OBSTETRICS

Clinical experience and follow-up with large scale single-nucleotide polymorphism–based noninvasive prenatal aneuploidy testing

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OBJECTIVE: We sought to report on laboratory and clinical experience following 6 months of clinical implementation of a single-nucleotide polymorphism–based noninvasive prenatal aneuploidy test in high- and low-risk women.

STUDY DESIGN: All samples received from March through September 2013 and drawn ≥9 weeks' gestation were included. Samples that passed quality control were analyzed for trisomy 21, trisomy 18, trisomy 13, and monosomy X. Results were reported as high or low risk for fetal aneuploidy for each interrogated chromosome. Relationships between fetal fraction and gestational age and maternal weight were analyzed. Follow-up on outcome was sought for a subset of high-risk cases. False-negative results were reported voluntarily by providers. Positive predictive value (PPV) was calculated from cases with an available prenatal or postnatal karyotype or clinical evaluation at birth.

RESULTS: Samples were received from 31,030 patients, 30,705 met study criteria, and 28,739 passed quality-control metrics and received a report detailing aneuploidy risk. Fetal fraction correlated positively with gestational age, and negatively with maternal weight. In all, 507 patients received a high-risk result for any of the 4 tested conditions (324 trisomy 21, 82 trisomy 18, 41 trisomy 13, 61 monosomy X; including 1 double aneuploidy case). Within the 17,885 cases included in follow-up analysis, 356 were high risk, and outcome information revealed 184 (51.7%) true positives, 38 (10.7%) false positives, 19 (5.3%) with ultrasound findings suggestive of aneuploidy, 36 (10.1%) spontaneous abortions without karyotype confirmation, 22 (6.2%) terminations without karyotype confirmation, and 57 (16.0%) lost to follow-up. This yielded an 82.9% PPV for all aneuploidies, and a 90.9% PPV for trisomy 21. The overall PPV for women aged ≥35 years was similar to the PPV for women aged <35 years. Two patients were reported as false negatives.

CONCLUSION: The data from this large-scale report on clinical application of a commercially available noninvasive prenatal test suggest that the clinical performance of this single-nucleotide polymorphism–based noninvasive prenatal test in a mixed high- and low-risk population is consistent with performance in validation studies.

Key words: low-risk, noninvasive prenatal testing, single-nucleotide polymorphism, trisomy 21

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Since becoming clinically available in late 2011, cell-free DNA (cfDNA)-based noninvasive prenatal testing (NIPT) for fetal aneuploidy has seen an unprecedented rapid adoption into clinical care.[1] This followed multiple publications on methodologies, validation, and test performance,[2-14] all demonstrating improved sensitivities

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and lower false-positive (FP) rates than current screening methods. Opinion statements by national and international professional societies support the clinical use of NIPT in pregnant women, with most recommending use restricted to women at high risk for fetal aneuploidy.[15-17]

Two approaches to NIPT have been developed and commercialized. In the first approach, fetal chromosome copy number is determined by comparing the number of sequence reads from the chromosome(s) of interest to those from reference chromosomes.[7,8,11-13,18-22] The second approach entails targeted amplification and sequencing of single-nucleotide polymorphisms (SNPs).[2-5,23,24] This approach requires a sophisticated informatics-based method to compute aneuploidy risk through SNP distribution. Validation of the SNP-based NIPT method at 11-13 weeks' gestation was recently reported, demonstrating high sensitivity and specificity for detection of trisomy 21, trisomy 18, trisomy 13, Turner syndrome (monosomy X), and triploidy.[2,3]

Despite hundreds of thousands of tests already having been performed worldwide, there are few large-scale reports describing performance of NIPT in actual clinical settings,[22,25] with most studies reporting on <1000 total patients.[26-29] Here, laboratory and clinical experience of >31,000 women who received prenatal screening with a SNP-based NIPT is reported.

TABLE 1
Demographics of commercial cases

Demographic	Whole cohort, n = 31,030	Follow-up cohort, n = 17,885
Maternal age, y[a]		
Mean	33.3 ± 6.0	33.7 ± 6.1
Median	35.0	35.0
Range	14.0–60.0	14.0–52.0
Gestational age, wk		
Mean	14.0 ± 4.4	14.5 ± 4.7
Median	12.6	13.0
Range	3.1–40.9	9.0–40.9[b]
Maternal weight, lb[c]		
Mean	158.4 ± 39.2	157.2 ± 37.9
Median	149.0	148.0
Range	83.0–425.0	83.0–385.0
Fetal fraction, %		
Mean	10.2 ± 4.5	10.8 ± 4.4
Median	9.6	10.1
Range	0.6–50.0	3.7–50.0[b]

[a] At estimated date of delivery; [b] As the follow-up cohort does not include any out-of-specification cases, or any cases that failed to receive a noninvasive prenatal testing result, minimum gestational age and fetal fraction are higher than in the whole cohort—however, mean values and SD are equivalent between the 2 cohorts; [c] Analysis of maternal weight was limited to centers and laboratories that provided this information, and samples originating from United States to avoid inconsistent weight units.

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Materials and Methods

This is a retrospective analysis of prospectively collected data on 31,030 cases received for commercial testing from March through September 2013. This study received a notification of exempt determination from an institutional review board (Albert Einstein College of Medicine Institutional Review Board: no. 2014-3307). Samples were classified as out of specification and excluded in cases of gestational age <9 weeks, multiple gestation, donor egg pregnancy, surrogate carrier, missing patient information, sample received >6 days after collection, insufficient blood volume (<13 mL), wrong collection tube used, or if the sample was damaged.

Analysis was performed for all samples on chromosomes 13, 18, 21, X, and Y, and included detection of trisomy 21, trisomy 18, trisomy 13, and monosomy X. All samples were processed and analyzed at Natera Inc's Clinical Laboratory Improvement Act (CLIA)-certified and College of American Pathologists (CAP)-accredited laboratory (San Carlos, CA). Laboratory testing was performed as previously described using validated methodologies for cfDNA isolation, polymerase chain reaction amplification targeting 19,488 SNPs, high-throughput sequencing, and analysis with the next-generation aneuploidy test using SNPs (NATUS) algorithm.[2-5] Samples were subject to a stringent set of quality-control metrics. A second blood draw (redraw) was requested if total input cfDNA, fetal cfDNA fraction, or signal-to-noise ratio did not meet quality metrics, or for poor fit of the data to the model. In cases of large regions (>25%) of loss of heterozygosity or suspected maternal or fetal mosaicism, redraw was not requested. Reports included a risk score for the 4 aneuploidies; when requested, reports included fetal sex. Risk scores were calculated by combining the maximum likelihood estimate generated by the NATUS algorithm with maternal and gestational age prior risks. All samples with a risk score ≥1/100 were reported as high risk for fetal aneuploidy and samples with risk scores <1/100 were considered low risk. For the purposes of this study, the high-risk results were further divided into a maximum-risk score of 99/100 or an intermediate-risk score of ≥1/100 and <99/100. The presence of >2 fetal haplotypes (indicative of either triploidy or multiple