Published in final edited form as:

Obstet Gynecol. 2014 August ; 124(2 0 1): 210–218. doi:10.1097/AOG.0000000000000363.

Single-Nucleotide Polymorphism–Based Noninvasive Prenatal Screening in a High-Risk and Low-Risk Cohort

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Abstract

Objective—To estimate performance of a single-nucleotide-polymorphism–based noninvasive prenatal screen for fetal aneuploidy in high-risk and low-risk populations upon single venopuncture.

Methods—One thousand sixty-four maternal blood samples from 7 weeks of gestation and beyond were included; one thousand fifty-one were within specifications, 518 (49.3%) low-risk. Cell-free DNA was amplified, sequenced, and analyzed using the Next-generation Aneuploidy Test Using SNPs algorithm. Samples were called as trisomies 21, 18, 13, or monosomy X, or euploid, and male or female.

Results—Nine hundred sixty-six samples (91.9%) successfully generated a cell-free DNA result. Among these, sensitivity was 100% for trisomy 21 (58/58, CI: 93.8–100%), trisomy 13 (12/12, CI: 73.5–100%), and fetal sex (358/358 female, CI:99.0–100%; 418/418 male, CI: 99.1–100%), 96.0% for trisomy 18 (24/25, CI: 79.7–99.9%), and 90% for monosomy X (9/10, CI: 55.5–99.8%). Specificity for trisomies 21 and 13 was 100% (905/905 [CI: 99.6–100%] and 953/953 [CI: 99.6–100%], respectively) and for trisomy 18 and monosomy X was 99.9% (938/939 [CI: 99.4–100%] and 953/954 [CI: 99.4–100%], respectively). However, 16% (20/125) of aneuploid samples did not return a result; 50% (10/20) had a fetal fraction below the 1.5th percentile of euploid pregnancies. Aneuploidy rate was significantly higher in these samples ($p < 0.001$, odds ratio: 9.2, CI: 4.4–19.0). Sensitivity and specificity did not differ in low-risk and high-risk populations.

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Presented at the American Society of Human Genetics meeting (October 22–26, 2013, Boston, MA).

Financial Disclosure: The following authors are employees of Natera and hold stock or options to hold stock in the company: Bernhard Zimmermann, Milena Banjevic, Matthew Hill, Styrmir Sigurjonsson, Allison Ryan, Megan P. Hall, Michael Dodd, Phil Lacroute, Melissa Savage, Nikhil Chopra, Nathan Hunkapiller, Dennis E. Prosen, Sallie McAdoo, Zachary Demko, Asim Siddiqui, and Matthew Rabinowitz. Eugene Pergament is an unpaid member of Natera's advisory board, and is a consultant to PerkinElmer. Howard Cuckle is a paid consultant to Natera, as well as a consultant to Ariosa Diagnostics and PerkinElmer.

Conclusions—This noninvasive prenatal screen performed with high sensitivity and specificity in high-risk and low-risk cohorts. Aneuploid samples were significantly more likely to not return a result; the number of aneuploidy samples was especially increased among samples with low fetal fraction. This underscores the importance of redraws or, in rare cases, invasive procedures based on low fetal fraction.

Introduction

The identification of fetal cell-free DNA in maternal plasma facilitated the development of noninvasive prenatal screening methods for fetal whole-chromosomal aneuploidies with improved detection rates and accuracies as compared to traditional screening methods (1–18). The most straightforward cell-free DNA-based approaches utilize nonspecific amplification followed by massively parallel shotgun sequencing, identifying abnormal amounts of DNA from the chromosome-of-interest relative to reference chromosomes (5–11). For trisomy 21 and trisomy 18, reported sensitivities and false positive rates were >99% and <0.2%, respectively (19); however, for trisomy 13 and monosomy X these rates ranged from 78.6–94.4%, with false positive rates as high as 1% (6, 8, 13, 20), partially attributed to amplification efficiency variation (21, 22). While GC-correction can improve chromosome 13 sensitivity (23), similar corrections have not been reported for the X chromosome. A related method (“Digital ANalysis of Selected Regions”, DANSR) introduced a targeted amplification step, increasing efficiency by interrogating only chromosomes-of-interest (12–14, 18).

Next-generation technology employs cell-free DNA-based methods that incorporate genotypic information by targeting single nucleotide polymorphisms (SNPs), determining allele identity and count (15–17, 24). SNP-based methods rely on consistent amplification across alleles at a locus, and therefore are expected to return accurate copy number calls across chromosomes. We recently reported smaller studies of a SNP-based noninvasive prenatal screen that employed sophisticated informatics, did not require reference chromosomes, and calculated sample-specific accuracies for each chromosome; this method accurately detected fetal trisomies 21, 18, and 13, 47,XXY, 47,XYY, monosomy X, fetal sex, and triploidy (15–17, 24). Here, we present the results of a larger study of this SNP-based and informatics-based noninvasive prenatal screen; the objective was to determine the sensitivity and specificity for detecting those outcomes included in the clinical offering at the time of analysis, namely trisomies 21, 18, and 13, monosomy X, and fetal sex, in high-risk and low-risk populations.

Materials and Methods

Pregnant women were enrolled at 36 prenatal care centers under several Institutional Review Board-approved protocols from each participating site (Appendix 1, available online at <http://links.lww.com/xxx>), pursuant to local regulations. Enrolled women were 18 years of age with a singleton pregnancy of at least 7 weeks of gestation, and signed an informed consent. Results were not disclosed to patients. 1,064 maternal blood samples were drawn and corresponding paternal genetic samples (blood or buccal) were collected for 512 (48.1%) samples. Copy number on all samples was verified using standard invasive testing

with confirmatory fluorescence *in situ* hybridization and/or cytogenetic karyotype analysis, or by genetic testing of cord blood, buccal sample, saliva, or products of conception. Karyotype information in 204 cases was collected using a protocol that did not include reporting the sex of the fetus to laboratory personnel.

At the time of analysis, the protocol had been validated for identification of trisomy 21, trisomy 18, trisomy 13, and monosomy X in singleton, non-egg-donor pregnancies. Samples were considered outside of the specifications for this study and excluded from the determination of sensitivity and specificity upon a single venopuncture (“draw”) based on any of the following criteria: confirmed sex chromosome abnormality (47,XXX/XXY/XXYY), confirmed triploidy, or confirmed fetal mosaicism (see Results). Although sex chromosome abnormalities (47,XXX/XXY/XXYY) were correctly identified by the algorithm, they were not included in the initial study goals and were identified only after the cohort had been unblinded; as such, they were excluded from calculations of test performance metrics. Triploidy samples are identified by the presence of an additional parental haplotype, and do not result in an algorithm-calculated risk score; as such, they were similarly excluded from test performance calculations. In contrast to previous studies (8, 11, 20, 25), cases of confined placental mosaicism were included in all analyses to more closely represent the clinical experience.

Of the 1,064 blood samples collected, 543 (51.0%) were defined as high-risk by any of the following criteria, alone or in combination: abnormal serum screen, ultrasound abnormality, and maternal age of ≥ 35 years (Figure 1, Appendix 2, available online at <http://links.lww.com/xxx>). Of these, 401 were prospective high-risk blood samples from women planning to undergo an invasive procedure (amniocentesis or CVS) who subsequently had a confirmatory invasive procedure (398, 60 aneuploid) or who subsequently miscarried and had confirmatory products-of-conception testing (3, 1 aneuploid); 71 (63 aneuploid) were blood samples drawn 4 days after an invasive diagnostic procedure; and 71 were blood samples from women with advanced maternal age (≥ 35) with either follow-up confirmatory sampling at birth (24, none aneuploid) or via confirmatory products-of-conception testing after elective termination (47, 2 aneuploid). Studies have indicated that fetal fractions increase immediately after invasive procedure (26, 27), raising the concern that fetomaternal transfusion could bias results. Preliminary analysis supports that there was no significant increase in fetal fraction at 1 day or 7 days post-invasive procedure (not shown); post-procedure aneuploid samples in this cohort were all drawn at least four days after invasive procedure, reducing the likelihood of procedure-related bias.

The remaining 521 (49.0%, 9 aneuploid) blood samples were defined as low-risk, with maternal age <35 and lacking any reported high-risk indication(s) (Figure 1). Of these, 405 were from women undergoing elective termination with follow-up confirmation by products-of-conception testing, and 116 were from normal pregnancies with follow-up confirmatory sample collection at birth.

Cell-free DNA was amplified, sequenced, and analyzed using the Next-generation Aneuploidy Test Using SNPs algorithm to determine fetal ploidy status. The algorithm was blinded to sample karyotype. The first 574 (53.9%) samples were used to refine the quality

control parameters for identifying samples that do not pass (“no-calls”), although not the algorithm that generated a copy number and an associated confidence estimate. These were subsequently reanalyzed in a blinded fashion and were termed “internally blinded” samples. For the remaining 490 (46.1%) “externally blinded” samples, knowledge of the karyotype was made available to laboratory personnel only after calls were reported to external collaborators.

Sample preparation, 19,488-plex PCR, the Next-generation Aneuploidy Test Using SNPs algorithm, and sequence alignment were described previously (15–17, 24). Here, 2×10mL tubes (a single draw) of blood from each subject were analyzed. When available, paternal samples were included in the analysis, although they were not required. An average of 1.22×10^7 reads were mapped for each sample when sequenced at normal depth-of-read. Samples not generating sufficient information (35.6%) were re-sequenced at a higher depth-of-read (average: 2.45×10^7 mapped reads per sample). To evaluate whether paternal DNA contribution affected the proportion of samples that did not return a result, samples with an accompanying paternal sample were reanalyzed without using the paternal data. Fetal sex was identified as presence or absence of the Y chromosome.

Except where otherwise stated, significance was determined using a chi-square test with Yates correction (SigmaPlot 12.5). A linear regression model (SigmaPlot 12.5) was used to determine the correlation between gestational age and fetal fraction, with 8–10, 11–20, and >20 weeks of gestation evaluated independently. Fetal fractions were expressed as multiples of the median (MoM) among unaffected pregnancies, based on linear regression of median fetal fraction against the median day of gestation, weighted for the number of pregnancies at each completed week of gestation; p-values were calculated using the Wilcoxon Rank Sum Test. To determine performance of the model-generated confidences, the Next-generation Aneuploidy Test Using SNPs algorithm calculated model fit on a per-SNP basis for each chromosome in each sample. For each SNP, a p-value was calculated for the observed-to-true heterozygosity rate given fetal fraction, depth-of-read, and noise parameters derived by the algorithm. The distribution of p-values was then compared using the Kolmogorov-Smirnov test. Odds ratios (OR) were calculated using the following equation: $OR = ad/bc$, where a, b, c, and d were defined as the number of samples with (a) low fetal fraction that were aneuploid, (b) low fetal fraction that were euploid, (c) normal fetal fraction that were aneuploid, and (d) normal fetal fraction that were euploid.

Samples failed quality control metrics and did not return a result (no-calls) for the following reasons: 1) low fetal fraction (<3.8% fetal fraction, or <8.0% fetal fraction with insufficient cell-free DNA signal-to-noise ratio for the algorithm to make a high confidence call), 2) low amount of input cell-free DNA (<1,500 genome equivalents), 3) contamination (>0.2% contaminant DNA for fetal fractions of <8% and >0.5% for fetal fractions above 8%), 4) the presence of regions of loss of heterozygosity (LOH) in maternal DNA exceeding 25% of the chromosome, or 5) poor fit of the data to the model (likely due to anomalous biology, see Discussion). In clinical practice, no-calls (on all five chromosomes or on a single chromosome) due to sample-specific issues like low fetal fraction trigger a request for a redraw and reanalysis; these redraws are not billed. As this was a single-draw study it is not possible to report results after repeat sample analysis.

Sensitivity and specificity were calculated as previously reported (5–18, 20) by excluding the samples that do not return a result (no-called samples). Unlike previous studies (5–18, 20), the number of aneuploidy samples that do not return a result is also reported herein.

As expected with SNP-based technology, abnormal results beyond trisomies 21, 18, and 13, as well as monosomy X and fetal sex in singleton, non-donor pregnancies were obtained, including 47,XXX, 47,XXY, 47,XYY, and triploidy. How these samples would fare using the current clinical offering, which includes detection of these abnormalities, are described in Appendixes 3–4, available online at <http://links.lww.com/xxx>. Clinicians should be aware that while such results are reported with the current tests, in this study these samples were excluded from reported performance metrics.

Results

Patient demographics are described in Appendix 2, <http://links.lww.com/xxx>. The overall median gestational age was 14.3 weeks (mean: 17.0, range: 7.6–40.6), 14.1 weeks (mean: 17.2, range: 7.6–40.6) for euploid samples, and 14.6 weeks (mean: 15.8, range: 8.0–38.9) for aneuploid samples. The overall median maternal age was 30.0 years (mean: 30.3, range: 18–47), 29.0 years (mean: 29.6, range: 18–47) for euploid samples, and 37.0 years (mean: 35.1, range: 18–46) for aneuploid samples.

The overall cohort included 1,064 samples: 926 euploid, 67 trisomy 21, 32 trisomy 18, 14 trisomy 13, 12 monosomy X, 2 47,XXY, 1 47,XXX, 1 47,XYY, 6 triploid, and three samples (1 trisomy 13, 2 monosomy X) with confirmed fetal mosaicism. Eight monosomy X samples were previously reported (15). Thirteen samples (2 47,XXY, 1 47,XXX, 1 47,XYY, 1 trisomy 13 fetal mosaic, 2 monosomy X fetal mosaics, 6 triploids) were excluded from performance metric calculations (Figure 1, see Materials and Methods).

Figure 1 depicts a flowchart of samples. Of the 1,051 samples considered within the specifications for testing, a result was obtained for 966 (91.9%) samples; this includes samples of less than 9 weeks of gestation, which have a significantly higher no-call rate (Table 1); as a result, these samples are not accepted for clinical testing. The 966 samples that returned results included 58 trisomy 21, 25 trisomy 18, 12 trisomy 13, and 10 monosomy X samples. Figure 1 and Appendix 5 (Appendix 5 is available online at <http://links.lww.com/xxx>) describe the samples that failed to pass quality control metrics on all five chromosomes. Eight additional samples received a result for four of five chromosomes (7 euploid and 1 trisomy 21); these were treated as successful calls for sample-based analyses, and as no-calls for analyses concerning the chromosome for which no result was obtained.

Results for identification of fetal trisomy 21, 18, and 13, as well as monosomy X and fetal sex are described in Table 2. This includes sensitivity and specificity for the 966 samples considered within the specifications for testing, passing quality control, and of sufficient fetal fraction. The Next-generation Aneuploidy Test Using SNPs algorithm correctly identified 58/58 trisomy 21 samples (sensitivity: 100%, CI: 93.8–100%), 24/25 trisomy 18 samples (sensitivity: 96%, CI: 79.7–99.9%), 12/12 trisomy 13 samples (sensitivity: 100%,

CI: 73.5–100%), and 9/10 monosomy X samples (sensitivity: 90%, CI: 55.5–99.8%) (Table 2). The algorithm reported two false negative results (monosomy X and trisomy 18) and two false positive results (monosomy X and trisomy 18). The trisomy 18 false negative was shown to have a 40% euploid placenta (Appendix 3, <http://links.lww.com/xxx>). The method accurately identified fetal sex in all cases that passed quality control (418/418 male, 358/358 female). The specificity was 100% (905/905, CI: 99.6–100%) for trisomy 21, 99.9% (938/939, CI: 99.4–99.98%) for trisomy 18, 100% (953/953, CI: 99.6–100%) for trisomy 13, and 99.9% (952/953, CI: 99.4–100%) for monosomy X. The combined specificity including all four syndromes was 99.76% (856/858, CI: 99.16–99.97%) on a per-sample basis, and the overall sensitivity was 98.1% (103/105, CI: 93.3–99.8%). Performance on the externally blinded cohort was better than that of the overall cohort (Appendix 6, available online at <http://links.lww.com/xxx>). The algorithm correctly identified 1 47,XXX, 1 47,XXY, and 1 47,XYY; the remaining 47,XXY was a no-call due to low fetal fraction (Appendices 3–4 <http://links.lww.com/xxx>], Materials and Methods). High-risk calls warrant follow-up confirmatory testing.

In contrast to previous studies (5–18, 20), the fraction of aneuploid samples that did not return a result was calculated. Here, 20 (16.0%) of the 125 aneuploid samples within the specifications for testing did not return a result. Additionally, aneuploidy incidence was increased (20/86, 23.3%, this includes one trisomy 21 sample that was a no-call only on chromosome 21) in the samples that did not return a call when compared to the aneuploidy incidence in samples with a call ($p=0.004$). This translated to an odds ratio of 2.5 (CI: 1.4–4.3), indicating that samples without a result were two-and-a-half times more likely to be aneuploid.

Importantly, 75.0% (15/20) of the aneuploid samples without a result were no-called due to low fetal fraction or a combination of low fetal fraction and insufficient data clarity (mean: 3.4%; range 1.4% to 5.8%). Additionally, 50% (10/20) of the aneuploidy samples that did not return a result were found to have an assigned fetal fraction below the 1.5th percentile, or 3.4%, of euploid fetal fractions. Together, this suggested that fetal fraction was inversely correlated to aneuploidy risk. Corroborating this, the aneuploidy rate was significantly higher (10/24, 41.7%, including 3 trisomy 21, 4 trisomy 18, 2 trisomy 13, 1 monosomy X) than the aneuploidy rate in samples above this threshold ($p<0.001$). This translated to an odds ratio of 5.7 (CI: 2.5–13.1), indicating that samples in this fetal fraction range were nearly 6 times more likely to be abnormal than those from pregnancies with a fetal fraction of greater than 3.4%. Analysis of the full cohort of 1,064 samples revealed that 17 of 31 (54.8%) samples at or below this threshold were aneuploid, significantly higher than the aneuploidy rate in samples above this threshold ($p<0.0001$). This translated to an odds ratio (OR) of 9.2 (CI: 4.4–19.0). Indeed, all six triploidy samples (excluded from the main cohort) were identified as low fetal fraction (Appendices 3–4, <http://links.lww.com/xxx>). This supported that, for samples with a low fetal fraction, aneuploidy risk and fetal fraction were inversely proportional.

Of the 474 (91.5%) low-risk samples that were within test specifications and which passed quality control, all calls were correct, including all five aneuploid samples (1 trisomy 21, 2 trisomy 13, and 2 monosomy X) with a result (Table 3); sensitivities were reported overall

due to the low incidence and large confidence intervals (Table 3). Sensitivity was 100% for fetal sex (474/474, CI: 99.2–100%). Specificity was 100% overall (468/468, CI: 99.2–100%) for all tested indications. Five of 6 aneuploid pregnancies were identified in the low-risk cohort on the first drawn blood sample (Table 3); this was comparable to the high-risk cohort. The slightly higher no-call rate in low-risk patients when compared to the overall cohort (8.5% versus 8.1%, respectively, $p=0.86$) is accountable by the lower gestational age when compared to the overall cohort (median of 12.9 versus 14.3 weeks, respectively). To determine whether low-risk samples showed systematic differences from high-risk samples, the model fit p -value distributions for chromosomes 13, 18, and 21 in each cohort were compared (Materials and Methods, Appendix 7 [available online at <http://links.lww.com/xxx>]). No significant difference was found for any of the three chromosomes between the low- and high-risk cohorts, indicating that Next-generation Aneuploidy Test Using SNPs algorithm performed the same in low-risk pregnancies as it did in high-risk pregnancies. This consistency in performance is reflected in the accuracy of the copy number calls for the reported cohorts.

Of the 966 samples with a result, paternal genetic information was available for 507 (48.3%) samples. Analysis of the cohort that was accompanied by a paternal genomic sample (78 aneuploids and 429 euploids) with and without the paternal genomic information found that excluding the paternal sample had no effect on sensitivity (100% for all four indications) or specificity (100% for trisomy 21, trisomy 13, and monosomy X; 99.8% for trisomy 18). However, including a paternal sample reduced the number of samples that did not pass quality control from 5.5% (28/507) to 2.8% (14/507) ($p=0.04$).

Fetal fraction was positively correlated with gestational age (Appendix 8, available online at <http://links.lww.com/xxx>). As expected, this corresponded to an increase in the number of samples returning a result (Table 1). Differences in fetal fraction were observed when samples were stratified by karyotype. Trisomy 21 and monosomy X samples were observed to have increased fetal fractions, although the increases were not statistically significant (1.04 MoM [$p=0.06$] and 1.11 MoM [$p=0.39$], respectively), whereas trisomy 18 and trisomy 13 samples were observed to have significantly decreased fetal fractions (0.70 MoM [$p=0.003$] and 0.71 MoM [$p=0.01$], respectively).

Discussion

This SNP-based noninvasive prenatal screen showed high sensitivity and specificity in detecting fetal trisomies 21, 18, and 13, monosomy X, and fetal sex in high-risk and low-risk patients. However, an increased aneuploidy rate was observed in samples that did not return a result, particularly in samples identified with a low fetal fraction. This underscores the importance of redraws for all noninvasive prenatal screening methods, when samples fail to return a result and for methods where accuracy is decreased at lower fetal fractions (7).

Comparison of this SNP-based method with quantitative methods (5–8, 10–14) identified a significant improvement in performance. The results of this study were combined with the results from an externally blinded study using the same methodology (17) and compared to the combined results of the quantitative methods (5–8, 10–14). Here, the combined

specificity for the three autosomal trisomies was 99.91% (1,103/1,104 total negative samples, CI: 99.5–100%) (17); the overall specificity of the combined quantitative methods was 99.32% (4,084/4,112, CI: 99.02–99.55%) (Appendices 9–11, available online at <http://links.lww.com/xxx>). This is a statistically significant difference ($p < 0.009$). A similar comparison showed improved sensitivity of the SNP-based method ($123/124 = 99.20\%$) compared to the quantitative methods ($808/820 = 98.54\%$), although this difference did not reach significance ($p = 0.43$). As quantitative methods have not reported sensitivities for sex chromosome aneuploidies as extensively, comparisons were limited to autosomal trisomies. P-values were calculated using the exact binomial distribution to account for the low error rate. “Unclassified” samples from Bianchi *et al.* were conservatively treated as no-calls; treating these samples as positives or negatives is less-favorable for the quantitative methods. Clinically, pregnancies with high-risk calls should be confirmed by invasive diagnostic testing.

Clinicians must be aware of sensitivity and specificity as well as the high rate of aneuploidy in no-called samples. This is the first study that highlights this issue. Although previous studies correctly excluded no-calls from sensitivity and specificity calculations (5–18, 20, 28), the no-call rate among aneuploidy samples was higher than for euploid samples (5, 6, 8, 9, 11, 13, 14, 20, 25); not all studies reported karyotypes of samples excluded due to low fetal fraction (8). Significantly, this study included a substantially higher percentage of samples drawn at earlier gestational ages than previous studies (5–8, 11, 13, 14, 20, 25, 28), which explains the increased no-call rate. Additionally, this is one of only two studies that examined all four indications (8); the majority of studies focused on subsets of abnormalities (5–7, 9–15, 18, 25, 28). Together, this confounds direct comparisons. The inherent trade-off between no-call rate and accuracy must also be noted; a no-call may be clinically preferred to an incorrect call.

Low fetal fraction may be due to small or dysfunctional placentas, as is often observed with some aneuploidies (29). Indeed, samples that were no-called and those samples with fetal fractions below the 1.5th percentile of euploid pregnancies were at increased risk of aneuploidy (odd ratios of 2.5 and 9.2, respectively). This corroborates a recent study (24) that adjusted fetal fraction for maternal weight and gestational age – not possible here due to the absence of maternal weight information for the overall cohort. Taking into account maternal weight and gestational age would further improve detection.

Clinically, although samples not returning a result due to low fetal fraction are at increased risk for aneuploidy, they cannot be considered high-risk *per se*. Preliminary evidence reported herein suggests that, for samples with a low fetal fraction, aneuploidy risk and fetal fraction were inversely proportional; thus, by taking into account fetal fraction, a modified risk score can be generated for those samples that do not return a result due to low fetal fraction. Because this modified risk would take into account prior risk, samples with a low fetal fraction cannot be automatically classified as either high-risk or low-risk. Studies are ongoing to calculate a precise risk adjustment based on fetal fraction. Because fetal fraction varies within the same pregnancy from day to day, a sample that does not return a result may resolve upon redraw, as was recently reported; without redraws test performance suffers (30, 31). Indeed, in clinical practice, roughly 98% of samples ultimately return a result after

redraws are analyzed, and results are reported in less than one calendar week for over 80% of samples. Thus, the ability to offer a modified risk score for low fetal fraction samples would allow clinicians to determine whether the patient should be counseled to submit a second sample or to undergo invasive diagnostic testing; without electing one of these options, some aneuploidies will be missed. This is a significant departure from traditional serum screening methods, where ambiguous results based on hormone levels rarely generate a redraw request, and thus rarely resolve; as such, they have traditionally been considered high-risk. Taken together, pregnancies that do not return a result due to low fetal fraction should be followed by repeat noninvasive prenatal screening, high-resolution ultrasound, or invasive testing; invasive testing should be considered in light of gestational age at testing, patient preference, modified risk (when available), and other indications. This underscores the importance of reporting fetal fraction, which may clarify revised risk and clinical management.

Few reports analyzed large low-risk cohorts (25, 30, 32–34), raising concerns about test performance consistency in the general pregnant population. Test and algorithm performance in the low-risk cohort here, which had a comparable aneuploidy rate to other low-risk studies (25, 30, 32–34), was consistent with performance in the entire cohort, addressing concerns with whether noninvasive prenatal screening technology validations are applicable to low-risk populations.

Published noninvasive prenatal screening studies excluded mosaics (8, 11, 20, 25), which can generate (and clinically may be considered) incorrect calls (9, 35). Indeed, undetected confined placental mosaicism was predicted to generate a 1% false negative rate and a 0.025–0.1% false positive rate in noninvasive prenatal screening (36). Significantly, trisomy 13 and trisomy 18 pregnancies have been associated with increased placental mosaicism (37–40), likely contributing to reduced performance of all noninvasive prenatal screening tests when compared to chromosome 21. Here, known mosaics were interpreted conservatively so as to under-represent sensitivities (Table 2). Excluding the mosaic trisomy 18 case resulted in a sensitivity of 100% (24/24, CI: 85.8–100%), and including the mosaic monosomy X cases resulted in a sensitivity of 91.7% (11/12, CI: 61.5–99.8%).

This SNP-based method resulted in improved overall performance over quantitative methods. Clinicians should be cognizant of elevated risk when no result is returned due to low fetal fraction; a fetal fraction-dependent modified risk may clarify pregnancy management for these patients. In clinical practice, a request for a second sample occurs approximately 5% of the time. The accuracy reported in this low-risk cohort suggests that SNP-based noninvasive prenatal screening may provide pregnant women, regardless of age, an accurate screen for whole-chromosomal fetal aneuploidies and fetal sex.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Bin Hoang, Nicholas Wayham, Phikhanh Vu, Patrick Lau, and Jason Tong for technical assistance. The authors would also like to thank the following for input on the sample collection protocol and for providing samples: Dr. Ronald J. Wapner (Columbia University Medical Center, New York, NY), Dr. Arend Sidow (Stanford University School of Medicine, Palo Alto, CA), Dr. Harbinder Brar (University of California at Riverside, Prenatal Diagnostic and Perinatal Center, Riverside, CA), George Washington University (Washington, D.C.), Dr. Alan E. Donnenfeld (Women's Healthcare Group of Pennsylvania, Wynnewood, PA), Dr. Steven D. Meltzer (Houston, TX), Dr. Tyrone C. Malloy (DBA Soapstone Center for Clinical Research, Decatur, GA), Dr. James D. Goldberg (San Francisco Perinatal Associates, San Francisco, CA), Dr. Lech Dudarewicz (Polish Mother's Memorial Hospital-Research Institute, Poland), GENNET (Prague, Czech Republic), Dr. Joanne Stone (Mount Sinai School of Medicine), Dr. Daniel Saltzman (Carnegie Hill Imaging for Women), Dr. Ignatia Van den Veyver (Baylor), Dr. Jane Chueh (Stanford University), Dr. Peer Dar (Einstein Montefiore Medical Center), Dr. Millie Ferres (Tufts University), Dr. Maurice Mahoney (Yale University), Dr. Christopher Plummer (Madonna Perinatal), Dr. Natan Haratz (New York Methodist Hospital), Dr. Hassan Wehbeh (New Beginnings Perinatal), Dr. Hugh Miller (Watching Over Mothers and Babies, Obstetrix), Dr. Jorge Gomez and Dr. Anthony Lai (South Florida Perinatal), Dr. Fergal Malone (Royal College of Surgeons, Ireland), Dr. Carmina Comas Gabriel (Institut Universitari Dexeus, Barcelona, Spain), Dr. Leroy Carhart (Bellevue Health and Emergency Center, Bellevue, NE), Dr. Daniel R. Bourque (Lafayette, LA), Kristen Mac Dougall (Pacific Fertility Center, San Francisco, CA), Dr. Isabelle Ryan (Pacific Fertility Center, San Francisco, CA), Dr. Donald Taylor (Suburban Maternal Fetal Medicine LLC, Hoffman Estates, IL), Dr. Eric Knudston (University of Oklahoma Health Science Center, Oklahoma City, OK), Megan Tucker (St. Vincent Hospital, Center for Prenatal Diagnosis, Indianapolis, IN), Dr. Joseph Hwang (Perinatal Center of Iowa, Waukee, IA), Dr. Ronald E. Kuseski (Mayfair Women's Center, Aurora, CO), Dr. Ardeshir Karimi (Maternal Fetal Medicine Group, San Gabriel, CA), Dr. Kaoru Suzumori (Fetal Life Science Center, Japan), Dr. Israel Aruh (International Kent Hospital, Izmir, Turkey, StemExpress (Placerville, CA), Desert West ObGyn (Glendale, AZ), Goodman and Partiridge ObGyn (AZ), and Southwest Fertility Center (Phoenix, AZ).

Supported in part by a grant from the National Institute of Health, National Institute of Child Health and Human Development (4R44HD062114-02). The remainder of the work reported herein was privately funded by the investors of Natera.

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Figure 1.

Flow chart of samples. *Excludes one trisomy 21 sample that was called on chromosomes 13, 18, X, and Y, but no-called on chromosome 21. Samples that were considered within the specifications for testing and that passed quality control parameters were considered “calls.” Samples that were considered within the specifications for testing but did not pass quality control parameters were considered “no-calls.”

Table 1

Proportion of Samples Returning a Result by Gestational Age

	Results Reported, N (%)
10 weeks (n=900)	847 (94.1%)
9 weeks (n=956)	897 (93.8%)
9.0–9.9 weeks (n=56)	50 (89.3%)
<9 weeks (n=95)	69 (72.6%)

Table 2

Aneuploidy and Fetal Sex Detection Sensitivities and Specificities

	Sensitivity ^a		Specificity ^a	
Overall	103/105	98.1% CI: 93.3–99.8%	858/860 ^b	99.8% CI: 99.1–99.9%
Trisomy 21	58/58	100% CI: 93.8–100%	905/905	100% CI: 99.6–100%
Trisomy 18^c	24/25	96% CI: 79.7–99.9%	938/939	99.9% CI: 99.4–100%
Trisomy 13	12/12	100% CI: 73.5–100%	953/953	100% CI: 99.6–100%
Monosomy X^d	9/10	90% CI: 55.5–99.8%	953/954	99.9% CI: 99.4–100%
Female	358/358	100% CI: 99.0–100%	418/418	100% CI: 99.1–100%
Male	418/418	100% CI: 99.1–100%	358/358	100% CI: 99.0–100%

^aExcludes no-called samples.

^bEuploid samples only.

^cThe trisomy 18 false negative had a 40% euploid placenta. Excluding this case resulted in a sensitivity of 100% (24/24, CI: 85.8–100%).

^dExcludes two cases of fetal mosaic monosomy X. Including these cases resulted in a sensitivity of 91.7% (11/12, CI: 61.5–99.8%).

Table 3

Aneuploidy Sensitivity and Specificity According to Prior Risk

	Sensitivity ^a		Specificity ^{a,b}	
Overall^c	103/105	98.1% CI: 93.3–99.8%	858/860	99.8% CI: 99.1–99.9%
High-risk^c	98/100	98.0% CI: 93.0–99.8%	389/391	99.5% CI: 98.2–99.9%
Low-risk^c	5/5	100% CI: 47.8–100%	469/469	100% CI: 99.2–100%

^aExcludes no-called samples.^bEuploid samples only.^cIncludes trisomies 21, 18, and 13, and monosomy X.