing libraries were processed on the NextSeq 550 DX platform (Illumina, San Diego, CA, U.S.) with ultra-deep exome sequencing achieving a coverage of >500X per sample. Advanced technological approaches, including unique molecular indexing (UMI), were applied to minimize background noise and ensure accurate detection of low-level fetal DNA variants.

Bioinformatic data analysis

A customized bioinformatics pipeline was developed for fetal copy number analysis, accurate variant calling and filtering, and error correction using unique molecular identifiers. The pipeline incorporated site-specific noise modeling and fetal fraction estimation, ensuring reliable identification of autosomal and sex chromosome aneuploidies, sub-chromosomal CNVs, and single-gene disorders.

Estimation of Fetal DNA Fraction

The fetal fraction (i.e., the proportion of cell-free DNA in a maternal blood sample that is of fetal origin) was calculated to confirm the presence of fetal DNA in maternal plasma. Single nucleotide polymorphisms (SNPs) inherited from the father were analyzed within the cfDNA to estimate fetal fraction and validate DNA detection accuracy.

The calculation was based on the following formula:

$$\text{Fetal DNA fraction} = D\not{f}_{etus}/(D_{mother}+D\not{f}_{etus})$$

where D_{mother} represents alleles shared between the mother and fetus, and $D\not{f}_{etus}$ represents fetal-specific alleles.[14]

Performance Assessment for Detection of Fetal-Specific Variants

To evaluate the test's ability to identify fetal-specific variants, a single nucleotide variant (SNV) was classified as fetal-specific if present in maternal plasma DNA or fetal genomic DNA (gDNA) but absent in maternal gDNA. *De novo* mutations, a specific type of fetal-specific variant, were defined as genetic changes not detected in either parental samples.

Fetal-specific variants were identified using SNV sites that were heterozygous in the fetus (e.g., CT) but homozygous in the mother (e.g., TT), where the C allele represented the fetal-specific component. The performance of the test for detecting *de novo* mutations was evaluated based on its accuracy in identifying these fetal-specific variants.

The test results obtained from cfDNA samples were compared to paired fetal gDNA samples[15], considered the gold standard for validation. The following metrics were used to evaluate performance:

$$\text{Sensitivity} = \frac{TP}{TP+FN}$$

$$\text{Specificity} = \frac{TN}{TN+FP}$$

$$\text{Positive Predictive Value (PPV)} = \frac{sensitivity \times prevalence}{sensitivity \times prevalence + (1-specificity) \times (1-prevalence)}$$

$$\text{Negative Predictive Value (NPV)} = \frac{specificity \times (1-prevalence)}{(1-sensitivity) \times prevalence + specificity \times (1-prevalence)}$$

Where:

- **TP (True Positives):** Variants identified in both plasma DNA and fetal gDNA.
- **FN (False Negatives):** Variants detected in fetal gDNA but absent in plasma DNA.
- **FP (False Positives):** Variants detected in plasma DNA but absent in fetal gDNA.
- **TN (True Negatives):** Variants correctly absent in both plasma and fetal gDNA.

The **Positive Predictive Value (PPV)** reflects the likelihood that an identified variant is genuinely present. The **Negative Predictive Value (NPV)** reflects the likelihood that a variant is genuinely not present when the test is negative.

RESULTS

Detection of Fetal Aneuploidy and Structural Chromosomal Abnormalities

A total of 250 frozen plasma samples were retrospectively analyzed to assess the test's effectiveness in detecting chromosomal abnormalities, including aneuploidies, segmental chromosomal imbalances, and microdeletion/microduplication syndromes. Gestational ages ranged from 10 to 22 weeks, with fetal fractions between 4% and 21%.

Compared to traditional NIPT, the niFES test demonstrated **superior sensitivity and specificity**, with significant reductions in false positives and false negatives (Figure 1). **Overall sensitivity** reached **100%** (vs. 98.4%, $p < 0.001$), and specificity improved to **91.2%** (vs. 68.0%, $p < 0.001$). Table 1 summarizes the performance parameters of the niFES test compared to traditional NIPT (see also Supplementary Table 1 and 2).

Detection of *De Novo* Mutations

The niFES test also showed **high sensitivity and specificity** for detecting paternally inherited and *de novo* single nucleotide variants (SNVs), the largest category of clinically significant mutations after aneuploidies.

Table 1: Overall performance parameters comparison between traditional NIPT and niFES

Performance parameters	Traditional NIPTs*	95% CI	niFES	95% CI
True Negatives	85		114	
False Negatives	2		0	
True Positives	123		125	
False Positives	40		11	
Sensitivity	98.4%	94.34% - 99.81%	100%	97.09% - 100%
Specificity	68.0%	59.07% - 76.06%	91.2%	84.80% - 95.52%
Positive predictive value	75.5%	70.41% - 79.90%	91.9%	86.60% - 95.23%
Negative predictive value	97.7%	91.45% - 99.41%	100%	96.82% - 100%

***Low resolution NIPT (~9.6M reads)**

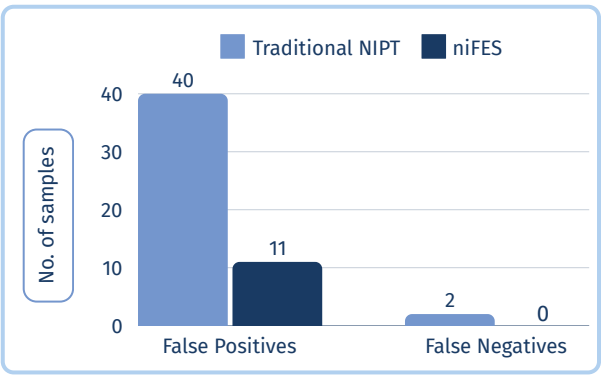

Figure 1: Overall false positive and false negative comparison between traditional NIPT and niFES

Among the 250 samples analyzed, the test correctly identified all disease-causing SNVs, achieving **100% sensitivity** (95% CI: 97.1–100%) and **specificity** (95% CI: 97.1–100%).

Notably, the niFES method resulted in zero false negatives and significantly fewer false positives compared to NIPT-SGD. Table 2 summarizes the performance parameters of the niFES test compared to targeted NIPT-SGD.

Table 2: performance parameters comparison between targeted NIPT-SGD and niFES

Performance parameters	Targeted NIPT-SGD	95% CI	niFES	95% CI
True Negatives	122		125	
False Negatives	0		0	
True Positives	125		125	
False Positives	3		0	
Sensitivity	100%	97.09% - 100%	100%	97.09% - 100%
Specificity	96.0%	93.15% - 99.50%	100%	97.09% - 100%
Positive predictive value	96.2%	93.15% - 99.50%	100%	97.09% - 100%
Negative predictive value	100%	97.02% - 100%	100%	97.09% - 100%

CONCLUSION

We have developed a groundbreaking platform for the simultaneous non-invasive prenatal detection of chromosomal abnormalities and monogenic diseases with analytical sensitivity and specificity exceeding 99.9%. The innovative niFES test, based on deep exome sequencing, offers a fast, comprehensive, and accurate method to identify a wide range of chromosomal and genetic disorders without imposing risks on the fetus or mother.

Our results demonstrate that niFES can reliably detect fetal chromosomal abnormalities, including rare aneuploidies, structural aberrations, and single nucleotide variants (SNVs). The platform also effectively identifies *de novo* and inherited variants. Additionally, it enables robust carrier screening for both parents to assess the risk of recessive genetic conditions.

By integrating niFES into routine prenatal care alongside fetal ultrasonography would significantly improve early detection rates of genetic disorders, reduce the number of invasive diagnostic procedures, and enable timely interventions.

This paradigm shift in prenatal screening offers a clearer and more complete picture of the genetic risks affecting pregnancies.

The findings from this study underscore the potential of niFES to advance prenatal care and support the broader adoption of deep exome sequencing for non-invasive screening. Future studies will further explore its capabilities for detecting pathogenic variants in different fetal conditions.

In conclusion, niFES represents a revolutionary step forward in prenatal genetic screening, offering unparalleled resolution and accuracy. By combining deep exome sequencing with a proprietary bioinformatics process and stringent variant interpretation strategies, this method bridges significant gaps in current NIPT technology and sets a new standard for non-invasive prenatal testing.

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Supplementary Table 1: performance parameters of traditional NIPT for detection of chromosomal abnormalities

Performance parameters	Trisomy 21	95% CI	Trisomy 18	95% CI	Trisomy 13	95% CI	Sex Chromosomes	95% CI	Rare aneuploidy	95% CI	Segmental aneuploidy	95% CI	Overall	95% CI
True Negatives	197		236		242		225		213		222		85	
False Negatives	0		0		1		0		0		1		2	
True Positives	49		11		4		20		27		12		123	
False Positives	4		3		3		5		10		15		40	
Sensitivity	100,0%	92.75% - 100.00%	100,0%	71.51% - 100.00%	80,0%	28.36% - 99.49%	100,0%	83.16% - 100.00%	100,0%	87.23% - 100.00%	92,3%	63.97% - 99.81%	98,4%	94.34% - 99.81%
Specificity	98,0%	94.98% - 99.46%	98,7%	96.38% - 99.74%	98,8%	96.46% - 99.75%	97,8%	95.30% - 99.33%	95,5%	91.91% - 97.83%	93,7%	89.78% - 96.41%	68,0%	59.07% - 76.06%
Positive predictive value	92,5%	82.28% - 97.00%	78,6%	54.36% - 91.86%	57,1%	28.51% - 81.68%	80,0%	62.69% - 90.50%	73,0%	59.57% - 83.19%	44,4%	32.36% - 57.23%	75,5%	70.41% - 79.90%
Negative predictive value	100,0%	98.14% - 100.00%	100,0%	98.45% - 100.00%	99,6%	97.67% t-o 99.93%	100,0%	98.47% - 100.00%	100,0%	98.28% - 100.00%	99,6%	97.12% - 99.93%	97,7%	91.45% - 99.41%

Supplementary Table 2: performance parameters of niFES for detection of chromosomal abnormalities

Performance parameters	Trisomy 21	95% CI	Trisomy 18	95% CI	Trisomy 13	95% CI	Sex Chromosomes	95% CI	Rare aneuploidy	95% CI	Segmental aneuploidy	95% CI	Overall	95% CI
True Negatives	199		238		245		228		220		234		114	
False Negatives	0		0		0		0		0		0		0	
True Positives	51		11		5		20		25		13		125	
False Positives	0		1		0		2		5		3		11	
Sensitivity	100,0%	93.02% - 100.00%	100,0%	71.51% - 100.00%	100,0%	47.82% - 100.00%	100,0%	83.16% - 100.00%	100,0%	86.28% - 100.00%	100,0%	75.29% - 100.00%	100,0%	97.09% - 100.00%
Specificity	100,0%	98.16% - 100.00%	99,6%	97.69% - 99.99%	100,0%	98.51% - 100.00%	99,1%	96.89% - 99.89%	97,8%	94.89% - 99.27%	98,7%	96.35% - 99.74%	91,2%	84.80% - 95.52%
Positive predictive value	100,0%	93.02% - 100.00%	91,7%	60.87% - 98.73%	100,0%	47.82% - 100.00%	90,9%	71.56% - 97.55%	83,3%	67.76% - 92.25%	81,3%	58.47% - 93.03%	91,9%	86.60% - 95.23%
Negative predictive value	100,0%	98.16% - 100.00%	100,0%	98.46% - 100.00%	100,0%	98.51% - 100.00%	100,0%	98.40% - 100.00%	100,0%	98.34% - 100.00%	100,0%	98.44% - 100.00%	100,0%	96.82% - 100.00%