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BMI-Stratified Mixed-Effects Modeling of Fetal DNA Fraction for NIPT Timing Optimization and Ensemble-Based Female Fetal

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Abstract: Non-invasive prenatal testing (NIPT) has gained widespread clinical adoption as an efficient and precise method for screening fetal chromosomal abnormalities. However, there remains room for optimization in balancing the timing of testing with diagnostic accuracy. This study employs multilevel modeling of clinical data from 267 pregnant women to propose a personalized testing timing optimization strategy based on multiple factors, including fetal cell-free DNA concentration and maternal body mass index (BMI). Using a mixed-effects model, we analyzed the relationship between BMI, gestational age, GC content, and fetal Y chromosome concentration, thereby establishing a predictive framework for optimal testing timing across different BMI groups. Experimental results indicate optimal testing timepoints of 10.0 weeks for low-BMI groups, 13.0 weeks for moderate-BMI groups, and 15.8 weeks for high-BMI groups. Compared to conventional methods, the model enables testing 3 to 6 weeks earlier. For determining chromosomal abnormalities in female fetuses, integrating multivariate Gaussian discriminant analysis with the RUSBoost ensemble learning algorithm successfully enhanced detection sensitivity and accuracy. Particularly when combining Z-scores with GC content, the approach effectively distinguished between normal and abnormal fetuses. Comprehensive analysis indicates this study provides scientific evidence for personalized optimization of NIPT testing timing and accurate diagnosis of chromosomal abnormalities in female fetuses.

Keywords: Non-invasive prenatal testing, Mixed-effects model, BMI grouping, Optimal testing timing, RUSBoost, Multivariate Gaussian discriminant analysis.

1. Introduction

Non-invasive prenatal testing (NIPT) [1, 2] has revolutionized prenatal screening by providing a highly accurate, safe, and early method for detecting fetal chromosomal abnormalities, particularly trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), and trisomy 13 (Patau syndrome). By analyzing cell-free fetal DNA (cffDNA) present in maternal blood, NIPT offers a non-invasive alternative to traditional invasive procedures like amniocentesis and chorionic villus sampling, which carry a small risk of miscarriage. The ability to detect these abnormalities early in pregnancy, with a high degree of accuracy, has made NIPT a cornerstone of modern prenatal care.

However, despite its success, there remain several challenges in optimizing the application of NIPT. One of the most significant factors affecting the accuracy of NIPT is the concentration of fetal DNA in maternal blood, also referred to as fetal fraction (FF). FF is influenced by several maternal and fetal factors, such as gestational age, maternal body mass index (BMI), and other clinical variables. The timing of the test is crucial, as performing the test too early in pregnancy may lead to insufficient fetal DNA concentration, while waiting too long could reduce the available time for effective intervention if an abnormality is detected.

Previous studies have established that both fetal DNA concentration and the detection thresholds for NIPT can vary significantly across different maternal characteristics. Maternal BMI, for example, has been shown to influence the fetal fraction, with higher BMI generally associated with lower fetal DNA concentrations, which can complicate the detection process. As a result, the need for personalized approaches to NIPT has emerged, where factors like BMI and gestational age are considered in determining the optimal timing for testing.

As a core quality control parameter for NIPT, FF concentration is significantly influenced by maternal BMI, gestational age, and other variables. Shree et al. [3] found that FF levels were significantly lower in obese pregnant women (BMI > 30) compared to the normal-weight group (9.2% vs. 12.5%, $p < 0.001$), with an indeterminate rate as high as 8.4% (vs. 1.7%). Hopkins et al. [4] noted that FF increases with gestational age, but the rate of increase is slower in obese individuals, necessitating repeat sampling to improve detection rates. Muzzey et al. [5] proposed that a "customized NIPS protocol" can maintain high sensitivity in low-FF scenarios.

Some studies indicate that data-driven risk stratification significantly outperforms traditional binary obesity classification. Lee et al. [6] developed an AI-driven NIPT algorithm (aiD-NIPT), which improved PPV to 88.4% in low-FF samples but did not resolve timing selection issues. Current ACMG guidelines recommend traditional screening for high-BMI individuals [7].

Due to the absence of Y-chromosome signals, detection of trisomy syndromes (T13/T18/T21) in female fetuses has long relied on indirect markers. Traditional Z-score methods exhibit only 75%–82% sensitivity for T13/T18 detection [8].

This study aims to address the critical issue of optimizing the detection timing for NIPT by using a data-driven approach based on hierarchical mixed-effects modeling. By analyzing data from 267 pregnant women, we examine the relationships between maternal BMI, gestational age, and fetal DNA

concentration to develop an individualized framework for determining the ideal time to perform NIPT. Additionally, we propose a novel method for improving the accuracy of fetal chromosomal abnormality detection in female fetuses, who lack a Y chromosome, using a combination of multivariate Gaussian discriminant analysis and ensemble learning algorithms, such as RUSBoost.

Through this research, we aim to provide a more robust and individualized approach to NIPT, ultimately improving the precision of prenatal screening and expanding its clinical applicability. By optimizing the testing window based on maternal characteristics and improving detection methods for female fetuses, our study aims to contribute valuable insights into the personalized application of NIPT, ensuring better outcomes for both mothers and their unborn children.

2. Methods

In this study, the primary objective was to optimize the detection timing and improve the accuracy of non-invasive prenatal testing (NIPT) for fetal chromosomal abnormalities, with a focus on fetal DNA concentrations, maternal BMI, and other clinical factors. The problem was approached using hierarchical modeling techniques and mixed-effects models to account for the nested structure of the data. We detail the methods employed for addressing the four research questions and provide the corresponding mathematical formulations.

2.1. Data Preprocessing and Structure

The dataset consists of 267 pregnant women, each with multiple blood draws and associated sequencing observations. The data was structured into a three-level hierarchy: Level 1—measurement observations, Level 2—blood draw events, and Level 3—pregnant women. This structure accounts for intra-individual correlation, ensuring that measurements from the same individual are not treated as independent. A total of 1068 sequencing observations from 1009 blood draw events were used for analysis. The Intraclass Correlation Coefficient (ICC) was calculated as 0.7089, indicating a significant within-subject correlation and justifying the use of mixed-effects models to analyze the data.

2.2. Mixed-Effects Modeling

To model the relationship between fetal DNA concentrations and maternal factors such as BMI, gestational age, and GC content, we employed a hierarchical mixed-effects model. This model accommodates the nested nature of the data, where multiple observations from the same individual may be correlated. The general form of the model is:

$$Y_{ijk} = \beta_0 + \beta_1 \mathrm{GA}_{ijk} + \beta_2 \mathrm{BMI}_{ijk} + \beta_3 \mathrm{Age}_{ijk} + \beta_4 \mathrm{GC}_{ijk} + u_i + e_{ijk}$$

Here, Y_{ijk} denotes the fetal Y-chromosome concentration for the k-th measurement of the j-th blood draw from the i-th pregnant woman. The model includes both fixed effects $(\beta_0, \beta_1, \beta_2, \beta_3, \beta_4)$ and random effects for individual women (u_i) to account for individual variability. The residual error term, e_{ijk}, represents random measurement error.

The fixed effects parameters were estimated using the maximum likelihood method, and the random effects were modeled using the Gaussian distribution. Significance of the fixed effects was assessed through p-values obtained from likelihood ratio tests. Pearson and Spearman correlation coefficients were calculated to investigate the relationships between fetal Y-chromosome concentrations and maternal BMI, gestational age, age, and GC content.

2.3. Optimal Timing for NIPT Detection

One of the main objectives was to determine the optimal timing for NIPT detection based on maternal BMI groups. We divided BMI values into three categories: low BMI (20.7-30.3 kg/m²), medium BMI (30.3-32.7 kg/m²), and high BMI (32.7-46.9 kg/m²). For each BMI group, we calculated the probability of achieving a fetal DNA concentration above the detection threshold (4%) at different gestational weeks. The detection success probability, P_{success}, was modeled using the cumulative distribution function (CDF) of a normal distribution:

$$P_{\text{success}}(t) = \Phi\left(\frac{Y(t) - 4\%}{\sigma_Y(t)}\right)$$

Where $Y(t)$ is the predicted fetal DNA concentration at time t, and $\sigma_Y(t)$ represents the standard deviation of the concentration at time t. A conservative threshold of 6.8% and an 80% success probability were used to determine the recommended detection times for each BMI group. A Monte Carlo sensitivity analysis was performed to simulate measurement errors and assess the robustness of the detection timing recommendations.

2.4. Multivariate Predictive Modeling

For more refined prediction, we extended the model to incorporate additional maternal characteristics such as age, weight, and height. This extension used a linear mixed-effects model (LME) to account for non-linear effects of gestational age. The updated model included polynomial terms for gestational age to capture its non-linear effect:

$$Y_{ijk} = \beta_0 + \beta_1 \mathrm{GA}_{ijk} + \beta_2 \mathrm{GA}^2_{ijk} + \beta_3 \mathrm{BMI}_{ijk} + \beta_4 \mathrm{Age}_{ijk} + u_i + e_{ijk}$$

K-means clustering was used to group women based on BMI and age, minimizing within-group variance and maximizing between-group differences. This approach allowed for the identification of risk groups that could guide the optimal timing for NIPT detection. For each risk group, we calculated the best detection time using two methods: probability of success and risk minimization, where the latter balances the risk of false negatives and delayed detection.

2.5. Abnormal Fetal Chromosome Detection for Female Fetuses

For female fetuses, the detection of chromosomal abnormalities (such as trisomy 13, 18, and 21) is more complex as they lack a Y chromosome. To address this, we employed a multivariate Gaussian discriminant analysis combined with the RUSBoost ensemble learning algorithm to classify abnormal chromosomal patterns. The feature set included Z-scores of chromosomes 13, 18, and 21, GC content, sequencing depth, and BMI. The classifier was trained on 537 normal and 67 abnormal samples, with the decision boundaries defined using Mahalanobis distance:

$$D_{\text{M}} = \sqrt{(X - \mu)^T \Sigma^{-1} (X - \mu)}$$

Where X is the feature vector for a sample, μ is the mean vector of normal samples, and Σ is the covariance matrix of the features. This approach allowed for the detection of

trisomy with high sensitivity, while minimizing false positive rates. The model was further validated using a three-tier clinical decision rule (negative result, recheck, and confirmation), based on the posterior probability of abnormality.

3. Experiments and Results

In this study, we employed multiple statistical modeling approaches based on real clinical data to conduct an in-depth analysis of timing selection for non-invasive prenatal testing (NIPT) and the detection of chromosomal abnormalities. The primary objectives were to optimize the timing for detecting fetal Y chromosome concentration and to enhance the accuracy of detecting chromosomal abnormalities in female fetuses. All analyses were conducted using mixed-effects models, multivariate Gaussian discriminant analysis, and ensemble learning algorithms. The experimental results demonstrate the performance of different models and methods in practical applications.

3.1. Data Preprocessing and Descriptive Statistics

Data were sourced from NIPT records of 267 pregnant women, encompassing 1,009 blood draws and 1,068 sequencing observations. The dataset included variables such as BMI, gestational age, and GC content, which are considered closely related to fetal cell-free DNA concentration. Quality control and missing value handling were performed to ensure data validity and reliability. After cleaning, 96.9% of the original data remained valid. Most pregnant women underwent multiple tests, with an average of 4 blood draws per individual.

Table 1 presents descriptive statistics for key variables. The mean fetal Y chromosome concentration was 7.79% with a standard deviation of 3.35%. Approximately 86.6% of samples exceeded the clinically accepted 4% detection threshold. Gestational age averaged 16.6 weeks, predominantly concentrated in the mid-pregnancy testing window (11–25 weeks). The mean BMI was 32.2, indicating that most pregnant women in the dataset belonged to the high BMI group. The mean GC content was 0.4006, falling within the normal sequencing range.

Table 1. Data Characteristics

Characteristic Data	Mean	Standard deviation	minimum value	maximum value	Characteristic Description
Y Chromosome Concentration	7.79%	3.35%	1.00%	23.42%	86.9% reached the 4% detection threshold
Gestational Age	16.6 weeks	3.9 weeks	11.0 weeks	24.9 weeks	Primarily concentrated in mid-term detection
BMI	32.2	3.8	20.7	43.0	Predominantly among individuals with high BMI
Age	28.9 years	3.6 years	21 years	43 years	Childbearing age cohort
GC Content	0.4006	0.0032	0.3862	0.4191	Technical Quality Indicators

3.2. Correlation Analysis

To explore relationships among variables, we first conducted Pearson and Spearman correlation analyses. Figures 1 and 2 present heatmaps of Pearson correlation coefficients and Spearman rank correlation coefficients between variables, respectively. Results indicate significant linear negative correlations between Y chromosome concentration and gestational age (-0.709) as well as BMI (-0.713). This implies that fetal Y chromosome concentration tends to increase with gestational age, while higher BMI may lead to reduced Y chromosome concentration. Furthermore, weaker correlations were observed between GC content, X chromosome concentration, and Y chromosome concentration, suggesting their lesser influence on Y concentration.

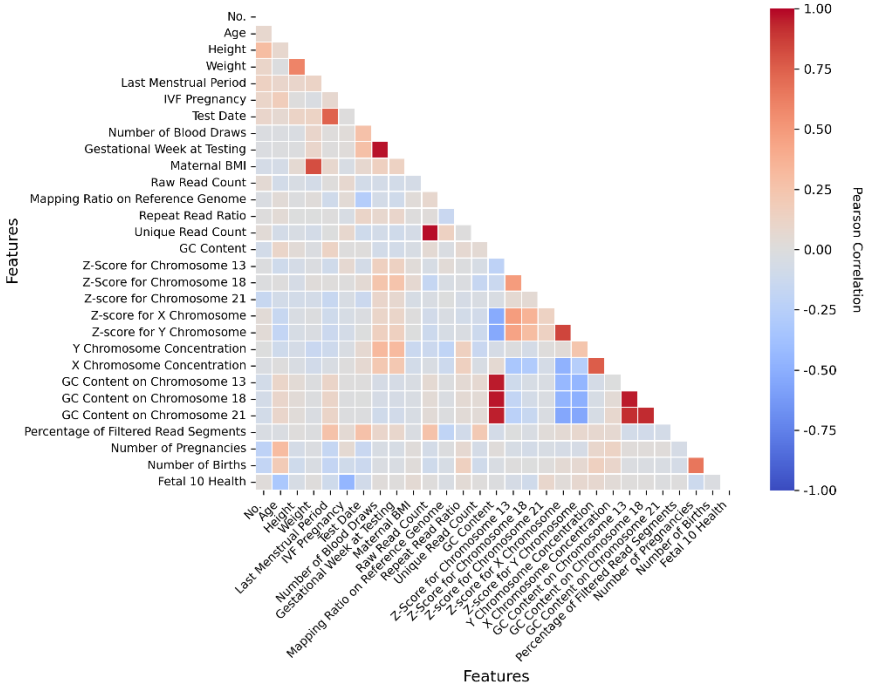

Figure 1. Heatmap of Pearson Correlation Coefficients

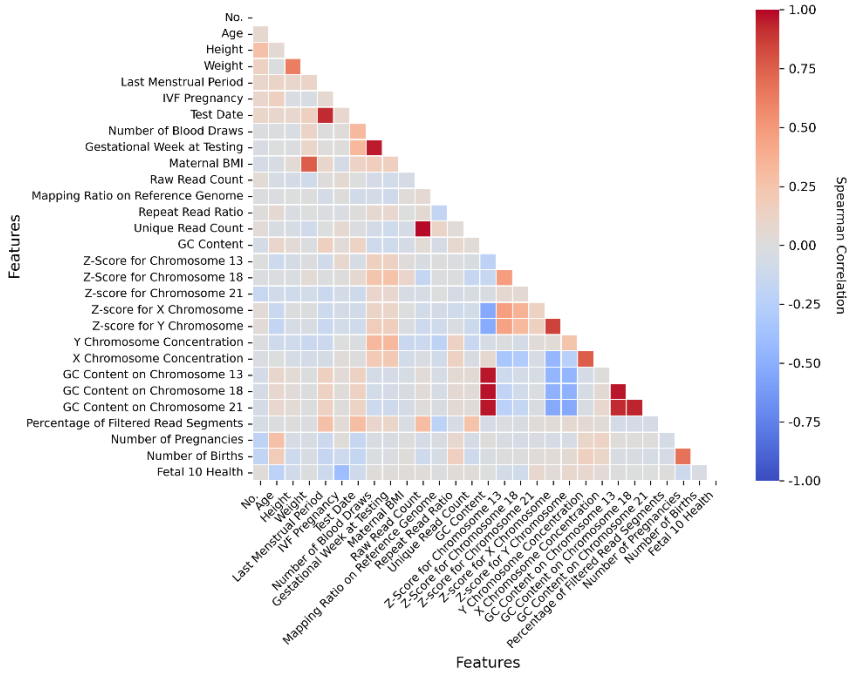

Figure 2. Spearman Correlation Heatmap

3.3. Model Selection and Fitting

Based on the above analysis, we selected a mixed-effects model to model the relationship between fetal Y chromosome concentration and factors such as gestational age and BMI. In model fitting, the pooled-mother random intercept model demonstrated superior fit, achieving a marginal R^2 of 0.8051. In contrast, the full two-level random effects model (accounting for random effects at both the mother and blood draw event levels) marginally improved model fit with a marginal R^2 of 0.8064. However, this model exhibited poorer stability, leading to the final selection of the pooled-mother random intercept model.

Table 2 summarizes the comparative performance of the two models. Significance testing revealed that gestational age and BMI were significant factors influencing fetal Y chromosome concentration ($p<0.001$).

Table 2. Performance Comparison Table of Two Models

Model Type	marginal R^2	Log-likelihood	Converged state
Aggregated_PregnantWomen_Random_Intercept	0.8051	2304.18	Stable
Complete_Two-Level_Random_Effects	0.8064	2458.43	Relatively stable

Based on this, further linear regression analysis confirmed the main effects of gestational age and BMI on Y chromosome concentration, along with their quadratic effects. Notably, the nonlinear effect of gestational age was better

modeled by introducing a quadratic term, indicating that the rate of increase in fetal DNA concentration accelerates as pregnancy progresses.

3.4. Recommended Optimal Testing Timepoints

Based on the pooled-pregnant-woman random intercept model, we further analyzed optimal NIPT testing timepoints across different BMI groups. According to the model-calculated probability of achieving detection thresholds, the optimal testing timepoint was 10.0 weeks for the low BMI group (20.7–30.3 kg/m²), the optimal timing for the moderate BMI group (30.3–32.7 kg/m²) is 13.0 weeks, while the high BMI group (32.7–46.9 kg/m²) is recommended to undergo testing at 15.8 weeks. The probability of achieving the Y chromosome concentration threshold across different BMI groups and gestational weeks. It can be observed that as BMI increases, the gestational week corresponding to the same probability of achieving the threshold gradually delays. This aligns with clinical observations, indicating that pregnant women with high BMI exhibit lower fetal cell-free DNA concentrations due to the blood dilution effect, thus requiring a longer period to reach the detection threshold.

To validate the robustness of this model, we conducted a Monte Carlo simulation sensitivity analysis. Even with a 2% measurement error, the success rate for each group remained above 76% (Figure 3), indicating the model's strong robustness under conditions of higher error. The results demonstrate that the model-based approach enables detection 3–6 weeks earlier than traditional methods, thereby providing a longer intervention window.

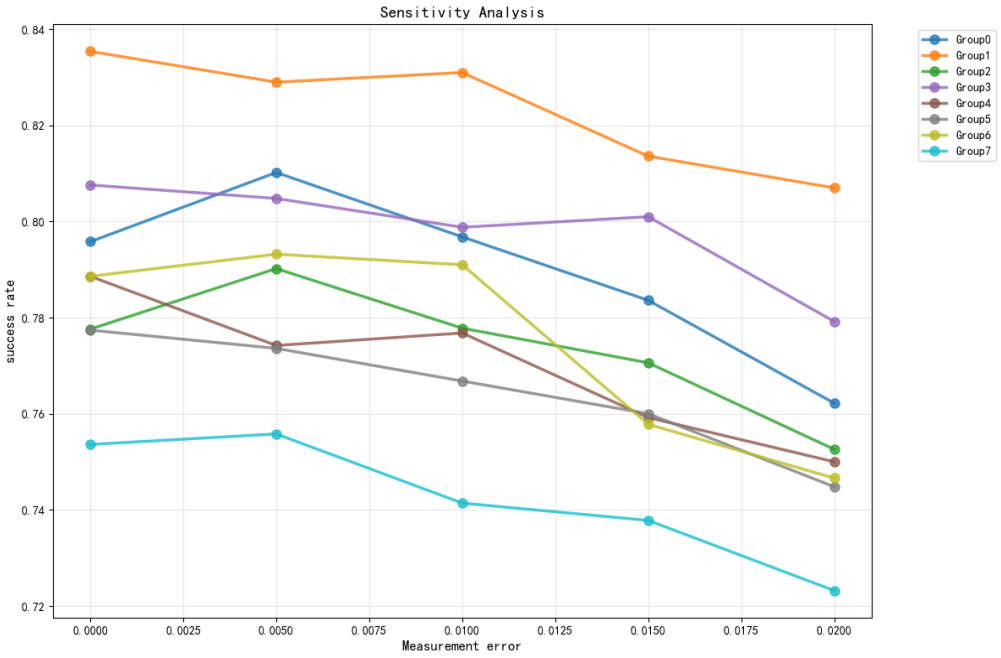

Figure 3. Sensitivity Analysis Chart

3.5. Chromosomal Abnormality Detection in Female Fetuses

For detecting chromosomal abnormalities in female fetuses, we employed a combined approach of multivariate Gaussian discriminant analysis and RUSBoost ensemble learning. Based on 604 valid samples, the model successfully distinguished features between normal fetuses and those with chromosomal abnormalities. Abnormality probability was calculated using Mahalanobis distance, and a multidimensional feature space was constructed for detection by integrating features such as Z-scores and GC content. Figure 4 illustrates the distribution of Z-scores across different chromosomes. Z18 exhibits optimal separation, while Z13 shows significant overlap, complicating interpretation. Based on the model's decision rules, we established a three-tiered reporting system: negative report, recommended retest, and confirmatory testing.

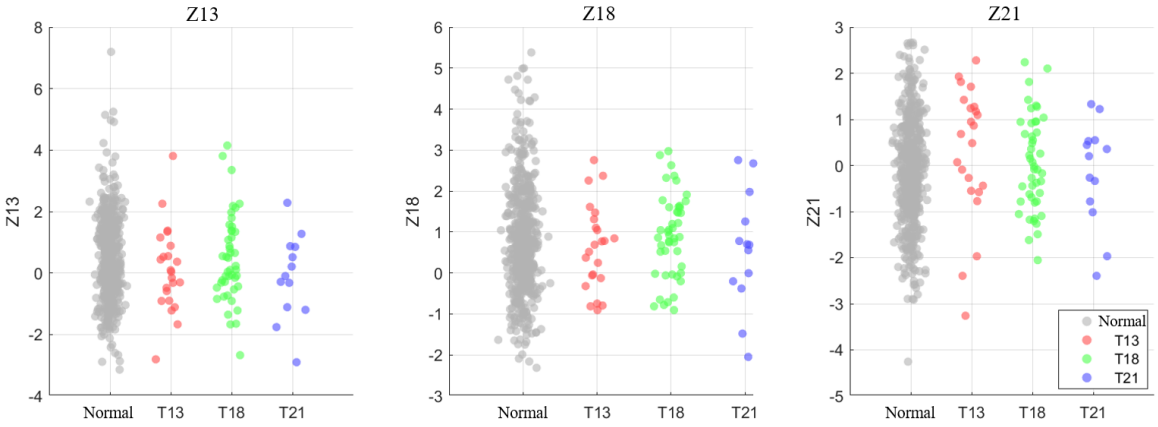

Figure 4. Z-Score Distribution Analysis

4. Conclusion

This study developed an optimized NIPT timing model based on clinical data, comprehensively considering multiple factors including maternal BMI and gestational age. It also proposed a novel solution for determining chromosomal abnormalities in female fetuses. Using a mixed-effects model, we identified significant linear and nonlinear relationships between fetal Y chromosome concentration and both gestational age and BMI, with the optimal testing window being significantly delayed for women with high BMI. Through further multivariate modeling and K-means clustering analysis, we provided personalized testing window recommendations for different BMI groups, thereby optimizing NIPT screening timing and enhancing both sensitivity and accuracy.

Furthermore, utilizing multivariate Gaussian discriminant analysis and the RUSBoost ensemble learning algorithm, we successfully enhanced detection accuracy for female fetal chromosomal abnormalities. Particularly when combining Z-scores with GC content, the approach effectively distinguished between normal and abnormal fetuses. Model validation demonstrated high stability and accuracy even under conditions of measurement error.

The findings of this study provide scientific support for the clinical application of NIPT, holding significant practical implications for optimizing personalized screening timepoints in high-BMI populations and accurately identifying chromosomal abnormalities in female fetuses. Future research may further expand sample sizes and explore the influence of additional clinical variables on NIPT detection to enhance the precision and universality of prenatal screening.

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