ORIGINAL ARTICLE

Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma

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ABSTRACT

Objective To determine the effects of gestational age and maternal weight on percent fetal cell-free DNA (cfDNA) in maternal plasma and the change in fetal cfDNA amounts within the same patient over time.

Methods The cfDNA was extracted from maternal plasma from 22 384 singleton pregnancies of at least 10 weeks gestation undergoing the Harmony™ Prenatal Test. The Harmony Prenatal Test determined fetal percentage via directed analysis of cfDNA.

Results At 10 weeks 0 days to 10 weeks 6 days gestation, the median percent fetal cfDNA was 10.2%. Between 10 and 21 weeks gestation, percent fetal increased 0.1% per week ($p < 0.0001$), and 2% of pregnancies were below 4% fetal cfDNA. Beyond 21 weeks gestation, fetal cfDNA increased 1% per week ($p < 0.0001$). Fetal cfDNA percentage was proportional to gestational age and inversely proportional to maternal weight ($p = 0.0016$). Of 135 samples that were redrawn because of insufficient fetal cfDNA of the initial sample, 76 (56%) had greater than 4% fetal cfDNA in the sample from the second draw.

Conclusion Fetal cfDNA increases with gestation, decreases with increasing maternal weight, and generally improves upon a blood redraw when the first attempt has insufficient fetal cfDNA. © 2013 John Wiley & Sons, Ltd.

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INTRODUCTION

Over this past year, non-invasive prenatal testing (NIPT) via the analysis of cell-free DNA (cfDNA) from maternal plasma for the detection of trisomy 21, 18, and 13 has been introduced into clinical practice. NIPT can detect fetal trisomy with high sensitivity (up to 99%) and at low false positive rates (less than 0.1%)[1–9] making it a promising alternative to current screening modalities that have trisomy detection rates of up to 95% and false positive rates of 5%.[10,11] Several different approaches have been reported for the analysis of cfDNA chromosomal fragments utilizing next-generation DNA sequencing technology, including directed or chromosome-selective sequencing of cfDNA, random analysis of cfDNA fragments by massively parallel shotgun sequencing, or sequencing of single-nucleotide polymorphisms (SNPs) in cfDNA.[2,6,12]

Regardless of the approach, the ability to complete an analysis and report out a reliable clinical result is related to the proportion of fetal to maternal cfDNA in maternal plasma, where the minimum fetal cfDNA needed for analysis is approximately 4%.[2,6] Although others have shown an increase in fetal cfDNA by using other technologies,[13] previous reports using next-generation DNA sequencing technology showed no statistical difference week to week (between 10 and 22 weeks gestation) in the percentage of fetal cfDNA in maternal plasma. The average fetal cfDNA percent was 11%–13.4%, although a large variance in percentage exists among patients.[2,6] In addition, the clinical factors of maternal age, prenatal screening results, and nuchal translucency measurement, commonly associated with a priori trisomy risk, do not appear to influence fetal cfDNA percentage.[14] Moreover, in high risk pregnancies between 11 and 13 weeks gestation, there is no correlation between percent fetal cfDNA and fetal karyotype, crown rump length, or other maternal characteristics. Fetal percentage in maternal blood cfDNA is associated with an increase with serum pregnancy-associated plasma protein A and free beta hCG, and decreases with increasing maternal weight.[15]

Although a small percentage of patients will not receive a NIPT result primarily because of low fetal cfDNA percentage, a specific threshold relative to maternal weight where results cannot be obtained has not been established. The objective of this study was to further define the relationship between gestational age and maternal weight on the percent fetal in maternal plasma cfDNA.

METHODS

The study set included 22 384 maternal plasma samples drawn between 20 July 2012 and 31 December 2012 from women with singleton pregnancies and a stated gestational age on the test requisition form of at least 10 weeks. Not all gestational ages were confirmed by ultrasound. Blood samples were collected into Cell-Free DNA BCT ™ tubes (Streck, Omaha, NE) and underwent testing with the Harmony Prenatal Test (Ariosa Diagnostics, San Jose, CA) if received within 5 days of being drawn. Samples were anonymized and used in compliance with all applicable laws.

Maternal plasma cfDNA was isolated and analyzed by using the DANSR™ assay (Ariosa Diagnostics, San Jose, CA, USA), which determines chromosome proportion and fetal cfDNA percentage simultaneously as previously described.[1,3] Measurement of fetal cfDNA percentage was determined against a set of 192 SNP-containing loci on chromosomes 1–12 to query each SNP. The HapMap 3 dataset was used to optimize the SNPs for minor allele frequency (http://hapmap.ncbi.nlm.nih.gov/). Based on measurements of informative polymorphic loci where the maternal alleles were different from the fetal alleles, a maximum likelihood estimate using the binomial distribution was utilized to determine the most likely fetal cfDNA percentage.

Linear regression was used for continuous variables and chi-squared test with 10 000 000 simulated replicates was used for categorical comparisons.

RESULTS

Fetal percentage of cfDNA was measured in 22 384 patients. The mean reported gestational age in the group was 15.8 weeks (range: 10–40 weeks), while the mean maternal weight was 73 kg (range: 32–284 kg) (Figure 1). At 10 weeks 0 days to 10 weeks 6 days gestation, the median percent fetal cfDNA was 10.2%. Between 10 and 21 weeks gestation, percent fetal increased at 0.10% per week ($p < 0.0001$) (Figure 2), and 2% of the pregnancies during this period were below 4% fetal cfDNA. Starting at 21 weeks gestation, the percent fetal cfDNA increased at a rate of 1% per week ($p < 0.0001$), a tenfold increase in the amount of percent fetal per week compared with the weeks between 10 and 21 weeks gestation (Figure 2).

Table 1 shows the fraction of samples having at least 4% fetal cfDNA in relation to maternal weight. The negative correlation between fetal cfDNA and maternal weight was statistically significant ($p = 0.0003$). Samples were grouped by weight categories or bins in 10 kg increments. Within the cohort, 95% of the samples came from women between the weight bin categories of <50–120 kg. Of women with maternal weight less than 90 kg, 99.1% had a fetal cfDNA greater than 4%. Both gestational age and maternal weight significantly influence the amount of fetal cfDNA, explaining as much as 27% of the variations seen in percent fetal ($p < 0.0001$).

In our study, approximately 1.9% of our tests resulted in redraw requests because of low fetal percent. At the close of this study, we have made 357 requests because of insufficient fetal cfDNA, of which 135 women underwent a second blood draw and sent the sample to our laboratory. As compared with the entire cohort, the patients who underwent a second blood draw were on average at a younger gestational age (13.9 weeks vs 15.8 weeks, $p < 0.0001$) and had higher mean maternal weight (103 kg vs 73 kg, $p < 0.0001$). We observed a mean 1% fetal cfDNA gain in the second draw as compared with the first draw with an average interval time between the two draws of 3.6 weeks. Of the 135 samples that had insufficient percent fetal cfDNA with the first blood draw, 76 (56%) resulted in greater than 4% fetal percent on the second blood draw. The proportion of women with sufficient fetal percent on the second draw decreased with increasing maternal weight (Table 2). If we accounted for gestational age between blood draws within the same pregnancy, the difference in percent fetal cfDNA between first draw and second draw is no longer significant with respect to maternal weight ($p = 0.76$), suggesting that waiting for a later gestational age could overcome low initial percent fetal cfDNA because of high maternal weight.

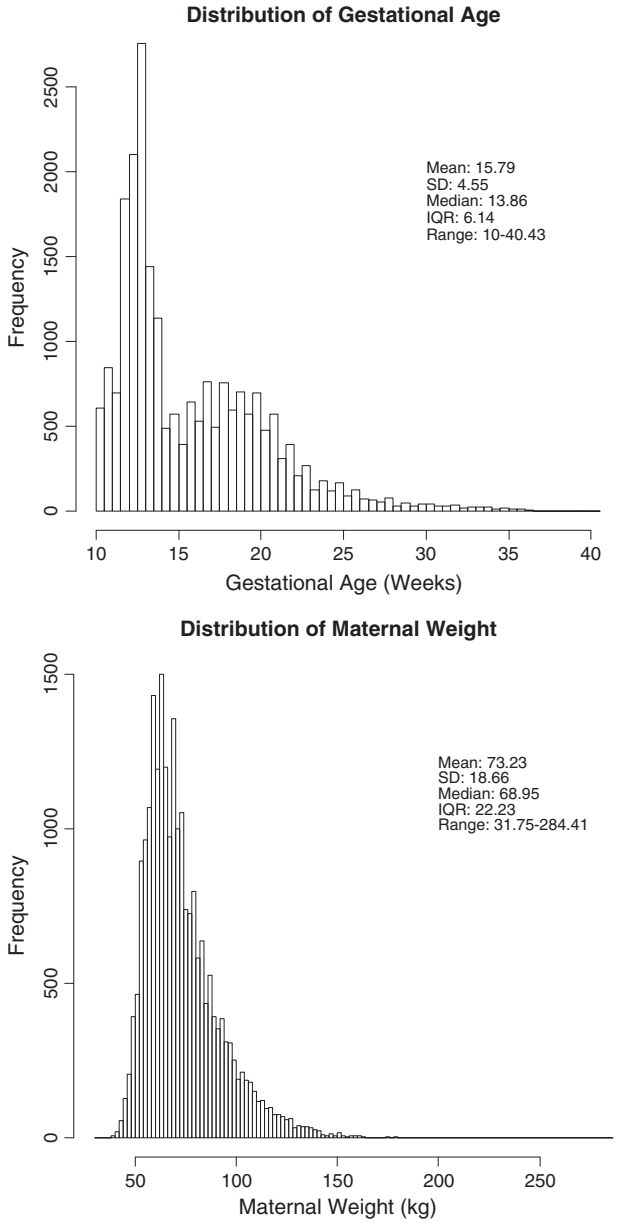

Figure 1 Distribution of gestational age and maternal weight

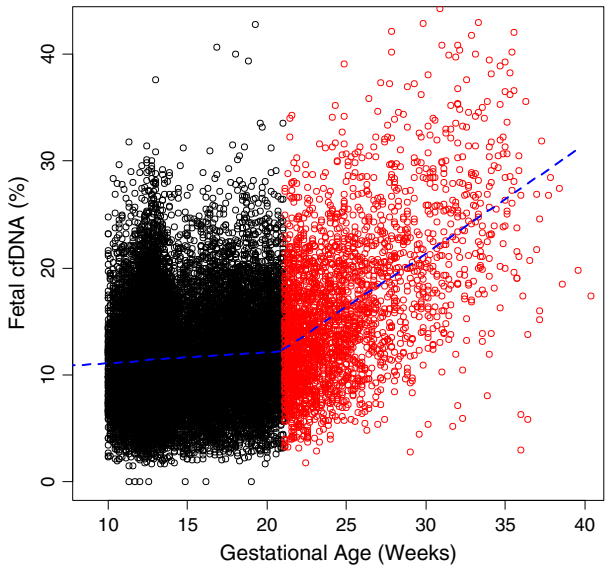

Figure 2 Relationship between percentage of fetal cfDNA and gestational age. Fetal cfDNA percentage for 22 384 pregnancies is plotted with respect to gestational age (weeks). At 10 weeks 0 days to 10 weeks 6 days gestation, the median fetal cfDNA percentage was 10.2%. Between 10 and 21 weeks gestation (black open circles), fetal cfDNA increased at 0.10% per week ($p < 0.0001$; blue dash line within black open circles) and 2% of the pregnancies during this period were below 4% fetal cfDNA. Starting at 21 weeks gestation (red open circles), the fetal cfDNA percentage increased at a rate of 1% per week ($p < 0.0001$; blue dash line within red open circles), a tenfold increase in the amount of fetal cfDNA percentage per week compared with the weeks between 10 and 21 weeks gestation

Table 1 Proportion of pregnancies with ≥4% fetal cell-free DNA on first blood draw

Maternal weight bin (kg)	*n*	Pregnancies with ≥4% fetal cell-free DNA (%)
<50	809	99.8
≥50 < 60	4825	99.6
≥60 < 70	6224	99.2
≥70 < 80	4313	98.8
≥80 < 90	2574	98.2
≥90 < 100	1608	96.3
≥100 < 110	921	93.9
≥110 < 120	508	89.8
≥120 < 130	298	87.9
≥130 < 140	172	81.4
≥140	132	71.2

DISCUSSION

This study represents the largest clinical data set on the percent fetal cfDNA related to both gestational age and maternal weight. We found that the fetal cfDNA increased incrementally between 10 and 21 weeks of gestation and that over this gestational age window, an overall 1% increase in total fetal percent can be anticipated. However, starting at 21 weeks of gestation, a greater weekly increase in fetal percent can be expected (1%). It is unlikely that a 0.1% average weekly increase would be clinically relevant and warrant a general change in blood draw protocol for the general population greater than 10 weeks gestational age.

Table 2 Proportion of women with ≥4% fetal cfDNA on repeat blood draw

Maternal weight bin (kg)	# ≥4% fetal on 2nd draw	# of total patients	% with ≥4% on 2nd draw
<90	30	42	71.4%
≥90 < 100	14	23	60.9%
≥100 < 110	13	22	59.1%
≥110 < 120	10	17	58.8%
≥120 < 130	2	7	28.6%
≥130 < 140	5	13	38.5%
≥140	2	11	18.2%

For those patients who had insufficient fetal cfDNA on their first blood draw and underwent a second blood draw, the maternal weight and gestational age characteristics were both significantly different from the entire cohort. These patients had higher maternal weight and were earlier in gestation. Although 56% of these pregnancies resulted in greater than 4% fetal upon redraw, it is puzzling why the rate of increase in fetal cfDNA in these samples were threefold higher than the expected, given their gestational age range (0.28% per week vs 0.1% per week observed in the general cohort). One simple explanation is that the rate of cfDNA increase is different when measured longitudinally in the same individual versus aggregating results from different individuals. However, when we looked at the entire cohort of redraws for various reasons, which includes these 135 pregnancies requested because of low initial percent fetal cfDNA, the rate of increase with respect to gestational age is 0.09% per week within the same pregnancy (data not shown). This rate is not dissimilar from the overall rate of 0.1% computed from the entire cohort of pregnancies. Alternatively, compounded with a higher maternal weight, it is entirely possible that some of these patients with low initial fetal percent did not have accurate dating of their pregnancies as not all dating was based on ultrasound. We have observed a significant decrease in fetal percent before 10 weeks gestation (data not shown) and the rate of fetal percent increase may be higher during this period. Therefore, in addition to the anticipated limitations of maternal weight, accurate gestational age dating at the time of first blood sample is critical to the likelihood of receiving a result and in determining when to schedule a redraw if necessary or desired.

This study confirms the previously reported relationship between increasing maternal weight and decreasing fetal percent.[15] However, the majority of women, based on this set of 22 000 commercial samples, will have greater than 4% fetal DNA, regardless of gestational age or maternal weight, and will be able to receive a result from NIPT. As the ability to detect trisomy depends on the precision of the assay and fetal

percent, these findings can be used by providers and their patients in making the decision as to whether or not to repeat NIPT or undergo traditional methods of prenatal testing for those patients who do not receive a result. As there exists a segment of the population who would not undergo invasive testing or termination under any circumstances but seek the information and reassurance NIPT can provide, the option of waiting until later in the second trimester for a repeat blood draw, when fetal percent increases more rapidly can also be presented. In our study, there were 1224 samples (5.5% of all samples) submitted after 24 weeks gestation, which suggests that there is interest in this information in the third trimester.

One limitation of our study is that we were not able to calculate body mass index (BMI) as we did not have maternal height for the calculation. Thus far, it is still unclear why fetal cfDNA percent decreases with increasing maternal weight. Having maternal height data and BMI with which to assess obesity might allow a more accurate predictor of assay success as compared with maternal weight alone; because in obese women, there is an increased turnover of adipocytes, thereby increasing the amount of maternal cfDNA and decreasing the fetal cfDNA percent. However, BMI does not allow for the analysis of body fat directly nor does it distinguish between different body types relative to fat or muscle. For example, BMI based on a strict height and weight calculation overestimates body fat in persons who are very muscular and can underestimate body fat in persons who have lost or have little muscle mass.[16,17] An alternate explanation is that increasing total blood volume may be correlated with decreasing percent fetal cfDNA (the diluting effect). In this case, the total blood volume in a tall person is also larger than in a small person, and height should certainly be taken into account. Future studies including covariates such as weight, height, BMI, and body type are warranted. Therefore, without additional studies, a simpler clinical standard around which to counsel regarding cfDNA analysis success likelihood might be just maternal weight. Data from future investigations that include weight, height, and body type might shed light on this aspect.

CONCLUSION

This study provides clinically useful data to prepare patients for the possibility of low fetal percent based on their gestational age and weight at the time of blood draw. The vast majority of clinical maternal plasma samples greater than 10 weeks gestational age contain an adequate fetal cfDNA proportion to allow for useful clinical results. However, attention should be paid to accurate gestational age determination—especially at early gestational ages—to ensure sufficient fetal percent for assay completion. Extremely high maternal weight is a primary correlative factor for low fetal cfDNA and thus no result from NIPT using cfDNA analysis. A repeat blood draw for NIPT for samples with low fetal percent may be worthwhile for patients, except in cases of high maternal weight. When possible, second attempts are most beneficial after waiting several weeks. If gestational age was estimated to be later than the actual gestational age, the waiting time to receive the first result may be long enough to increase fetal percent above the 4% cutoff. In other cases, the waiting time may need to be longer because of the slow rate of increase in fetal percent until about 21 weeks gestation. In addition to maternal weight and gestational age, there remains a substantial amount of unexplained variation in fetal percent in the population. Other clinical factors that influence fetal percent remain an area for future investigation.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Previous literature indicates no significant change in average percentage of fetal cell-free DNA between 10 and 22 weeks when using Next-Generation Sequencing technology.

WHAT DOES THIS STUDY ADD?

- This is the largest sample set reporting changes in percent fetal cell-free DNA in relation to maternal weight and gestational age.

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