GENETICS

Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18

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OBJECTIVE: We sought to evaluate performance of a noninvasive prenatal test for fetal trisomy 21 (T21) and trisomy 18 (T18).

STUDY DESIGN: A multicenter cohort study was performed whereby cell-free DNA from maternal plasma was analyzed. Chromosome-selective sequencing on chromosomes 21 and 18 was performed with reporting of an aneuploidy risk (High Risk or Low Risk) for each subject.

RESULTS: Of the 81 T21 cases, all were classified as High Risk for T21 and there was 1 false-positive result among the 2888 normal cases, for a sensitivity of 100% (95% confidence interval [CI], 95.5–100%) and a false-positive rate of 0.03% (95% CI, 0.002–0.20%). Of the 38 T18 cases, 37 were classified as High Risk and there were 2 false-positive results among the 2888 normal cases, for a sensitivity of 97.4% (95% CI, 86.5–99.9%) and a false-positive rate of 0.07% (95% CI, 0.02–0.25%).

CONCLUSION: Chromosome-selective sequencing of cell-free DNA and application of an individualized risk algorithm is effective in the detection of fetal T21 and T18.

Key words: aneuploidy detection, cell-free fetal DNA, Down syndrome, noninvasive prenatal diagnosis, trisomy

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Currently, the most effective and commonly used prenatal screening tests for fetal aneuploidy use a combination of maternal age, sonographic measurement of the fetal nuchal translucency, and measurement of maternal serum

★ EDITORS' CHOICE ★

screening markers in the first and second trimesters.[1-4] Although prenatal screening tests have greatly improved in the past decade, the best performing screening tests have false-positive rates of 2-3% and false-negative rates of ≥5%.[1-4] Positive screening results require confirmation with diagnostic testing (eg, chorionic villus sampling [CVS] or amniocentesis); these tests carry fetal loss rates of approximately 1

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in 300 procedures.[5,6] Current screening paradigms are not uniform, with multiple algorithms available for use at various stages of pregnancy, and therefore can be confusing to incorporate into clinical practice.[7]

The presence of fetal and maternal cell-free DNA (cfDNA) circulating in maternal plasma is now widely appreciated, and several groups have demonstrated fetal trisomy 21 (T21) detection using massively parallel DNA shotgun sequencing (MPSS) in case-control studies.[8-12] This technique sequences cfDNA fragments to determine their specific chromosomal origin; a slightly higher than expected percentage of chromosome 21 fragments indicates that the fetus has a third chromosome 21.[8,9] In addition to detecting T21, several studies have reported on the use of MPSS in assaying trisomy 18 (T18) and trisomy 13 (T13).[11-14]

Despite these promising results, MPSS randomly analyzes DNA from the entire genome, resulting in higher cost and complexity than is practical for widespread clinical adoption. Recent studies have reported on an alternative assay, Digital ANalysis of Selected Regions (DANSR), that selectively evaluates specific genomic fragments from cfDNA, providing more efficient use of sequencing and potentially reduced costs when compared to MPSS.[15-17] This process of chromosome-selective sequencing has been extended to enable simultaneous determination of the fraction of fetal cfDNA in the maternal plasma as well as the chromosome proportion by assaying polymorphic and nonpolymorphic loci.[16] When combined with a novel analysis algorithm, the Fetal-fraction Optimized Risk of Trisomy Evaluation (FORTE), this information can provide an individualized assessment of trisomy risk.[16] In a recently published blinded independent study, the use of DANSR and FORTE was found to separate all cases of T21 and 98% of cases of T18 from euploid pregnancies in 400 singleton pregnancies at 11-13 weeks' gestation.[17]

This report describes the results of a multicenter study designed to evaluate the performance of this noninvasive prenatal assay and algorithm in a large cohort of women prior to invasive prenatal diagnostic testing.

MATERIALS AND METHODS

Study population

This was a prospective, cohort study comprising pregnant women aged ≥18 years, at gestational age ≥10 weeks, with a singleton pregnancy, who were planning to undergo invasive prenatal diagnosis for any indication. Subjects who were pregnant with >1 fetus, or who themselves had a known aneuploidy, had active malignancy or a history of metastatic cancer, or had already undergone CVS or amniocentesis during the current pregnancy were excluded.

Subjects were prospectively enrolled after providing informed consent at selected prenatal care centers in the United States, The Netherlands, and Sweden. Institutional review board approval was obtained at all participating centers.

Sample collection and preparation

Approximately 20 mL of blood was collected from each subject prior to any invasive procedure into a Cell-free BCT tube (Streck, Omaha, NE). Samples were sent directly to the laboratory without processing and needed to be received within 7 days of collection with no temperature excursions indicating freezing. Plasma was isolated from blood via a double centrifugation protocol. cfDNA was isolated from plasma using the Dynabeads Viral NA DNA purification kit (Dynal, Grand Island, NY) protocol, with minor modifications, and each sample was arrayed into individual wells of a 96-well microtiter plate.

Test methods

Each subject's cfDNA sample was isolated and quantified using the DANSR assay, which has been described previously.[15] Briefly, this method uses ligation of locus-specific oligonucleotides to produce a sequencing template only from selected genomic loci, thus reducing the amount of DNA sequencing needed. The FORTE algorithm, also previously described in detail,[16] was used to estimate the risk of aneuploidy for chromosomes 21 and 18 in each sample. The FORTE risk score is determined by calculating the odds ratio for trisomy based on chromosome 21 and 18 cfDNA counts, and fraction of fetal cfDNA in the sample, then applying this as a likelihood ratio to the a priori trisomy risk based on the maternal age and gestational age.[16] A predefined cutoff value of 1 in 100 (1%) was designated as the threshold for classifying a sample as High Risk vs Low Risk. The cutoff value was determined based on previous analyses that demonstrated an optimal separation between trisomy and euploid samples. Samples that did not generate a result were classified as low (<4%) fraction of fetal cfDNA, inability to measure fraction of fetal cfDNA, unusually high variation in cfDNA counts, and failed sequencing.

The laboratory personnel who performed the analyses were blinded to the clinical information associated with each sample. Finalized results were transferred to an independent data management center (Advance Research Associates, Mountain View, CA) for merging of assay and clinical data, and unblinding.

Data analysis

Sample size was calculated based on obtaining sufficient cases of T21 to achieve lower bound 95% confidence intervals (CI) for sensitivity and specificity that were comparable or superior to current prenatal screening tests. The target performance for the DANSR and FORTE method was anticipated to be ≥98% for both sensitivity and specificity based on previous data.[15-17] Using this estimate, at least 60 cases of T21 would be required to provide a lower 95% CI of 90% for sensitivity. Assuming a T21 prevalence of 1 in 50 in the study cohort, based on a typical population of women undergoing invasive prenatal diagnosis, at least 3000 eligible subjects would be required. Categorical variables were summarized by the number and percentage of subjects in each category. Continuous variables were summarized as total number, mean, SD, minimum, median, and maximum values. We used χ^2 tests with Bonferroni correction when comparing categorical variables and proportions. Linear regression models were used to test the correlation between continuous variables (eg, percent fetal and gestational age) with the null hypothesis that the slope between 2 continuous variables is 0. Multivariate lo-