Fetal fraction-based risk algorithm for non-invasive prenatal testing: screening for trisomies 13 and 18 and triploidy in women with low cell-free fetal DNA

T. MCKANNA1#, A. RYAN1#, S. KRINSHPUN1, S. KAREHT1, K. MARCHAND2, C. GRABARITS3, M. ALI4, A. MCELHENY5, K. GARDINER6, K. LECHIEN7, M. HSU8, D. SALTZMAN9, M. STOSIC1, K. MARTIN1 and P. BENN10

1 Natera Inc., San Carlos, CA, USA; 2 Beth Israel Deaconess Medical Center, Boston, MA, USA; 3 Vanderbilt University Medical Center, Nashville, TN, USA; 4 Weill Cornell Medicine, New York, NY, USA; 5 St Louis University School of Medicine, St Louis, MO, USA; 6 LifeLabs Genetics, Toronto, ON, Canada; 7 Mercy Hospital, St Louis, MO, USA; 8 Northshore University Health System, Chicago, IL, USA; 9 Icahn School of Medicine at Mount Sinai, New York, NY, USA; 10 UConn Health, Farmington, CT, USA

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ABSTRACT

Objective To identify pregnancies at increased risk for trisomy 13, trisomy 18 or triploidy attributable to low fetal fraction (FF).

Methods A FF-based risk (FFBR) model was built using data from more than 165,000 singleton pregnancies referred for single-nucleotide polymorphism (SNP)-based non-invasive prenatal testing (NIPT). Based on maternal weight and gestational age (GA), FF distributions for normal, trisomy 13, trisomy 18 and triploid pregnancies were constructed and used to adjust prior risks for these abnormalities. A risk cut-off of ≥1% was chosen to define pregnancies at high risk for trisomy 13, trisomy 18 or triploidy (high FFBR score). The model was evaluated on an independent blinded set of pregnancies for which SNP-based NIPT did not return a result, and for which pregnancy outcome information was gathered retrospectively.

Results The evaluation cohort comprised 1148 cases, of which approximately half received a high FFBR score. Compared with rates expected based on maternal age (MA) and GA, cases with a high FFBR score had a significantly increased rate of trisomy 13, trisomy 18 or triploidy combined (5.7% vs 0.7%; P < 0.001) and also of unexplained pregnancy loss (14.7% vs 10.4%; P < 0.001). For cases that did not receive a high FFBR score, the incidence of a chromosomal abnormality or pregnancy loss was not significantly different from that expected based on MA and GA. In this study cohort, the sensitivity of the FFBR model for detection of trisomy 13, trisomy 18 or triploidy was 91.4% (95% CI, 76.9–98.2%) with a positive predictive value of 5.7% (32/564; 95% CI, 3.9–7.9%).

Conclusions For pregnancies with a FF too low to receive a result on standard NIPT, the FFBR algorithm identified a subset of cases at increased risk for trisomy 13, trisomy 18 or triploidy. For the remainder of cases, the risk of a fetal chromosomal abnormality was unchanged from that expected based on MA and GA.

INTRODUCTION

Non-invasive prenatal testing (NIPT) screens for common fetal chromosomal abnormalities by analyzing cell-free DNA in maternal plasma. Accurate testing is facilitated by a high proportion of fetal cell-free DNA, referred to as the fetal fraction (FF). Single-nucleotide polymorphism (SNP)-based NIPT evaluates more than 13,000 SNPs to assess risk for chromosomal abnormalities and simultaneously determines FF. A ‘no-result’ report is generated when the NIPT algorithm cannot make a high-confidence call, usually because the FF is too low. FF increases with gestational age (GA), but decreases with...
increasing maternal weight (MW)\textsuperscript{7–9}. FF is lower in cases of trisomy 13, trisomy 18 and digynic (maternal) triploidy (all of which tend to have smaller placentas)\textsuperscript{8,10–14}. Professional society guidelines\textsuperscript{15–17} recommend that women who receive a ‘no result’ on NIPT due to low FF should be referred for ultrasound examination, be offered diagnostic testing or consider alternative forms of prenatal screening, due to an elevated residual risk of aneuploidy. However, there are many causes for low FF, and not all women with low FF are at high risk for trisomy 13, trisomy 18 or triploidy.

To address this, we developed a FF-based risk (FFBR) algorithm for singleton pregnancies. This new algorithm is distinct from the standard SNP-based NIPT algorithm in that it considers FF, GA, MW and prior risk to identify ‘no-result’ cases at increased risk for trisomy 13, trisomy 18 or triploidy. The FFBR algorithm was evaluated in a large independent cohort of women for which the SNP-based NIPT did not return a result due to low FF or inability to make a high-confidence call.

METHODS

The FFBR algorithm provides a mathematical estimate of an individual’s risk for trisomy 13, trisomy 18 or triploidy. To compute a patient-specific FFBR score for each pregnancy, the prior risk is adjusted according to their observed FF. The calculation from the observed FF considers the relative probability that the sample was derived from a pregnancy with each of the different possible fetal karyotypes. The prior probability of each hypothesis is defined by the population-based risk before testing. Prior risks for trisomy 13 and trisomy 18 were based on maternal age (MA) and GA\textsuperscript{18}. For digynic triploidy, prior risk was set at 1/5505 based on reported incidence\textsuperscript{19–22}.

The mathematical models for FF forming the basis of the FFBR computation were constructed from a predominantly normal reference dataset of approximately 165 000 singleton-pregnancy SNP-based NIPT tests performed between 2013 and 2015\textsuperscript{23}. The FF SD varied significantly over the range of MW and GA indicating that the use of multiples of the median (MoM) was not an appropriate approach. We therefore used data binned by MW and GA to establish expected FF distributions. Affected pregnancy FF values were derived from: a validation study\textsuperscript{3}; samples collected under a research protocol to support product enhancements\textsuperscript{3}; a previous effort to collect clinical follow-up on cases with failed NIPT testing due to low FF; and a study focused on triploidy\textsuperscript{24}. Compared with a normal case, an average case with trisomy 13 or 18 would have an expected FF approximately 63% as high and an average case with digynic triploidy would have expected FF about 20% as high. Additional information on the construction of the models is provided in Appendix S1.

An observed patient-specific FF was compared with each of three models (for hypotheses of normal karyotype, trisomy 13 or 18, or digynic triploidy) to determine the likelihoods. The likelihoods were combined with the corresponding prior risks to calculate posterior probabilities using the standard Bayesian approach\textsuperscript{25}. The overall case-specific FFBR score represents the estimated probability (risk) that the pregnancy is affected by trisomy 13, trisomy 18 or digynic triploidy, and is the sum of the posterior probabilities corresponding to the trisomy and triploidy models. FFBR scores were computed based only on the FF, GA, MW and prior risk for each case. A high FFBR score was defined as a risk ≥1% of trisomy 13, trisomy 18 or triploidy. This cut-off was defined before the evaluation data were collected and was chosen for consistency with the high-risk threshold used in the existing SNP-based method for trisomies 21, 18 and 13 and sex chromosome abnormalities\textsuperscript{7}.

To evaluate the performance of the algorithm, outcome data were collected for NIPT performed on singleton pregnancies between December 2013 and December 2016 that were reported as ‘no result’ due to low FF or low-confidence call. This dataset did not include any of the affected cases used in the algorithm construction. Two methods were used to obtain these follow-up data. Firstly, several large maternal–fetal medicine and general obstetrical practices with access to electronic medical records that had undergone internal Institutional Review Board (IRB) review were provided with case identification, a password-protected outcome registry and instructions for completion. For a second cohort of women at smaller centers, following IRB approval, outcomes were obtained either by telephone by Natera genetic counselors or collected by genetics staff at a commercial partner’s laboratory and recorded in an internal registry database (Appendix S1).

Following data collection and classification of fetal outcomes, cases were assessed to determine whether all the data required to calculate FFBR (i.e. MA, GA, FF and MW) were available. MW was that at the time of NIPT blood draw or the date closest to the blood draw. For cases with sufficient data, FFBR scores were computed by comparing the observed FF with that expected from the three previously described models (Figure 1). Prior risk for pregnancy loss was modeled as a function of MA
and GA based on published data; it was not, however, incorporated into the FFBR calculation.

After assigning FFBR scores and categorizing patients according to the ≥1% risk cut-off, statistical tests were performed for each risk cohort to compare chromosomal abnormality rates and pregnancy loss rates, with those expected based on prior risk. Among the outcomes, there were 12 independent significance tests (six outcomes across two risk sub-cohorts). Therefore, a 5% overall significance level corresponds to a P-value of 0.004 for each individual test. Given the wide range of prior risks contained in each cohort, standard tests assuming a single population rate (such as chi-squared) were not suitable, so a simulation-based method was used to compute distributions of expected numbers of affected cases (Appendix S1). A P-value < 0.004 implied that the observed result was significantly inconsistent with prior risk.

RESULTS

Of 1350 patients in the study cohort who received a ‘no result’ due to low FF or a low-confidence result, 195 (14.4%) were lost to follow-up (e.g. patient delivered at out-of-network hospital or changed obstetrician, or medical records were unobtainable). Seven additional cases (pregnancy loss (n = 1), trisomy 21 (n = 1), unaffected (n = 5)) lacked information (e.g. MW or FF) required to calculate a FFBR score. Therefore, a cohort of 1148 (85.0%) cases were included in the analysis. Average MA was 34.0 (range, 17–48) years, average MW was 1148 (85.0%) cases were included in the analysis. Average GA was 95.0 (range, 42.7–200.9) kg, average FF was 3.1 (range, 0.5–15.8) % and average GA at the time of the blood draw was 12.3 weeks (Table S1). FF was reported with a lower limit of 0.5% based on measurement precision. The FF distribution is shown in Figure S1. The primary reasons for NIPT referral were advanced MA (AMA) (55.0%) and routine average risk aneuploidy screening (33.9%); 4.2% of cases had an abnormal maternal serum screen, 3.7% an abnormal ultrasound examination and 3.2% were referred due to family history (Table S2).

Clinical follow-up of the cohort eligible for analysis (n = 1148) identified 48 (4.2% (95% CI, 3.1–5.5%)) cases that had a confirmed chromosomal abnormality, a rate significantly higher than that expected based on MA and GA alone (expected rate, 1.5%; P < 0.001) (Table 1). The incidence of triploidy (1.8% (95% CI, 1.1–2.8%)) exceeded the expected rate (0.02%) of digynic triploidy by 90-fold and the rate for trisomy 18 was also significantly elevated (1.0% (95% CI, 0.5–1.7%) vs 0.3% expected; P < 0.001). Although the rate of trisomy 13 was higher than expected (0.3% (95% CI, 0.05–0.8%) vs 0.1% expected) it did not reach statistical significance (P = 0.069). The number of cases with trisomy 21 and monosomy X were similar to that expected.

FFBR scores were calculated for the analysis cohort; 49.1% (564/1148) received a high FFBR score, indicating they had an estimated risk ≥1% for trisomy 13, trisomy 18 or triploidy. The distribution of FFBR scores is shown in Figure 2a. The rate of high FFBR scores was 63.7% in the AMA referral group, 41.7% in cases with abnormal maternal serum screen, 39.5% for those with an abnormal ultrasound examination, 40.5% for those with family history and 28.3% in the low-risk referral group (Table S2). The rate of trisomy 13, trisomy 18 or triploidy among the cases with a high FFBR score was significantly higher than expected based on a-priori risk (5.7% (95% CI, 3.9–7.9%) observed vs 0.7% expected; P < 0.001).

| Table 1 Observed abnormal outcome of study cohort compared with expected outcome based on prior risk, according to fetal fraction-based risk (FFBR) category |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Outcome** | **FFBR ≥ 1% (High FFBR score) (n = 564)** | **FFBR < 1% (n = 584)** | **Total (n = 1148)** |
| **Chromosomal abnormality** | **Observed** | **Expected** | **P** | **Observed** | **Expected** | **P** | **Observed** | **Expected** | **P** |
| All* | 40 (7.1) | 12.1 (2.2) | < 0.001 | 8 (1.4) | 5.2 (0.9) | 0.119 | 48 (4.2)† | 17.3 (1.5) | < 0.001 |
| T13, T18 or triploidy | 32 (5.7)† | 3.7 (0.7) | < 0.001 | 3 (0.5) | 1.3 (0.2) | 0.087 | 35 (3.0)† | 5.0 (0.4) | < 0.001 |
| T13 | 3 (0.53) | 0.87 (0.2) | 0.034 | 0 | 0.28 (0.05) | 0.38 | 3 (0.3) | 1.2 (0.1) | 0.069 |
| T18 | 9 (1.6) | 2.7 (0.5) | 0.001 | 2 (0.3) | 0.88 (0.15) | 0.14 | 11 (1.0) | 3.6 (0.3) | < 0.001 |
| Triploidy | 20 (3.6) | 10.0 (0.2) | < 0.001 | 1 (0.2) | 0.11 (0.02) | 0.053 | 21 (1.8) | 0.21 (0.02) | < 0.001 |
| T21 | 5 (0.9) | 6.5 (1.2) | 0.293 | 3 (0.5) | 2.1 (0.4) | 0.26 | 8 (0.70) | 8.6 (0.8) | 0.436 |
| Monosomy X | 2 (0.4) | 1.9 (0.3) | 0.432 | 0 | 1.8 (0.3) | 0.080 | 2 (0.2) | 3.7 (0.3) | 0.197 |
| Other | 1 (0.2)‡ | — — | — — | 2 (0.3)§ | — — | — — | 3 (0.3)‡ | — — | — — |
| Pregnancy loss | — — | — — | — — | 22 (3.8)** | 22.4 (3.8) | 0.48 | 120 (10.5)†† | 88.0 (7.0) | < 0.001 |
| All* | 98 (17.4)¶ | 58.4 (10.4) | < 0.001 | 16 (2.7) | 22.4 (3.8) | 0.078 | 99 (8.6) | 88.0 (7.0) | 0.016 |
| With normal or unknown karyotype | 83 (14.7) | 58.4 (10.4) | < 0.001 | 24 (4.1) | — — | — — | 147 (12.8) | — — | — — |

Data are presented as n (%). *Includes pregnancy loss with confirmed chromosomal abnormality. †Excludes cases with suspected but not proven chromosomal abnormality (on NIPT redraw). ‡4q terminal deletion. §Trisomy X (n = 1), 22q duplication (n = 1). ¶Includes 15 cases with confirmed chromosomal abnormality. **Includes six cases with confirmed chromosomal abnormality. ††Includes 21 cases that also showed a chromosomal abnormality, FF, fetal fraction; T13, trisomy 13; T18, trisomy 18; T21, trisomy 21.
Distribution of fetal fraction-based risk (FFBR) scores in cases for which single-nucleotide polymorphism-based non-invasive prenatal testing did not return a result, in entire cohort (n = 1148) (a), and in cases with FFBR score ≥ 25% (n = 78) (b). Unaffected pregnancies (■), and cases with trisomy 18 (■) and triploidy (■) are shown. Pregnancy losses include only those of unknown karyotype (■).

Figure 2

Table 1. In cases that did not receive a high FFBR score, the incidence of trisomy 13, trisomy 18 or triploidy was not significantly different from that expected (0.5% (95% CI, 0.1–1.5%) vs 0.2% expected; P = 0.087). The three cases of trisomy 13, trisomy 18 and triploidy that did not receive a high FFBR score had multiple ultrasound anomalies at the time of NIPT.

Of the 35 cases affected by trisomy 13, trisomy 18 or triploidy, 32 (91.4%) received a high FFBR score. Thus, the sensitivity of the FFBR model was 91.4% (95% CI, 76.9–98.2%) for trisomy 13, trisomy 18 and triploidy combined, with a positive predictive value (PPV) of 5.7% (32/564; 95% CI, 3.9–7.9%). Of the 78 cases with FFBR scores ≥ 25%, there were 14 cases of triploidy, one case of trisomy 18 and 31 pregnancy losses. For the 15 cases with FFBR scores ≥ 98%, there was one case of triploidy and 11 pregnancy losses (Figure 2b). Cases with triploidy tended to have lower FF and higher FFBR scores compared with cases with trisomy (Figure S2).

Because prior risk for trisomy 18 and trisomy 13 increases with MA, the FF threshold for receiving a high-risk FFBR score also varies with MA (as well as with GA and MW). To illustrate this, the mathematical relationship between MW and FF at ≥ 1% risk, the cut-off for a high FFBR score used in this study, is presented in Figure 3. Figure S3 illustrates how changing the threshold for a high-risk FFBR result would affect our findings. A receiver–operating characteristics curve for the FFBR algorithm (detection rate vs false-positive rate) is presented in Figure S4, which demonstrates the effect of changing the high-risk threshold.

Although the FFBR model was designed to evaluate the combined risk for trisomy 18, trisomy 13 or triploidy, given the unexpectedly high rate of triploidy (n = 21), further analysis of this group was performed. Of the 21 cases of triploidy in the analysis cohort, 20 (95% CI, 76.2–99.9%) were identified using the high FFBR cut-off of ≥ 1% (Table 1). The only case of triploidy that did not receive a high FFBR score was tested at a gestational age of 27 weeks after multiple congenital anomalies (intrauterine growth restriction, congenital heart disease, Dandy–Walker malformation, and hydrops) were identified. Triploidy cases had the lowest mean FF (2.3%; range, 0.8–5.8%) of the three FF-related chromosomal abnormalities (Table S1). Clinical parameters and outcomes of the triploidy cases are detailed in Figure S5. With the exception of the single case that did not receive a high FFBR score, all other cases underwent NIPT prior to 22 weeks’ gestation, with 13 (62%) cases having NIPT prior to 14 weeks. Notably, 16 (76%) of the triploidy cases were confirmed to be alive in the second trimester, including 78% (7/9) of the pregnancies that were terminated. Of the triploidy cases confirmed by cytogenetic analysis, four were of the digynic type. The remainder were of unknown type.
After 14 weeks' gestation 36/564 (6.4) 17/584 (2.9)

Without confirmed chromosomal abnormality

2.7–6.1%) (Table 1).

this combined rate was less than 1 in 20 (4.1%; 95% CI, 18.5–25.5%) cases. For cases without a high FFBR score, the high FFBR cohort exceeded 1 in 5 (21.8%; 95% CI, 18.5–25.5%) cases. For cases without a high FF score, this combined rate was less than 1 in 20 (4.1%; 95% CI, 2.7–6.1%) (Table 1).

DISCUSSION

Although low FF has been associated with increased risk of chromosomal abnormalities, and there are many causes of low FF (including early GA, higher MW, and aneuploidy), until now there was no way to quantify this risk when reporting NIPT results. The FFBR algorithm was developed as part of an ongoing effort to improve the ability of SNP-based NIPT to identify cases at increased risk of chromosomal abnormalities. It adjusts risk for trisomy 13, trisomy 18 or triploidy for cases receiving ‘no result’ on SNP-based NIPT by accounting for the effects of GA and MW on FF, and identifies cases with a low adjusted FF.

In this study, the FFBR algorithm allocated 1148 cases with an initial ‘no result’ on NIPT into two approximately equally-sized groups: one with high FFBR (a risk of ≥ 1% for trisomy 13, trisomy 18 or triploidy) and one for which risk remained unchanged. Although some studies have suggested that the rate of trisomy 21 and monosomy X might be elevated among cases with failed test or with low FF3,28, we observed no such association.

Diandric (paternal) triploidy, which tends to have an elevated FF, is detected with good sensitivity by SNP-based NIPT24,29. On the other hand, digynic triploidy, which is associated with a small placenta and low FF, often yields ‘no result’ on NIPT due to low FF23. Consistent with that, in this cohort, the incidence of triploidy was significantly more frequent than expected, confirming the association between low FF and fetal triploidy. Although not conclusively established through follow-up testing, these cases were most likely digynic. Thus, it appears that by adding the FFBR algorithm to the SNP-based NIPT algorithm, both types of triploidy can be identified with good sensitivity. In contrast, NIPT based on counting methodologies cannot detect either type of triploidy.

The overall rate of pregnancy loss in the study cohort was also significantly higher than expected (10.5% vs 7.0%), and the rate was higher still for women who received a high FFBR score (17.4% vs 10.4%). The finding that pregnancy loss is more frequent among cases with low FF would be expected for those cases with a chromosomal abnormality.

A limitation of the FFBR algorithm is that its construction was based on only a limited number of affected cases. Despite this limitation, our validation data indicated that we could effectively modify risk for an affected pregnancy. Use of body mass index instead of weight or adjustments for complications or medications that affect FF might enhance the algorithm. Our observations of increased risk for pregnancy loss in the cohort need to be interpreted cautiously. There is a paucity of data on MA-specific and GA-specific loss rates, and our expected rates are projected from data indicated that we could effectively modify risk for an affected pregnancy. Use of body mass index instead of weight or adjustments for complications or medications that affect FF might enhance the algorithm. Our observations of increased risk for pregnancy loss in the cohort need to be interpreted cautiously. There is a paucity of data on MA-specific and GA-specific loss rates, and our expected rates are projected from a relatively small dataset26. Because pregnancy losses do not routinely receive karyotyping, there is a possibility that we underestimated the number of chromosomally abnormal losses in the study cohort. A further limitation of our study is that we were unable to look for correlations between FF and maternal serum markers. Both first- and second-trimester serum markers are known to be low in trisomy 18, trisomy 13 and digynic triploidy30–32. The extent to which a combination of FF measurement and
biochemistry might contribute to identification of affected pregnancies remains to be determined.

Early NIPT literature raised concerns that chromosomal abnormalities were over-represented in low-FF or ‘no-result’ cohorts. Moreover, FF has been demonstrated to be lower than normal in pregnancies affected by trisomy 13 or trisomy 18. In response, professional society guidelines recommend that all cases receiving a ‘no result’ on NIPT should be followed up with invasive testing. In this study, we found an over-representation of chromosomal abnormalities associated with low FF and pregnancy loss, but not trisomy 21 or monosomy X. Given the high rate of fetal loss observed among cases receiving high FFBR scores, and the fact that most cases of trisomy 13, trisomy 18 and triploidy can be detected by ultrasound examination, clinical management of women receiving high FFBR scores should include confirmation of fetal viability and GA, genetic counseling, anatomy scan and the offer of diagnostic testing. These results also suggest that care plans take into consideration the residual risk for pregnancy loss. For women who did not receive a high FFBR score, we found no evidence of increased risk for any of these conditions (beyond risk based on MA and GA at the time of NIPT referral).

As the FFBR method can identify pregnancies at risk for trisomy 13, trisomy 18 and triploidy in cases for which the FFBR algorithm is otherwise not sufficient for analysis, the FFBR model is distinct from the previously published SNP-based algorithm. Because the underlying screening methods for low FF are the same regardless of the screening criterion used, so long as an NIPT method can accurately measure FF, the FFBR approach could be applied. Each laboratory would need to develop and validate their own model based on their measurement of FF and criteria for calling results at low FF. This is preferable to using strict FF cut-offs that do not account for the significant effects of MW or GA.

Overall, the FFBR model identified 32 of the 35 cases of trisomy 13, trisomy 18 and triploidy in the analysis cohort; a sensitivity of 91.4%, with a PPV of 5.7%. The overall rate of any chromosomal abnormality or pregnancy loss in the high FFBR group was 21.8%. We believe this performance justifies use of this information in NIPT reporting and integration into overall pregnancy management.

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Risk and fetal fraction

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*SUPPORTING INFORMATION ON THE INTERNET*

The following supporting information may be found in the online version of this article:

- Appendix S1 Supplemental methods
  - Table S1 Maternal and pregnancy characteristics by fetal outcome and fetal fraction-based risk (FFBR) cohort
  - Table S2 Reasons for non-invasive prenatal testing referral
  - Figure S1 Distribution of fetal fraction (FF) for the analysis cohort (*n* = 1148).
  - Figure S2 Fetal fraction (FF) and FF-based risk (FFBR) scores in trisomy and triploidy cases.
  - Figure S3 Effect of sliding cut-off on fetal fraction-based risk (FFBR) threshold. X-axis is on a log scale. As the threshold for a high FFBR score increases from 0 to 100%, the fraction of cases classified as high FFBR decreased (red). Conversely, as the threshold for a high FFBR score increases, the positive predictive value (PPV) for high FFBR cases (blue) and the rate of pregnancy loss (green) also increased.
  - Figure S4 Receiver–operating characteristic curve showing the effect of adjusting the high FFBR risk threshold from 0.5% to 90%.
  - Figure S5 Clinical outcomes for triploidy cases (*n* = 21).
  - Figure S6 Expected fetal fraction (FF) distributions by karyotype for women at the cohort median age (33 years) and median gestational age (11 weeks) for two different maternal weights (MW), 68.0 kg and 113.4 kg. Upper panels: 0 to 25%; lower panels: 0 to 5%.

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