CORRESPONDENCE

# Accurate Description of DNA-Based Noninvasive Prenatal Screening

**TO THE EDITOR:** Cell-free DNA–based noninvasive prenatal screening (which has also been called noninvasive prenatal diagnosis and noninvasive prenatal testing) is now offered by several companies that use various analytic methods. This screening has been reported to have higher detection rates for trisomy 21 (Down's syndrome) (99.0%), trisomy 18 (96.8%), and trisomy 13 (92.1%) than previous noninvasive prenatal screening methods.[1] However, evidence of the actual performance of noninvasive prenatal screening in the general population is limited. Furthermore, discordant results between noninvasive prenatal screening and traditional cytogenetic analysis have been observed,[2] despite the high sensitivity and specificity of the assay.

We are aware anecdotally of patients who have terminated karyotypically normal pregnancies on the basis of false positive results of noninvasive prenatal screening. As companies are expanding noninvasive prenatal screening to include the detection of rarer chromosomal abnormalities in low-risk pregnancies, the potential for harm is increasing. To accurately convey the capacity of the assay, we believe it should be referred to only as "DNA-based noninvasive prenatal screening" because it is not a test that provides a diagnosis.

We evaluated the performance of noninvasive prenatal screening in a multicenter cohort of women who had positive results on this screening and were referred for invasive prenatal diagnostic testing to confirm the presence of fetal aneuploidy. Most of the results of the noninvasive prenatal screening were reported by the following laboratories: Ariosa Diagnostics, BGI, Natera, Sequenom, and Illumina (Table S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). Confirmatory cytogenetic studies were performed by the cytogenetics laboratories with which we are affiliated.

Of 307 samples that were positive on noninvasive prenatal screening, this screening correctly detected 238 of the 294 cases (81%) that were later found to have a nonmosaic karyotype (Table 1). However, 9% of the women who received positive screening results for trisomy 21, 23% for trisomy 18, 46% for trisomy 13, 62% for monosomy X, and 17% for XXX, XXY, or XYY abnormalities were carrying fetuses with normal karyotypes. In addition, another fetus was found to have trisomy 21 and one fetus was found to have monosomy X after routine second-trimester ultrasonography showed fetal abnormalities, although previous results of noninvasive prenatal screening were normal.

Of the 15 false positive cases that were identified at the Chinese University of Hong Kong, 8 (5 with trisomy 21, 1 with trisomy 18, and 2 with trisomy 13) are known to have resulted in the live birth of a newborn with no apparent abnormalities as determined through examination by a pediatrician. We do not know the outcomes of the remaining 7 pregnancies. Since we work at reference laboratories, we are not typically informed of the clinical outcome data on pregnancies for which we perform prenatal diagnosis. That said, a discrepancy between the results of

**Table 1. True and False Positive Cases with Nonmosaic Karyotypes.**

| Chromosomal Abnormality | True Positive Result (N=238) | False Positive Result (N=56) |
|---|---|---|
| | *no./total no. (%)* | |
| Trisomy 13 | 14/26 (54) | 12/26 (46) |
| Trisomy 18 | 40/52 (77) | 12/52 (23) |
| Trisomy 21 | 161/177 (91) | 16/177 (9) |
| Monosomy X | 8/21 (38) | 13/21 (62) |
| XXX or XXY | 15/17 (88) | 2/17 (12) |
| XYY | 0/1 | 1/1 (100) |

prenatal diagnostic testing and pregnancy outcome would probably be brought to our attention by the referring center. No such discrepancies have been reported to us.

Circulating cell-free DNA is derived from both maternal and placental tissues, so intrinsic biologic factors such as somatic mosaicism, confined placental mosaicism, and maternal copy-number imbalance[2,3] can influence the accuracy of noninvasive prenatal screening. Thirteen cases of mosaicism were detected in our study (Table S2 in the Supplementary Appendix). Two of the false positive cases (one with trisomy 18 and one with monosomy X) showed mosaicism on fluorescence in situ hybridization analysis of cells obtained by means of chorionic-villus sampling and amniocentesis, respectively, but they were confirmed to be normal in an analysis of metaphase cells from amniotic-fluid culture. These data, together with data from other recent studies,[2-4] validate the role of these biologic factors as a true source of false positive and false negative results of noninvasive prenatal screening. As recommended by the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine,[5] positive findings on noninvasive prenatal screening must be followed by invasive prenatal diagnostic testing before any irreversible decisions are made.

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Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

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**1.** Gil MM, Akolekar R, Quezada MS, Bregant B, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. Fetal Diagn Ther 2014;35:156-73.
**2.** Snyder MW, Simmons LE, Kitzman JO, et al. Copy-number variation and false positive prenatal aneuploidy screening results. N Engl J Med. DOI: 10.1056/NEJMoa1408408.
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**5.** American College of Obstetricians and Gynecologists Committee on Genetics. Committee Opinion No. 545: noninvasive prenatal testing for fetal aneuploidy. Obstet Gynecol 2012;120:1532-4.

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