OBSTETRICS

Sequencing shorter cfDNA fragments improves the fetal DNA fraction in noninvasive prenatal testing

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BACKGROUND: Sequencing cell-free DNA in maternal plasma is an effective noninvasive prenatal testing technique that has been used in fetal aneuploidy screening worldwide. However, its clinical application is limited by the low fetal fraction (<4%) of cell-free DNA in many singleton pregnancies, which usually results in screen failures or no calls. In addition, dizygotic twin contributions of cell-free DNA into the maternal circulation can vary by 2-fold, complicating the quantitative diagnosis of fetal aneuploidy.

OBJECTIVE: We performed semiconductor sequencing of shorter fragments (107−145 bp) of circulating cell-free DNA to improve the fetal DNA fraction at lower uniquely mapped reads (1−8.5 MB) to reduce the probability of no calls.

STUDY DESIGN: We identified 2903 plasma samples from pregnant women, including 86 dizygotic twin pregnancy, that were collected at a single prenatal diagnostic center between October 2015 and July 2018. Size-selection noninvasive prenatal testing for fetal aneuploidy was applied to 2817 plasma samples (1409 male and 1408 female fetuses) and 86 dizygotic twins using noninvasive prenatal testing with and without size selection. Shorter fragment size was the key factor affecting fetal fraction in multivariable linear regression models as well as to validate the accuracy of the size selection for noninvasive prenatal testing.

RESULTS: Analysis of 1409 male fetuses by multivariable linear regression showed that maternal age, body mass index, number of pregnancies, average cell-free DNA size, maternal plasma cell-free DNA concentration, library concentration, and multiple gestation were negatively correlated with fetal fraction. Conversely, gestational age and uniquely mapped reads were positively correlated with fetal fraction. Compared with ≤120 bp cell-free DNA fragments, mean fetal fraction differences were −3.57% (95% confidence interval, −5.95% to −1.19%), for 121−130 bp, −9.52% (95% confidence interval, −11.89% to −7.14%) for 131−140 bp, and −14.47% (95% confidence interval, −18.37% to −10.58%) for ≥141 bp (P_{trend} < .0001). These results were statistically significant after multivariable adjustments in models for fetal fraction. Meanwhile, results from 86 dizygotic twins showed that the size selection increased the fetal fraction by ∼3.2-fold, with 98.8% of samples reaching a fetal fraction >10%. Improved detection accuracy was also achieved.

CONCLUSION: Sequencing shorter cell-free DNA fragments is a reasonable strategy to reduce the probability of no calls results because of low fetal fraction and should be recommended to pregnant subjects.

Key words: body mass index, cell-free DNA, fetal aneuploidy, fetal fraction, gestational age, multivariable linear regression models, noninvasive prenatal testing, size selection

Massively parallel sequencing (MPS) of cell-free DNA (cfDNA) in maternal plasma is a noninvasive prenatal testing (NIPT) method that has been applied for aneuploidy screening.[1−3] In 2016, the American College of Medical Genetics and Genomics proposed that this novel technology could replace conventional screening for genetic disorders such as Edwards, Patau, and Down syndromes across the maternal age spectrum for a continuum of gestational age beginning at 9-10 weeks and for patients who are not significantly obese(the failure rate of NIPT will increase).[4]

Still, cfDNA screen failures or no calls occur in 0.5−3% of samples and are frustrating for both patients and clinicians; the major reason for this failure is low fetal fraction.[5−7] For dizygotic twin pregnancies, the median fetal fraction, defined as median proportion of total cfDNA that is fetal in origin, is 7.9−8.7%,[8,9] which is significantly lower compared with singletons (typically ∼10%[10]).

At the first sampling, 5.6% of these subjects have a low fetal cfDNA fraction (<4%).[8] Each fetus can contribute different amounts of cfDNA into maternal circulation, with up to 2-fold variation.[9,11,12] That is, if the dizygotic twin fetal fraction was >8%, the contribution by each fetus is theoretically greater than the threshold of 4%. The 5.6% fetal fraction rate of dizygotic twins is therefore an underestimate.

In addition, a low fetal fraction in maternal circulation has been associated with an increased risk of fetal aneuploidies[4,13]; triploidy was most common (31%) and trisomy 21 was seen in 23% of cases of low fetal fraction.[13]

Previous studies reported that maternal-derived DNA molecules are longer than fetal-derived ones, which suggests that we may semiquantitatively enrich fetal-derived DNA from maternal plasma at shorter size for NIPT screening.[14,15] High-resolution plasma DNA size profiling has revealed that the most significant difference between fetal and maternal DNA in maternal plasma is the reduction in the 166 bp peak relative to the 143 bp peak.[16]

Recent investigations into the relative proportions of short (100−150 bp) and

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AJOG at a Glance

Why was this study conducted?

This study was conducted to increase fetal fraction of cell-free DNA to avoid screen failures in a prenatal diagnosis of many singleton and dizygotic twin pregnancies.

Key findings

We have demonstrated that shorter fragments were the key factors affecting fetal fraction of cell-free DNA in maternal plasma. Compared with longer cell-free DNA fragments (≥141 bp), sequencing of shorter ones (≤120 bp) significantly increased the fetal fraction both in singleton and in dizygotic twin pregnancies and therefore reduced the probability of a no-calls result.

What does this add to what is known?

Low fetal fraction (<4%) of cell-free DNA in many singleton and dizygotic twin pregnancies is known to cause screen failures or no calls; this study provides a strategy to increase fetal fraction.

long (163−169 bp) DNA fragments have suggested that sizes <143 bp are richer in fetal cDNA.[17−19] Our preliminary result of much smaller sample study, which suggested that fetal fraction was most abundant in the 125−135 bp fragment.[20] The current study focused on NIPT based on improving the fetal DNA fraction by sequencing of shorter cfDNA fragments (107−145 bp).

In this study, we examined the effect of different factors, including maternal age, body mass index (BMI), number of pregnancies, average size of cfDNA, maternal plasma cfDNA concentration, library concentration, singleton pregnancies or twins, gestational age, and uniquely mapped reads on the fetal fraction. We also analyzed the trend of fetal fraction in each 10 bp size change of cfDNA. Finally, we estimated the sequencing of shorter cfDNA fragments in dizygotic twin pregnancies.

Materials and Methods

A total of 2817 plasma samples from pregnant women (1409 male and 1408 female fetuses) were collected at a single prenatal diagnostic center between October 2015 and July 2018. The mean age, BMI, and gestational age of pregnant women were 30.6 years (range, 18−49 years), 22.4 kg/m^2 (range, 14.9−44.2 kg/m^2), and 17.4 weeks (range, 12−27 weeks) (Table 1).

We also collected whole blood from subjects pregnant with dizygotic twin (cfDNA obtained from 600 μL plasma in 86 patients) and 34 dizygotic twin (obtained from 1200 μL plasma in 34 patients) to assess the accuracy of size selection NIPT. Chorionicity was determined by examining the intertwin membrane at its junction with the placenta by ultrasound at 10−14 weeks of gestation.[21] All pregnant women underwent ultrasound to identify singletons or twins before NIPT. All samples had fetal karyotypes or clinical follow-up results.

The Reproductive Medicine Ethics Committee of Suzhou Municipal Hospital approved this study (approval number K901001).

Protocol for size selection NIPT

Blood samples were first collected into EDTA tubes and then centrifuged at 1600 g at 4°C for 10 minutes and subsequently at 16,000 g for 10 minutes to produce cell-free plasma. We used 600 μL plasma aliquots from 2817 singletons and 86 dizygotic twin pregnancies for cfDNA extraction using TIANamp Micro DNA purification kits (Tiangen Biotech, Beijing, China).

To determine whether it is possible to use size selection and increased plasma volume to increase the fetal fraction, additional cfDNA was extracted from 1200 μL plasma samples collected from 34 dizygotic twins.

The DNA library was constructed with an Ion Plus fragment library kit (Thermo Fisher Scientific, Waltham, MA). Polymerase chain reaction products were chosen from 197 to 235 bp (insert DNA ranging from 107 to 145 bp) using E-Gel EX 2% gels (Invitrogen, Carlsbad, CA) (Figure 1).

The selected fragments were sequenced using the Ion Proton system; all sequencing data were mapped to the h19 human reference genome (Figure 2), and fetal DNA concentrations were evaluated by calculating the proportion of reads from chromosome Y as previously described.[22] Fetal aneuploidy status for whole chromosomes was determined by Z-scores (normal, −3<Z<3).

Statistical analysis

The distribution of fetal fraction had an approximately normal distribution on a normal Q-Q plot. Multivariable linear regression models were used to evaluate the association between fetal fraction and maternal age, BMI, number of pregnancies, average cfDNA size, maternal plasma cfDNA concentration, library concentration, singleton or twin pregnancies, gestational age, and uniquely mapped reads.

The variables reported in the previous text that were considered clinically relevant or showed a univariate relationship with the outcome were entered into multivariable linear regression models. To test for linear trends, we used average cfDNA size as a continuous variable. High-resolution DNA size profiling revealed that fetal- and maternal-derived DNA exhibited a series of peaks, including one at 143 bp and a 10-bp periodicity below 143 bp.[13]

We also generated a categorical variable for average cfDNA size (≤120 bp vs 121−130 bp, 131−140 bp, ≥141 bp). Relative to the ≤120 bp reference category, we computed estimates and 95% confidence intervals (CIs) for the mean differences in fetal fraction for each category of average cfDNA size.

Model 1 in univariate linear regression analyzes the relationship between

average cfDNA size and fetal fraction. Model 2 was adjusted for gestational age and BMI (both continuous numerical variables). Model 3 was adjusted for maternal age (continuous numerical variable), BMI, number of pregnancies (continuous numerical variable, means number of pregnancies that a given mother has had), maternal plasma cfDNA concentration (continuous numerical variable), library concentration (continuous numerical variable), multiple gestation (categorical variable), gestational age (continuous numerical variable), and uniquely mapped reads (continuous numerical variable).

All analyses were performed using SPSS version 24.0 (IBM Corp, Armonk, NY,). All P values are 2 sided, and $P < .05$ was considered statistically significant.

Results

Sample characteristics

The results from 2817 (1409 male and 1408 female fetuses) size selection NIPT showed that fragment size varied between 107 and 145 bp (Figure 1). In addition, mapping of the human reference genome was the same in both size selection NIPT (Figure 2B) and NIPT (Figure 2A) may be suggesting that there is no significant deletion in genome by size selection. Study population characteristics are presented in Table 1.

The results from 1409 male fetuses showed that the mean uniquely mapped reads, cfDNA size, and fetal DNA concentration were 2.3 Mb (range, 1.1−8.5 Mb), 130 bp (range, 107−145 bp), and 31% (range, 5.8−93.6%), respectively. Compared with the traditional method,[5] size selection NIPT increased the fetal fraction and reduced the uniquely mapped reads, which suggested that this method was effective and convenient.

With and without enrichment were performed in 179 samples. Supplemental Figure 1 shows the Z-score for chromosome 21 of these samples. In one case, the Z-score increased of positive sample by size selection enrichment. In one missed diagnosis case, normal NIPT was negative and positive in size selection NIPT. However, a negative sample was false positive after size selection enrichment.

TABLE 1
Maternal and fetal characteristics of the study population (n = 2817)

Characteristics	All	Male fetus	Female fetus
Sample size	2817	1409	1408
Maternal age, y	30.6 (18.0−49.0)	29.8 (18.0−47.0)	31.0 (19.0−49.0)
Maternal weight, kg	59.8 (109−35.4)	59.8 (40−109)	60 (35.4−92.5)
Maternal height, cm	160.8 (145−179)	160.8 (145−179)	160 (145−176)
Body mass index, kg/m^2	22.4 (14.9−44.2)	22.5 (15.4−44.2)	22.3 (14.3−34.9)
Gestational age, wks	17.4 (12−27)	17.4 (12−26)	17.5 (12−27)
Number of pregnancies	2.4 (1−9)	2.5 (1−9)	2.4 (1−8)
Uniquely mapped reads, Mb	2.2 (1−8.5)	2.3 (1.1−8.5)	2.2 (1−4.4)
Average size of cell-free DNA, bp	130.2 (107−145)	130.2 (107−145)	130.3 (114−143)
Fetal DNA concentration, %	NA	30.8 (5.8−93.6)	NA
Maternal plasma cell-free DNA concentration, ng/μL	NA	0.17 (0.04−0.71)	NA
Library concentration, ng/μL	NA	8.0 (0.05−21.7)	NA
Singleton pregnancies	NA	1327	NA
Monochorionic twins	NA	7	NA
Dizygotic twins	NA	75	NA

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Multiple factors affecting fetal fraction

Fetal fraction was negatively correlated with average cfDNA size in both uni- and multivariate analysis (multivariate estimate −0.695% per 1 bp, $P < .0001$, Figure 3). Notably, when cfDNA sizes were between 107 and 130 bp, the mean fetal DNA concentration was significantly higher than 30% (Figure 4A), suggesting that a relative enrichment of fetal-derived DNA in the shorter cfDNA fragments.

Consistent with previous studies, we also found a negative correlation between fetal fraction and maternal BMI (multivariate estimate, −0.541% per 1 kg/m^2, $P < .0001$, Figure 3) and a positive correlation between fetal fraction and gestational age (multivariate estimate, 0.959% per 1 week, $P < .0001$, Figure 3) in both uni- and multivariate analyses.

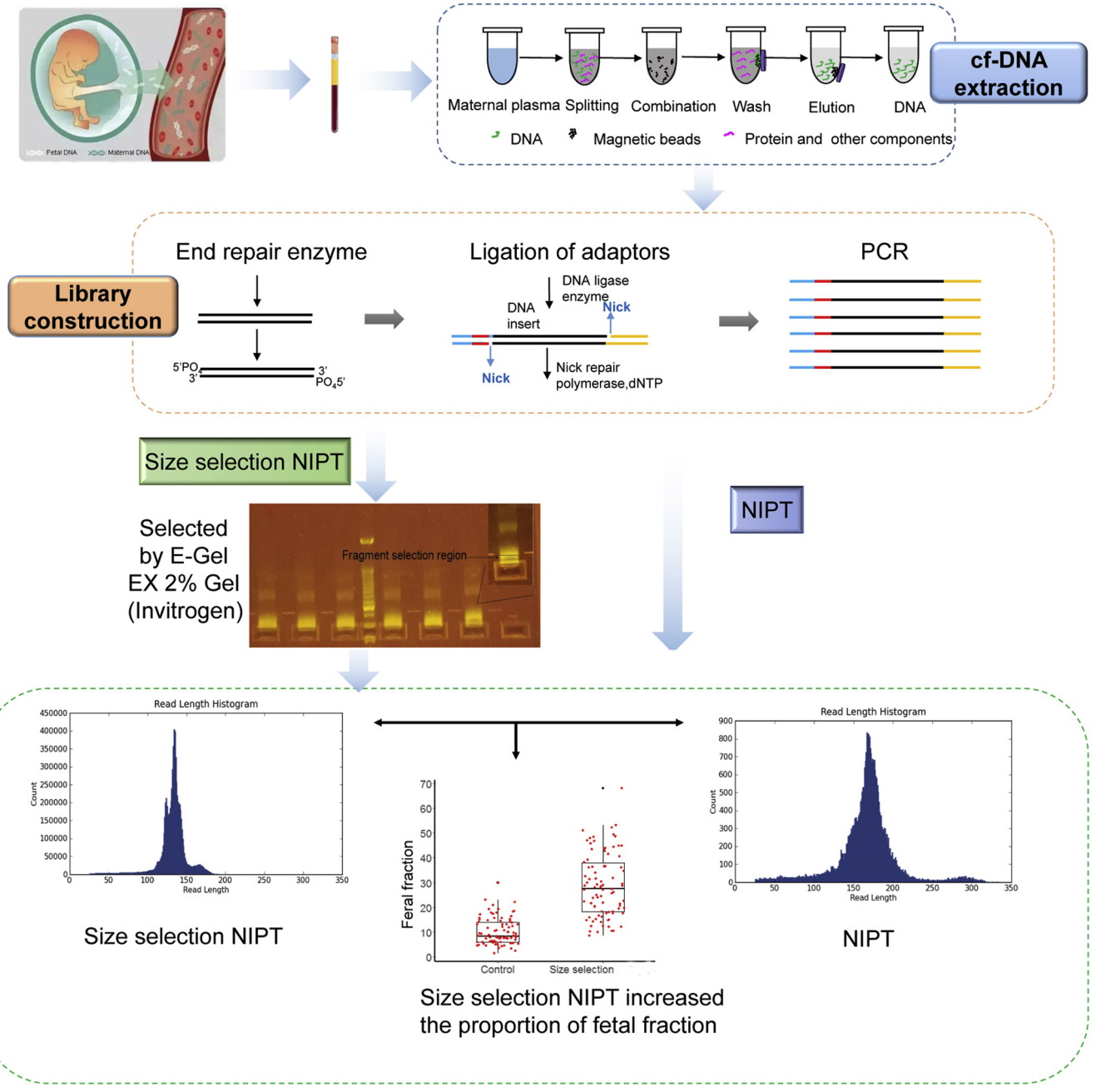
The mean fetal fraction was 22.0% in pregnant women with a high BMI (>30 kg/m^2, Figure 4B) and 26% among pregnant women with earlier gestational age (12−13 weeks, Figure 4C), suggesting a significantly higher fetal fraction in subjects with a high BMI and earlier gestational age.

As shown in Figure 3, a multivariable linear regression analysis revealed 6 significant independent predictors of fetal fraction: number of pregnancies (multivariate estimate, 0.661% per 1 pregnancy, $P = .0016$), uniquely mapped reads (multivariate estimate, 2.292% per 1 Mb, $P < .0001$), maternal age (multivariate estimate, −0.202% per 1 year, $P = .0005$), maternal plasma cfDNA concentration (multivariate estimate, −2.017% per 1 ng/μL, $P < .0001$), library concentration (multivariate estimate, −0.578% per 1 ng/μL, $P < .0001$), and multiple gestation (multivariate estimate, −4.575% per 1 kg/m^2, $P = .0008$).

cfDNA fragment size and fetal fraction

The crude and adjusted associations between cfDNA fragment size categories and fetal fraction are presented in Figure 5. The multivariable-adjusted

FIGURE 1
Flowchart depicting NIPT and size selection NIPT



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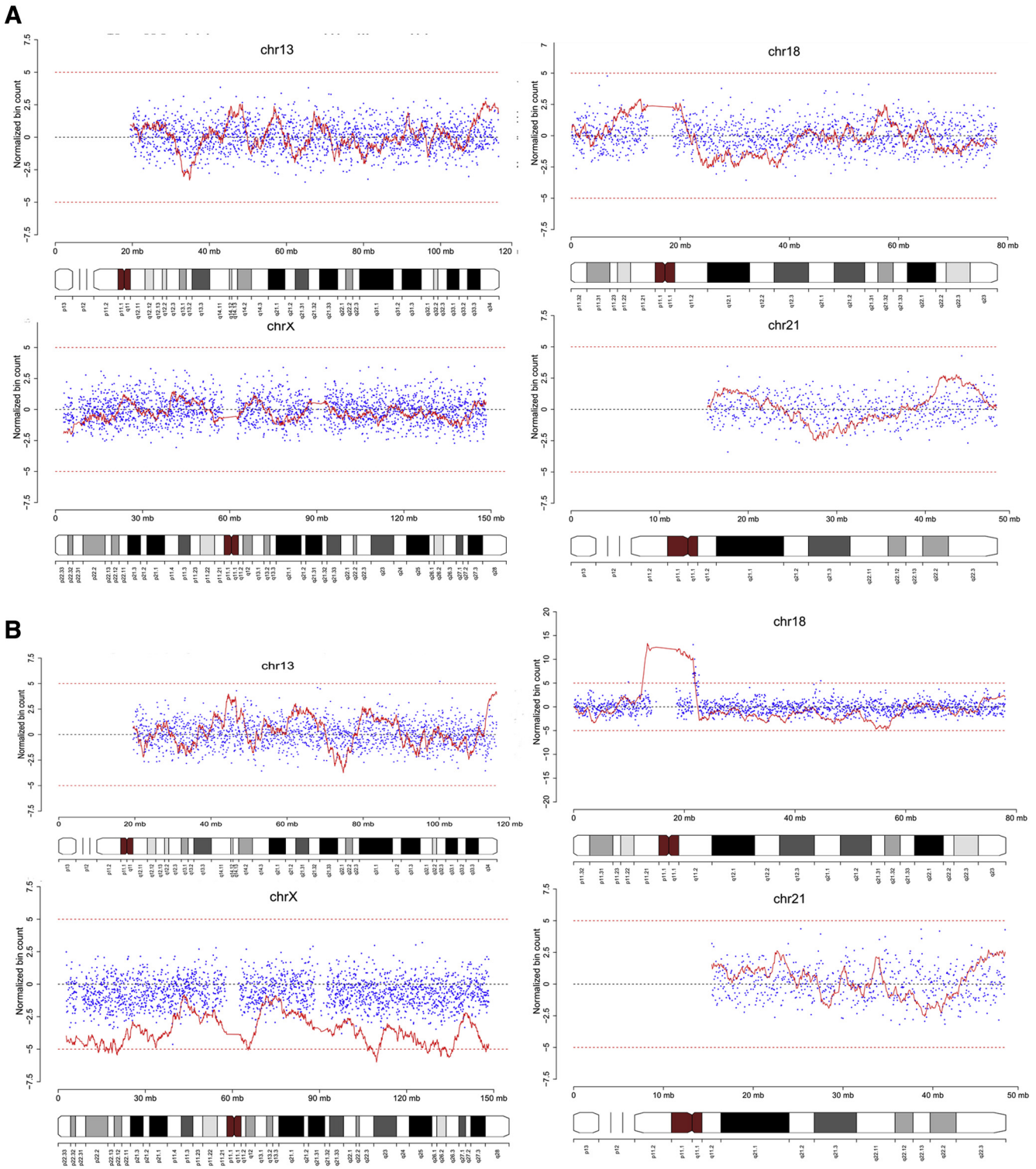
mean differences of fetal fraction across the categories were −3.57% (95% CI, −5.95% to −1.19%) for 121−130 bp, −9.52% (95% CI, −11.89% to −7.14%) for 131−140 bp, and −14.47% (95% CI, −18.37% to −10.58%) for ≥141 bp compared with sizes ≤120 bp (P_{trend} < .0001).

Improved fetal fraction in dizygotic twins

Previous studies have shown that samples from dizygotic twins have a significantly lower median fetal fraction (7.9−8.7%) compared with singletons (11.7%).[8,9] We confirmed that cfDNA fragment size is key factor affecting fetal fraction, suggesting that size selection could improve the yield from dizygotic twins.

Our results showed that the mean fetal fraction for size selection NIPT was 29% compared with 10% for NIPT (P < .0001, Figure 6A and Table 2), with 98.8% of size selection samples yielding a

FIGURE 2
NIPT and size selection NIPT



NIPT **(A)** and size selection NIPT **(B)** were mapped to the h19 human reference genome. **A,** The Z values of chromosomes 21, 18, 13, and X were 0.08, −0.11, −1.05, and −0.5, respectively. The average cfDNA size was 162 bp, and the fetal fraction was 11%. **B,** The Z values of chromosomes 21, 18, 13, and X were 0.23, 1.73, 0.17, and −0.09, respectively. The average cfDNA size was 131 bp, and the fetal fraction was 31.8%.

cfDNA, cell-free DNA.

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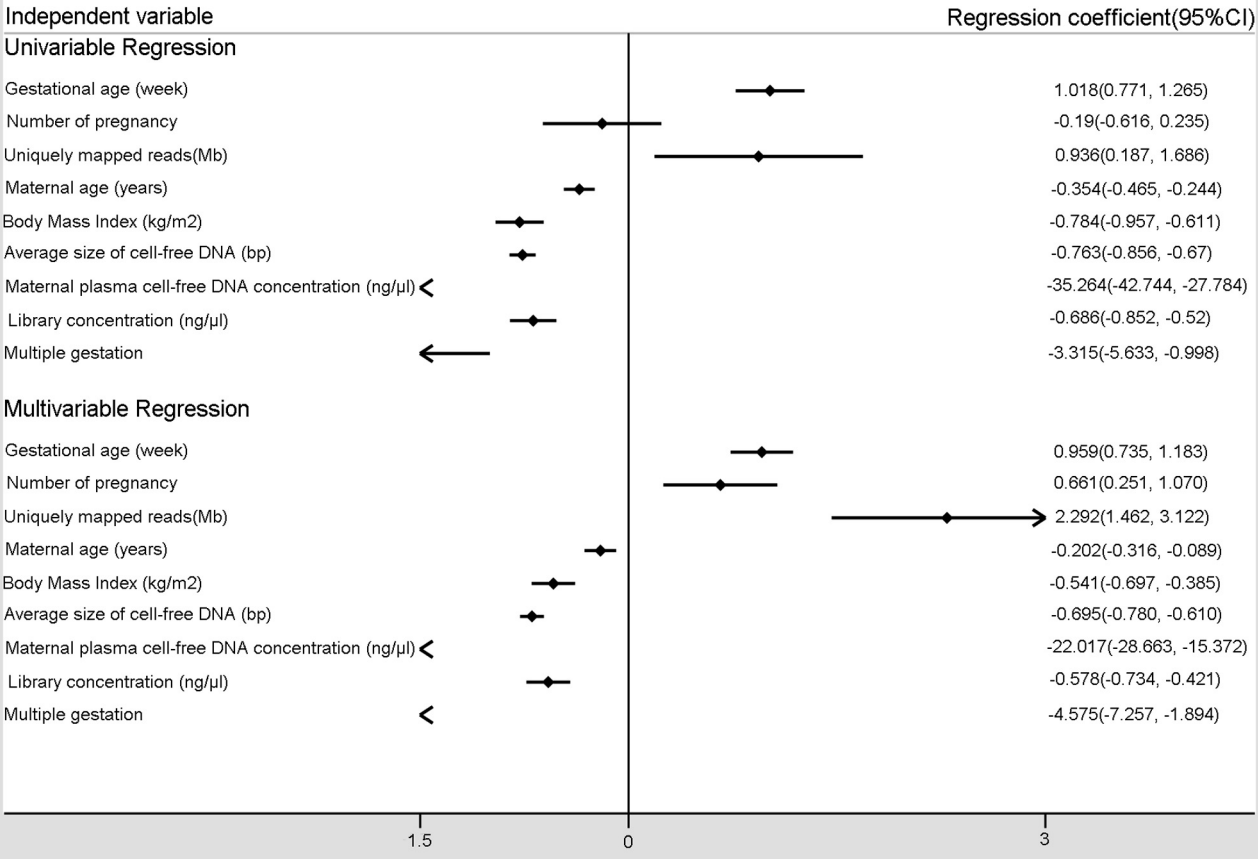
FIGURE 3
Regression analysis for predicting fetal fraction in maternal plasma cfDNA



Maternal age, BMI, number of pregnancies, average cfDNA size, maternal plasma cfDNA concentration, library concentration, and multiple gestations were negatively correlated with fetal fraction, while gestational age and uniquely mapped reads were positively correlated with fetal fraction.

BMI, body mass index; *cfDNA*, cell-free DNA.

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fetal fraction >10%. Notably, when the size was <130 bp, the fetal fraction was significantly higher with a median increase of 3.4-fold (vs >130 bp, 2.5-fold, $P < .0001$, Figure 6B). We did not find a significant association between fetal fraction and increased plasma extraction cfDNA ($P = .3$, Figure 6A).

Test performance

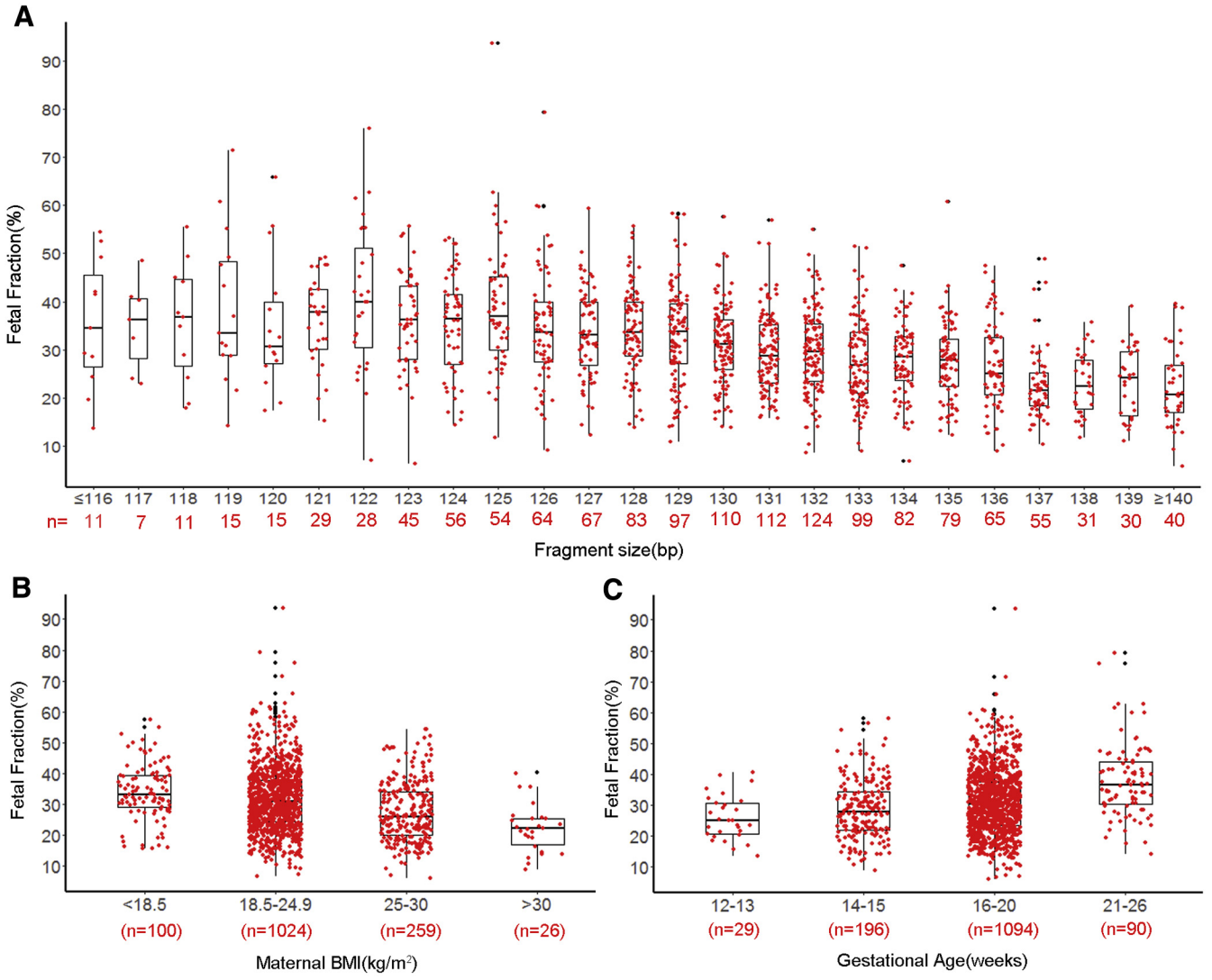
A total of 2817 size selection NIPT results showed that this method detected all cases of true aneuploidy (trisomy 21 = 11, trisomy 18 = 6, trisomy 13 = 1). The false-positive rates for trisomies 21, 18, and 13 were 0.036%, 0.036%, and 0.071%, respectively. Results from 86 dizygotic twins were the same as those obtained from untreated NIPT and on clinical follow-up.

Comment

Sufficient fetal fraction in maternal plasma is required for accurate aneuploidy detection by NIPT. It is therefore important to identify which factors affect fetal fraction. Our results indicate that maternal age, BMI, number of pregnancies, average cfDNA size, maternal plasma cfDNA concentration, library concentration, and multiple gestation are negatively correlated with fetal fraction, while gestational age and uniquely mapped reads are positively correlated with fetal fraction.

Previous studies suggested that the mean fetal fraction in women with a high BMI ($\geq$25 kg/m^2) is ~5%, and the low fetal fraction may increase the no-calls rate.[23,24] Maternal obesity affects 1 in 5 pregnant women in the United States, and this proportion is on the rise.[25,26] In

FIGURE 4
Relationships between fetal fraction and different variables



A, Fetal fraction was negatively correlated with average cfDNA size. When cfDNA size was between 107 and 130 bp, the mean fetal DNA concentration was significantly higher than 30%. **B,** Fetal fraction was negatively correlated with BMI. **C,** Gestational age was positively correlated with fetal fraction.

BMI, body mass index; *cfDNA*, cell-free DNA.

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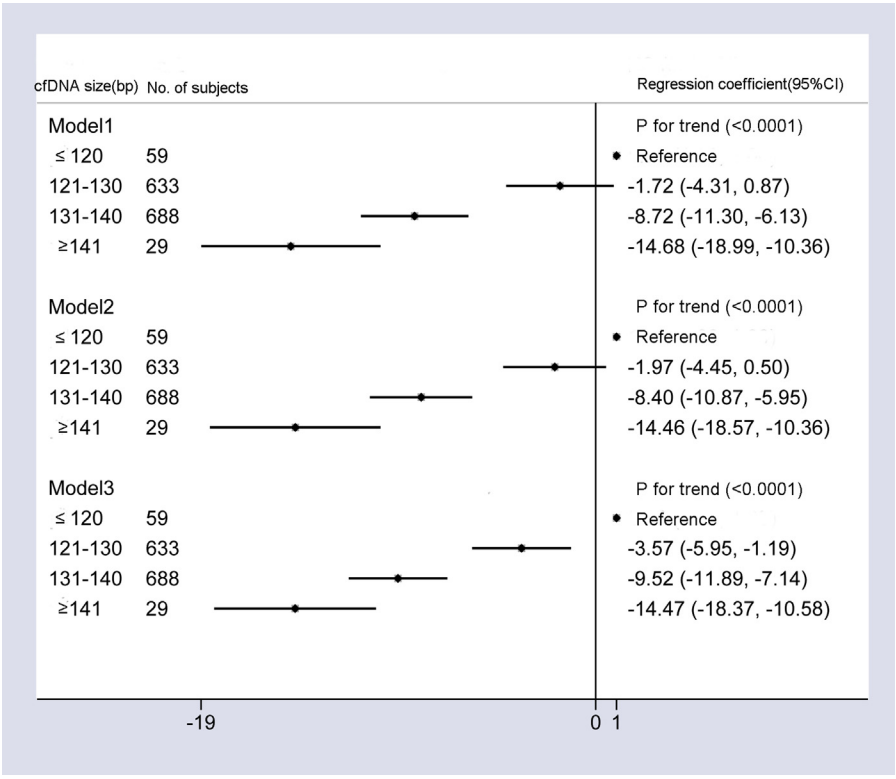
addition, a low fetal fraction was associated with an increased risk of fetal aneuploidy.[4]

We found that the fetal fraction was reduced by 0.541% for every BMI increase of 1 kg/m^2. The mean fetal fraction was 22.0% in the maternal group with a high BMI (>30 kg/m^2, Figure 3B), which is significantly higher compared with normal NIPT (typically ~10%), suggesting that sequencing of shorter cfDNA fragments may be an ideal method for improving fetal fraction in the high-BMI group.

Gestational age is another key factor affecting fetal fraction. We found an increase of 0.959% per week between 12 and 26 weeks' gestation, which was inconsistent with a previous study.[24] This may be due to other confounding factors affecting fetal fraction in shorter fragments, such as average cfDNA size. The mean fetal fraction was 26% among the maternal group with earlier gestational age (12−13 weeks, Figure 3C), suggesting that 9 or 10 weeks[4] (the earliest NIPT gestational age recommended by the American College of Medical Genetics and Genomics) may not be used as a threshold for gestational age in size selection NIPT.

This new technology may be implemented at an earlier gestational age (<9 weeks). These findings imply that sequencing shorter cfDNA fragments can improve the low fetal fraction in

FIGURE 5
Differences in fetal fraction according to size for cfDNA range



Model 1, crude model. Model 2, adjusted for gestational age and BMI. Model 3, adjusted for maternal age, BMI, number of pregnancies, maternal plasma cfDNA concentration, library concentration, multiple gestation, gestational age, and uniquely mapped reads.

BMI, body mass index; *cfDNA*, cell-free DNA.

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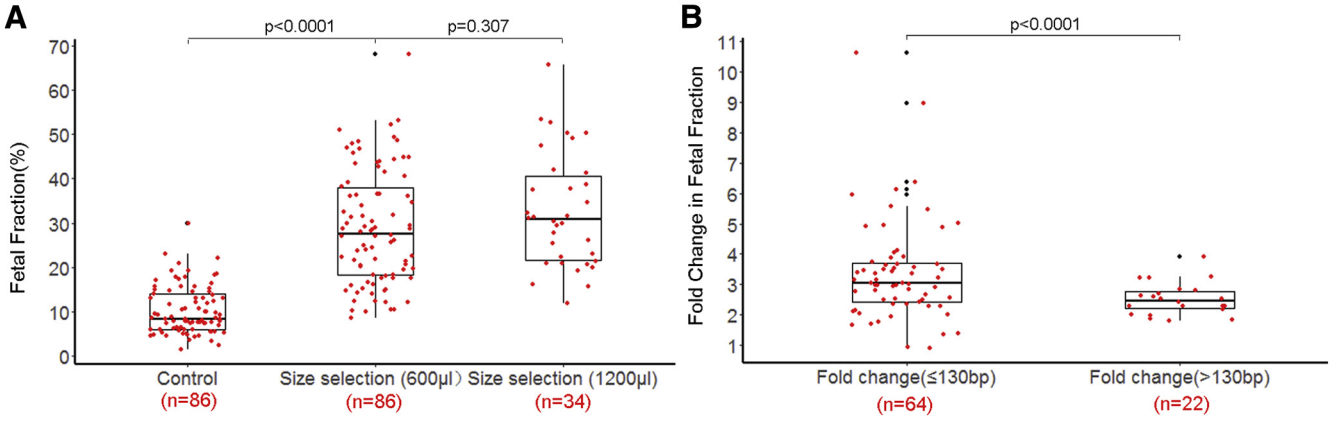
subjects with higher BMIs and earlier gestational ages. Next, we determined whether the same degrees of reliability and accuracy can be achieved earlier in gestation (<9 weeks) and in subjects with higher BMI (>30 kg/m^2) according to large sample clinical trials.

Fetal fraction is related to multiple factors. These variables can be used to study the relationship between cfDNA fragment size and fetal fraction. We adjusted for BMI and gestational age in model 2 and found that it did not significantly affect the association between cfDNA fragment size and fetal fraction, indicating that these were not major confounding factors in our study. We subsequently adjusted for factors associated with maternity such as BMI, gestational age, maternal age, number of pregnancies, and multiple gestation as well as factors associated with sequencing parameters such as uniquely mapped reads, maternal plasma cfDNA concentration, and library concentration (model 3). A more significant association was observed after these adjustments. We also noticed that for cfDNA fragments <130 bp, the magnitudes of mean fetal fraction differences were smaller compared with sizes ≤120 bp. Therefore, enriching cfDNA fragments shorter than 130 bp is recommended

FIGURE 6
Relationship between fetal fraction and size selection in dizygotic twins



A, The effect of size selection on fetal fraction after DNA extraction from different volumes of plasma. **B,** Fold change in fetal fraction with different fragment sizes after extracting cfDNA with 600 μL plasma.

cfDNA, cell-free DNA.

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to achieve sufficient fetal cfDNA for NIPT.

For dizygotic twins, the fetal fraction contributed by each individual would need to reach the 4% threshold to adequately assess the chromosomal representation of each twin by MPS-NIPT.[12] Each fetus can contribute different amounts of cfDNA, and the difference can be nearly 2-fold.[9,11,12]

In addition, the median fetal fraction of 7.9−8.7% is lower than that for singleton pregnancies (11.7%). NIPT performance might therefore be worse for dizygotic twins.[4] We found that the mean fetal fraction of size selection NIPT was significantly higher (29%) compared with NIPT (10%, P<0.0001, Figure 5A), with 98.8% fetal fractions achieving >10%.

For cfDNA fragment sizes <130 bp, the increase in fetal fraction was even more obvious. We hypothesize that a high fetal fraction ensures that each dizygotic twin is contributing >4%, which improves NIPT performance accuracy to the level observed in singletons.

This study has several strengths. First, this was an exploration of the relationship between cfDNA fragment size and fetal fraction adjusted for several potential confounders in a large maternal population. Second, we evaluated size selection NIPT performance in singletons and dizygotic twins. Third, the double-blind design reduced experimental and analytical bias.

Our study also suffers from limitations. Although we adjusted for many confounding factors, such as anticoagulation therapy,[27] in vitro fertilization conceptions[28] and other relevant factors may have been overlooked. In addition, only a small number of size selection NIPT samples were available from subjects with higher BMIs, earlier gestational ages, and dizygotic twins, so further studies are required to validate our results.

Simultaneously, NIPT testing is therefore able to identify pregnancies at high risk only for certain conditions and confirmatory testing such as chorionic villus sampling or amniocentesis is required for definitive diagnosis.[29]

TABLE 2
Maternal and fetal characteristics of twin pregnancies

Characteristic	1200 μL plasma size select	600 μL plasma size select
Sample size	34	86
Maternal age, y	29.2 (21.0−39.0)	30.1 (20.0−39.0)
Maternal weight, kg	59.6 (45−81)	57.5 (44.0−81.0)
Maternal height, cm	160.2 (145−179)	161.0 (150.0−172.0)
Body mass index, kg/m^2	23.2 (17.6−30.5)	22.2 (17−31.6)
Gestational age, wks	16.8 (12−22)	16.7 (13−21)
Number of pregnancies	2.0 (1−6)	2.0 (1.0−6.0)
Uniquely mapped reads, Mb	4.1 (1.3−6.0)	4.5 (1.1−9)
Average size of cell-free DNA, bp	125.6 (112−145)	125.8 (109−144)
Fetal DNA concentration, %	32.6 (11.9−65.6)	28.8 (8.6−68)
Library concentration, ng/μL	13.6 (0.2−21.7)	13.8 (3.1−21.1)

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However, a recent study[30] would support our conclusion about shorter fragments and fraction detection, although it is about circulating tumor DNA, which has shown that selecting fragments between 90 and 150 bp improved detection of tumor DNA, with more than 2-fold median enrichment in >95% of cases and more than 4-fold enrichment in >10% of cases.

In summary, our results suggest that sequencing shorter cfDNA fragments (especially those <130 bp) can improve the fetal DNA fraction. We also showed that sequencing shorter cfDNA fragments during NIPT yields a significantly lower false-positive rate and higher sensitivity for trisomies 21, 18, and 13 at lower uniquely mapped reads in singletons and dizygotic twins. By size selection, this effective method reduced the amount of sequencing data for each sample and reduced the cost simultaneously. It may be used as a common aneuploidy screening method.

This effective method can also be used in subjects with early gestational age, higher BMI, placental mosaicism, vanished twins,[31] and other scenarios associated with low quantities of fetal cfDNA. It may be used on different platforms, such as massively parallel sequencing and single-nucleotide polymorphisms. Moreover, this technique has great application potential beyond NIPT for investigating monogenic diseases, identifying chromosomal insertion/deletion, and determining paternity. ■

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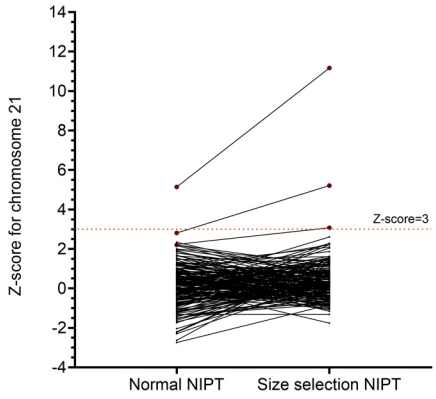
SUPPLEMENTAL FIGURE

Z-score for chromosome 21 in the same samples



NIPT, noninvasive prenatal testing

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