Original Research

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

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

METHODS: One thousand sixty-four maternal blood samples from 7 weeks of gestation and beyond were included; 1,051 were within specifications and 518 (49.3%) were 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, confidence interval [CI] 93.8–100%), trisomy 13 (12/12, CI 73.5–

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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 ($P<.001$, odds ratio 9.2, CI 4.4–19.0). Sensitivity and specificity did not differ in low-risk and high-risk populations.

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.

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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 with traditional screening methods.[1–18] The most straightforward cell-free DNA-based approaches use 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 greater than 99% and less than 0.2%, respectively[19]; however, for trisomy 13 and monosomy X, these rates ranged from 78.6 to 94.4% with false-positive rates as high as 1%,[6,8,13,20] partially attributed to amplification efficiency variation.[21,22] Although 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") introduced a targeted amplification step, increasing efficiency by interrogating only chromosomes of interest.[12–14,18]

Next-generation technology uses cell-free DNA-based methods that incorporate genotypic information by targeting single-nucleotide polymorphisms (SNPs), determining allele identity and count.[15–17,24] Single-nucleotide polymorphism–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 used 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] 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/AOG/A535) pursuant to local regulations. Enrolled women were 18 years of age or older with a singleton pregnancy of at least 7 weeks of gestation and signed an informed consent. Results were not disclosed to patients. One thousand sixty-four 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 or cytogenetic karyotype analysis, or by genetic testing of cord blood, buccal sample, saliva, or products of conception. Karyotype information in 204 participants 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, nonegg-donor pregnancies. Samples were considered outside of the specifications for this study and excluded from the determination of sensitivity and specificity on a single venopuncture ("draw") based on any of the following criteria: confirmed sex chromosome abnormality (47,XXX, XXY, XYY), confirmed triploidy, or confirmed fetal mosaicism (see "Results"). Although sex chromosome abnormalities (47,XXX, XXY, XYY) 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 or older (Fig. 1; Appendix 2, available online at http://links.lww.com/AOG/A535). Of these, 401 were prospective high-risk blood samples from women planning to undergo an invasive procedure (amniocentesis or chorionic villous sampling) who subsequently had a confirmatory invasive procedure (398; 60 aneuploid) or who subsequently miscarried and had confirmatory products-of-conception testing (three; one aneuploid); 71 (63 aneuploid) were blood samples drawn 4 days or later after an invasive diagnostic procedure; and 71 were blood samples from women with advanced maternal age (35 years or older) with either follow-up confirmatory sampling at birth (24; none aneuploid) or through confirmatory products-of-conception testing after elective termination (47; two 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 postinvasive procedure (not shown); postprocedure aneuploid samples in this cohort were all drawn at least 4 days after invasive procedure, reducing the likelihood of procedure-related bias.

The remaining 521 (49.0%; nine aneuploid) blood samples were defined as low risk with maternal age younger than 35 years and lacking any reported high-risk indication(s) (Fig. 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.

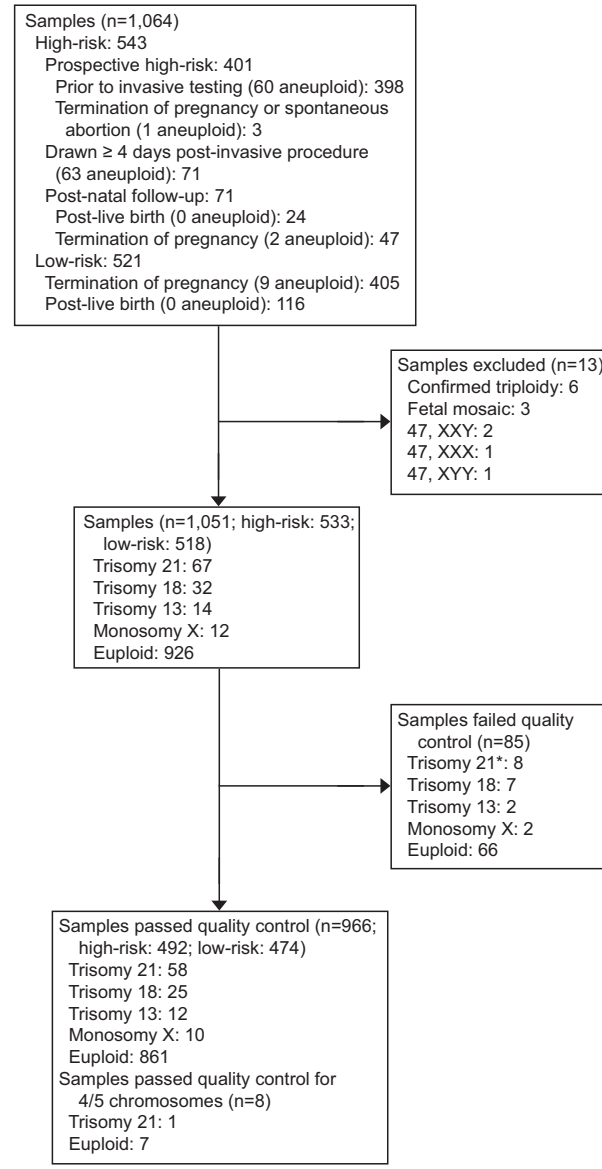

Fig. 1. Flow chart of samples. *Excludes one trisomy 21 sample that was called on chromosomes 13, 18, X, and Y, but no-called on chromosome 21. Samples that were considered within the specifications for testing and that passed quality control parameters were considered "calls." Samples that were considered within the specifications for testing but did not pass quality control parameters were considered "no-calls."

Pergament. Detecting Fetal Aneuploidy Through SNP Testing. Obstet Gynecol 2014.

Sample preparation, 19,488-plex polymerase chain reaction, the Next-generation Aneuploidy Test Using SNPs algorithm, and sequence alignment were described previously.[15–17,24] Here, two×10-mL tubes (a single draw) of blood from each participant were analyzed. When available, paternal samples were included in the analysis, although they were not required. An average of 1.22×10^7 reads was mapped for each sample when sequenced at normal depth of read. Samples not generating sufficient information (35.6%) were resequenced 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 χ^2 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 greater than 20 weeks of gestation evaluated independently. Fetal fractions were expressed as multiples of the median 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 (ORs) 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 (less than 3.8% fetal fraction or less than 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 (less than 1,500 genome equivalents); 3) contamination (greater than 0.2% contaminant DNA for fetal fractions of less than 8% and greater than 0.5% for fetal fractions above 8%); 4) the presence of regions of loss of heterozygosity in maternal DNA exceeding 25% of the chromosome; or 5) poor fit of the data to the model (likely as a result of anomalous biology; see "Discussion"). In clinical practice, no-calls (on all five chromosomes or on a single chromosome) as a result of sample-specific issues like low fetal fraction trigger a request for a redraw and reanalysis; these redraws are not billed. Because 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 here.

As expected with SNP-based technology, abnormal results beyond trisomies 21, 18, and 13 as well as monosomy X and fetal sex in singleton nondonor 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, is described in Appendices 3 and 4 (available online at http://links.lww.com/AOG/A535). Clinicians should be aware that although 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 (available online at http://links.lww.com/AOG/A535). The overall median gestational age was 14.3 weeks (mean 17.0 weeks, range 7.6–40.6 weeks), 14.1 weeks (mean 17.2 weeks, range 7.6–40.6 weeks) for euploid samples, and 14.6 weeks (mean 15.8 weeks, range 8.0–38.9 weeks) for aneuploid samples. The overall median maternal age was 30.0 years (mean 30.3 years, range 18–47 years), 29.0 years (mean 29.6 years, range 18–47 years) for euploid samples, and 37.0 years (mean 35.1 years, range 18–46 years) 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, one 47,XXX, one 47,XYY, six triploid, and three samples (one trisomy 13, two monosomy X) with confirmed fetal mosaicism. Eight monosomy X samples were previously reported.[15] Thirteen samples (two 47,XXY, one 47,XXX, one 47,XYY, one trisomy 13 fetal mosaic, two monosomy X fetal mosaics, six triploids) were

excluded from performance metric calculations (Fig. 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 (available online at http://links.lww.com/AOG/A535) 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 (seven euploid and one 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 Tables 2 and 3. 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 of 58 trisomy 21 samples (sensitivity 100%, confidence interval [CI] 93.8–100%), 24 of 25 trisomy 18 samples (sensitivity: 96%, CI 79.7–99.9%), 12 of 12 trisomy 13 samples (sensitivity: 100%, CI 73.5–100%), and 9 of 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/AOG/A535). 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/AOG/A535). The algorithm correctly identified one 47,XXX, one 47,XXY, and one 47,XYY; the remaining 47,XXY was a no-call as a result of low fetal fraction (Appendices 3 and 4, http://links.lww.com/AOG/A535; "Materials and Methods"). High-risk calls warrant follow-up confirmatory testing.

Table 1. Proportion of Samples Returning a Result, by Gestational Age

Gestational Age (wk)	Results Reported
10 or more (n=900)	847 (94.1)
9 or more (n=956)	897 (93.8)
9.0–9.9 (n=56)	50 (89.3)
<9 (n=95)	69 (72.6)

Data are n (%).

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 with the aneuploidy incidence in samples with a call (105/966 [10.9%], P=.004). This translated to an OR of 2.5 (CI 1.4–4.3), indicating that samples without a result were 2.5 times more likely to be aneuploid.

Importantly, 75.0% (15/20) of the aneuploid samples without a result were no-called as a result of low fetal fraction or a combination of low fetal fraction and insufficient data clarity (mean 3.4%, range 1.4–5.8%). 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 three trisomy 21, four trisomy 18, two trisomy 13, one monosomy X) than the aneuploidy rate in samples above this threshold (113/1,009, P<.001). This translated to an OR of 5.7 (CI 2.5–13.1), indicating that samples in this fetal fraction range were nearly six 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 (119/1,015, P<.001). This translated to an OR of 9.2 (CI 4.4–19.0). Indeed, all six triploidy samples (excluded

Table 2. Aneuploidy and Fetal Sex Detection Sensitivities and Specificities

	Sensitivity*		Specificity*	
Overall	103/105	98.1, CI 93.3–99.8	858/860[†]	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[‡]	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[§]	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

Data are n/N or %, 95% confidence interval.
* Excludes no-called samples.
† Euploid samples only.
‡ The trisomy 18 false negative had a 40% euploid placenta. Excluding this case resulted in a sensitivity of 100% (24/24, CI 85.8–100%).
§ Excludes two cases of fetal mosaic monosomy X. Including these cases resulted in a sensitivity of 91.7% (11/12, CI 61.5–99.8%).

from the main cohort) were identified as low fetal fraction (Appendices 3 and 4, http://links.lww.com/AOG/A535). 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 (one trisomy 21, two trisomy 13, and two monosomy X) with a result (Table 3); sensitivities were reported overall as a result of the low incidence and large CIs (Table 3). Sensitivity was 100% for fetal sex (474/474, CI 99.2–100%). Specificity was 100% overall (469/469, CI 99.2–100%) for all tested indications. Five of six 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 with the overall cohort (8.5% compared with 8.1%, respectively, $P=.86$) is accountable by the lower gestational age when compared with the overall cohort (median 12.9 compared with 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/AOG/A535). No significant difference was found for any of the three chromosomes between the low- and high-risk cohorts, indicating the 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 (52.5%) 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) ($P=.04$).

Fetal fraction was positively correlated with gestational age (Appendix 8, available online at http://links.lww.com/AOG/A535). 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

Table 3. Aneuploidy Sensitivity and Specificity According to Prior Risk

	Sensitivity*		Specificity*,[†]	
Overall[‡]	103/105	98.1, CI 93.3–99.8	858/860	99.8, CI 99.1–99.9
High risk[‡]	98/100	98.0, CI 93.0–99.8	389/391	99.5, CI 98.2–99.9
Low risk[‡]	5/5	100, CI 47.8–100	469/469	100, CI 99.2–100

Data are n/N or %, 95% confidence interval.
* Excludes no-called samples.
† Euploid samples only.
‡ Includes trisomies 21, 18, and 13, and monosomy X.

karyotype. Trisomy 21 and monosomy X samples were observed to have increased fetal fractions, although the increases were not statistically significant (1.04 multiples of the median [P=.06] and 1.11 multiples of the median [P=.39], respectively), whereas trisomy 18 and trisomy 13 samples were observed to have significantly decreased fetal fractions (0.70 multiples of the median [P=.003] and 0.71 multiples of the median [P=.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 with 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 through 11, available online at http://links.lww.com/AOG/A535). This is a statistically significant difference (P<.009). A similar comparison showed improved sensitivity of the SNP-based method (123/124 [99.20%]) compared with the quantitative methods (808/820 [98.54%]), although this difference did not reach significance (P=.43). Because 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[8] 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 as a result of 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 tradeoff 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 the result of a small or dysfunctional placenta 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 (ORs 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 as a result of 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 attributable to low fetal fraction are at increased risk for aneuploidy, they cannot be considered high risk per se. Preliminary evidence reported here 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 as a result of 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 on 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 1 calendar week for more than 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, in which 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 ultrasonography, 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 with chromosome 21. Here, known mosaics were interpreted conservatively so as to underrepresent 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 as a result of 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.

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