# Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18

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**OBJECTIVE:** We sought to develop a novel biochemical assay and algorithm for the prenatal evaluation of risk for fetal trisomy 21 (T21) and trisomy 18 (T18) using cell-free DNA obtained from maternal blood.

**STUDY DESIGN:** We assayed cell-free DNA from a training set and a blinded validation set of pregnant women, comprising 250 disomy, 72 T21, and 16 T18 pregnancies. We used digital analysis of selected regions in combination with a novel algorithm, fetal-fraction optimized risk of trisomy evaluation (FORTE), to determine trisomy risk for each subject.

**RESULTS:** In all, 163/171 subjects in the training set passed quality control criteria. Using a Z statistic, 35/35 T21 cases and 7/7 T18 cases

had Z statistic >3 and 120/121 disomic cases had Z statistic <3. FORTE produced an individualized trisomy risk score for each subject, and correctly discriminated all T21 and T18 cases from disomic cases. All 167 subjects in the blinded validation set passed quality control and FORTE performance matched that observed in the training set correctly discriminating 36/36 T21 cases and 8/8 T18 cases from 123/123 disomic cases.

**CONCLUSION:** Digital analysis of selected regions and FORTE enable accurate, scalable noninvasive fetal aneuploidy detection.

## BACKGROUND AND OBJECTIVE

The American Congress of Obstetricians and Gynecologists recommends that pregnant women be offered noninvasive screening for fetal chromosomal abnormalities. However, existing screening methods have detection rates in the range of 90–95% and false-positive rates in the range of 3–5%. To address these limitations, several groups have pursued noninvasive methods for detecting fetal aneuploidy.

Massively parallel shotgun sequencing (MPSS) has been used to quantify precisely cell-free DNA (cfDNA) fragments

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## ★ EDITORS' CHOICE ★

for fetal trisomy detection. The chromosomal dosage resulting from fetal aneuploidy is directly related to the fraction of fetal cfDNA. Because MPSS is indiscriminate with respect to chromosomal origin and chromosome 21 (chr21) represents ~1.5% of the human genome, ~6.3 million uniquely mapped reads are required to ensure sufficient chr21 counts. Given typical MPSS mapping yields of ~25%, this translates to 25 million raw sequencing reads per sample. This requirement constrains the throughput, cost efficiency, and clinical utility of MPSS for aneuploidy detection. Selective sequencing of relevant chromosomes can address these constraints.

We extended digital analysis of selected regions (DANSR) to assay simultaneously polymorphic and nonpolymorphic loci in a single reaction, enabling estimation of chromosome proportion and fetal fraction. We describe a novel analysis algorithm, the fetal-fraction optimized risk of trisomy evaluation (FORTE), which uses this information to compute the likelihood of fetal trisomy in each subject.

## MATERIALS AND METHODS

Subjects were prospectively enrolled. A subset of enrolled subjects consisting of

250 women with disomic pregnancies, 72 with trisomy 21 (T21) pregnancies, and 16 with trisomy 18 (T18) pregnancies was selected. Subjects were randomized into a training set consisting of 127 disomic pregnancies, 36 T21 pregnancies, and 8 T18 pregnancies and a validation set of 123 disomic pregnancies, 36 T21 pregnancies, and 8 T18 pregnancies.

To assess chromosome proportion, we designed DANSR assays against 576 nonpolymorphic loci on chromosome 18 and 576 such loci on chr21. To assess fetal fraction, we designed assays against a set of 192 single nucleotide polymorphism–containing loci on chromosomes 1-12. DANSR product was generated from each subject sample.

The FORTE algorithm estimates the risk of aneuploidy using an odds ratio comparing a model assuming a disomic fetal chromosome and a model assuming a trisomic fetal chromosome. Prior risk was taken from well-established tables capturing the risk of trisomy associated with maternal and gestational age.

## RESULTS

The mean maternal and gestational ages of disomic, T21, and T18 subjects were not significantly different between training and validation sets (all $t$ test $P > .05$).

**FIGURE**
## Validation set FORTE risk vs fetal fraction



A

B
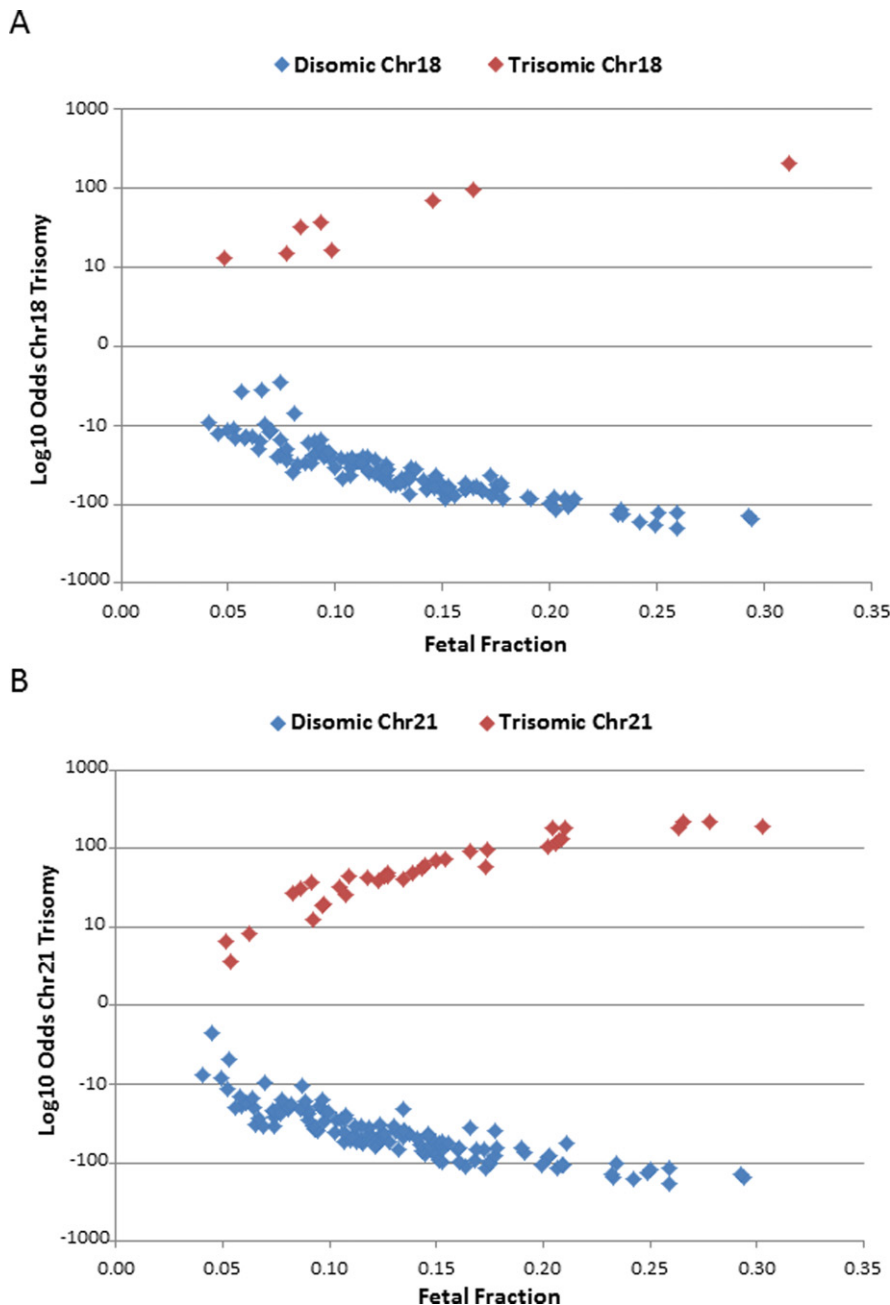
FORTE-computed odds of trisomy vs disomy for **A,** chromosome 18 (chr18) or **B,** chromosome 21 (chr21) is plotted vs fraction of fetal DNA for each blinded validation set subject. Disomic subjects are represented as *blue diamonds,* trisomic subjects as *red.* As in training set, FORTE-computed odds of trisomy increases with increasing fetal fraction among trisomic subjects and decreases with increasing fetal fraction among disomic samples. Thus, FORTE metric reflects increase in certainty resulting from increased fetal fraction in both disomic and trisomic samples.

*FORTE,* fetal-fraction optimized risk of trisomy evaluation.

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This training set consisted of 127 normal, 36 T21, and 8 T18 pregnancies. Six normal, 1 T18, and 1 T21 samples (8/171; 5%) did not meet quality control criteria. In all, 120/121 (99.2%) disomic samples had Z statistics <3; 1 disomic sample had a chr21 Z statistic of 3.5. All T21 (35/35) and T18 (7/7) samples had chromosome proportion Z statistics >3. Thus, using Z statistic analysis, DANSR exhibited 99.2% specificity and 100% sensitivity for T21 and 100% specificity and 100% sensitivity for T18.

As expected, the FORTE odds demonstrated a response to fetal fraction in both trisomic and disomic samples. FORTE correctly differentiated all euploid from aneuploid samples. The difference between the lowest aneuploid odds and the euploid odds was $>10^{12}$. All aneuploidy samples had odds $>10^{10}$.

To test the performance of the DANSR/FORTE assay in an independent set of subjects, we assayed a blinded validation set consisting of 123 normal, 36 T21, and 8 T18 pregnancies. All samples passed quality control criteria (Figure). FORTE correctly differentiated all trisomy from disomy subjects. The difference between the lowest aneuploid odds and the highest euploid odds was $10^{3.9}$. All 36 T21 and 8 T18 samples had trisomy odds $>10^{2.67}$ (>99.8% risk of trisomy).

## COMMENT

This study demonstrates the analytical performance of DANSR and FORTE in detecting fetal T21 and T18 in pregnant women of ≥10 weeks' gestational age. The combination of DANSR and FORTE correctly identified all 36 cases of T21 and 8 cases of T18 as having >99% risk for each trisomy in a blinded analysis. The separation in the risk score between trisomic and disomic samples was at least 1000-fold.

DANSR enables genotyping of individual polymorphic loci, which is not possible with current MPSS approaches. DANSR allowed us to develop an integrated assay to assess polymorphic as well as nonpolymorphic loci, thereby permitting simultaneous determination of fetal fraction and chromosome proportion.

FORTE is a novel algorithm that incorporates multiple risk factors to generate an individualized odds score for trisomy. While the result format of FORTE is similar to that of current prenatal screening results, the combination of DANSR and FORTE yields greatly improved performance.

Because DANSR enables directed analysis of specific genomic regions, DANSR could potentially be used to evaluate genetic conditions besides trisomy, such as subchromosomal conditions (eg, microdeletions). Because the FORTE algorithm can incorporate multiple clinical risk factors, incorporation of additional risk information, such as from ultrasonography, warrants investigation. Current cfDNA analysis turnaround times of 7-10 days are likely to be reduced with further technical advances in DNA sequencing. Should noninvasive testing via cfDNA analysis become affordable and widely accessible, current prenatal screening and testing options may evolve to a simpler model in which cfDNA analysis and ultrasound become a new standard for all pregnant women.

### CLINICAL IMPLICATIONS

■ Digital analysis of selected regions is a directed and efficient method of cell-free DNA analysis for noninvasive prenatal detection of fetal trisomy 21 and trisomy 18 and therefore has the potential to be an affordable blood test for pregnant women of ≥10 weeks' gestational age.

■ The fetal-fraction optimized risk of trisomy evaluation is a novel algorithm using data from cell-free DNA analysis in conjunction with other clinical risk factors to provide individualized risk estimates of fetal trisomy similar to that of current prenatal screening.

■ Combining the digital analysis of selected regions assay with the fetal-fraction optimized risk of trisomy evaluation algorithm provides a robust and accurate assessment of fetal trisomy risk.

■ Current prenatal screening and testing options may evolve to a simpler model in which cell-free DNA analysis and ultrasound become a new standard for all pregnant women.  ■