CORRESPONDENCE

High-Resolution and Noninvasive Fetal Exome Screening

TO THE EDITOR: Fetal genetic diagnosis can guide clinical management and provide a basis for precision medicine. Noninvasive prenatal testing has transformed fetal screening by enabling aneuploidy detection and targeting a small number of variants relevant to prenatal diagnosis[1,2]; however, comprehensive genetic testing requires an invasive procedure such as amniocentesis. Here, we describe a potentially scalable and high-resolution noninvasive approach to prenatal screening to survey the entire fetal exome from circulating cell-free DNA (cfDNA) (Fig. 1 and Fig. S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org).

We evaluated the feasibility of high-resolution noninvasive prenatal screening in 51 pregnancies that were representative of the pregnant population receiving care at the recruitment hospital (Tables S1 and S2). Gestational ages spanned the trimesters, with 5 pregnancies in the first trimester, 9 in the second, and 37 in the third. The fetal fractions (i.e., the proportion of cell-free DNA derived from the placenta or fetus) ranged from 6 to 51% (Table S3). We captured and sequenced 22,995 genes to a mean coverage of 210× (Table S4). We discovered single-nucleotide variants (SNVs) and insertions or deletions (indels) using a machine-learning–based pipeline that modeled fetal fraction and other sequence features of cfDNA (see the Supplementary Methods section) and explored read-depth profiles for copy-number variants (CNVs).

Figure 1 (facing page). Workflow for Noninvasive Fetal Exome Screening with High-Resolution Noninvasive Prenatal Testing.

Panel A shows the process for extracting cell-free DNA (cfDNA) from maternal plasma followed by exome capture (see the Supplementary Appendix). We generated 51 libraries across gestational ages and calculated the fetal fraction (fetal cfDNA÷total cfDNA) in each sample. In the box-and-whisker plot on the right, for each trimester, the bold horizontal line indicates the median, the box the interquartile range, and the circles individual data points; whiskers indicate the minimum and maximum values within the range (defined by subtracting 1.5 times the interquartile range from the first quartile and adding the same value to the third quartile). Panel B highlights the methods for detecting novel variants that were developed to account for fetal fraction and shows the corresponding unique allele fractions at each site according to the maternal and fetal genotype combinations present in cfDNA. Each cluster represents a unique maternal–fetal genotype combination. Clusters are colored according to genotypes generated from direct exome sequencing of maternal and fetal DNA. These data highlight the merging of clusters at heterozygous sites in the mother at lower coverage and fetal fraction and the clearly resolved de novo and paternally derived variants (fetal 0/1; maternal 0/0) regardless of fetal fraction. Panel C describes a proof-of-principle application of high-resolution noninvasive fetal screening (NIFS) to 14 pregnancies referred for invasive testing and representative variants of clinical interest, including a likely pathogenic splice variant in *COL2A1* (NC_000012.12:g.47982610C→T) in a fetus with micrognathia consistent with autosomal dominant Stickler's syndrome type 1, a 4-MB pathogenic deletion on chromosome 7 (NC_000007.14:g.155368937-159327017del) in a fetus with multiple congenital anomalies, and a carrier *PAH* variant (NC_000012.12:g.102866632C→T) that is associated with a risk of phenylketonuria. All the variants were orthogonally validated. WES denotes whole-exome sequencing.

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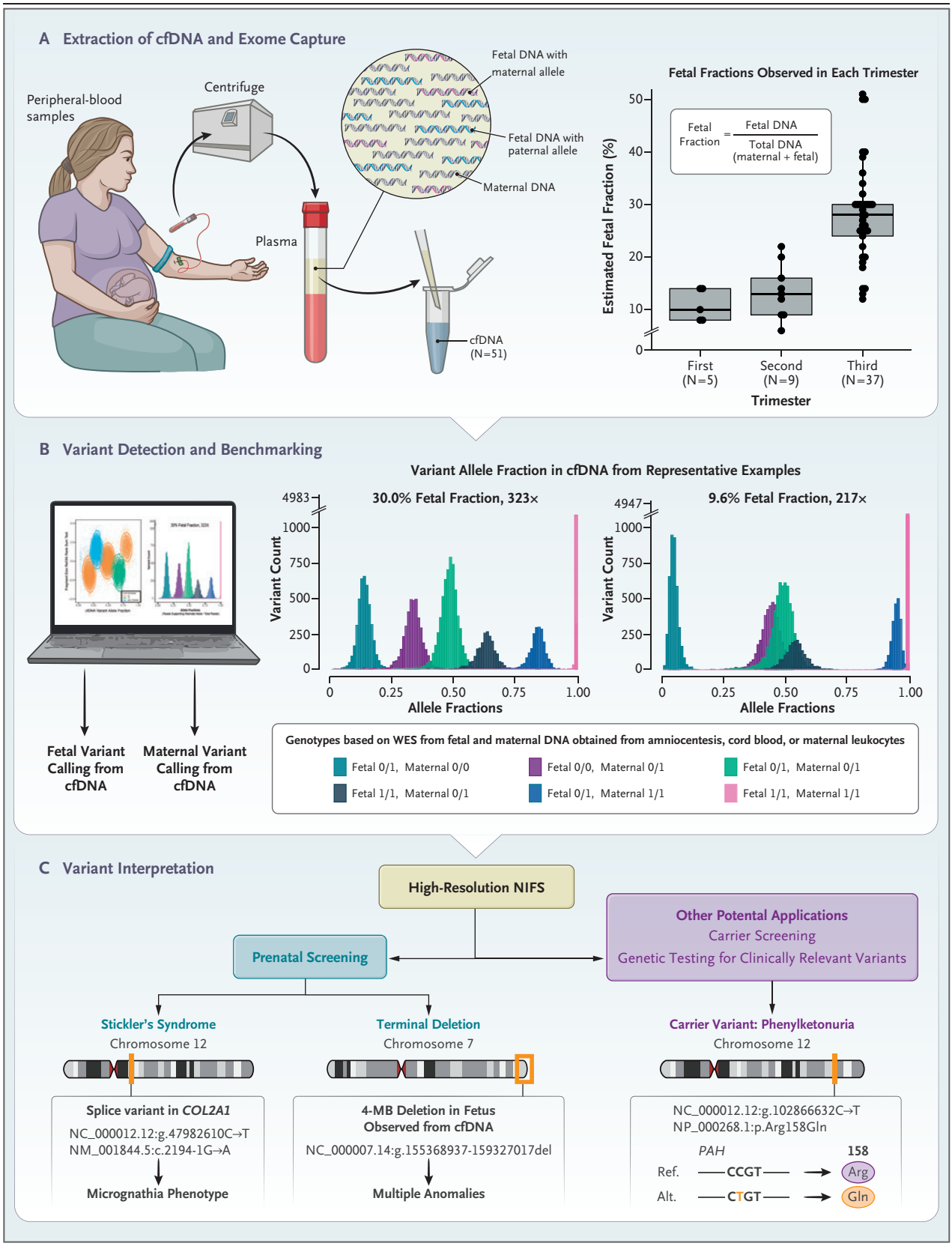

We compared the discovery of variants from high-resolution noninvasive prenatal screening in 11 fetuses and 28 mothers with matching germline exome sequencing. The method was highly sensitive for the discovery of SNVs, particularly de novo SNVs — the largest class of clinically significant variants after aneuploidies — and paternally inherited SNVs (median sensitivity, 96.3%) (Tables S5, S6, and S7). This result was robust to fetal fraction (sensitivity, 92.6 to 97.1%; positive predictive value, 87.9 to 92.2%). Overall, fetal SNV genotypes could be distinguished from maternal genotypes with relatively high sensitivity, but we found that accuracy decayed at lower fetal fractions if the mother was heterozygous (range, 65.6 to 93.2%) (Fig. S2 and Tables S8 and S9). Considering indels, which have higher false discovery rates than SNVs in both high-resolution noninvasive prenatal screening and standard exome sequencing,[3] we found that the median sensitivity was 81.9% (range, 66.4 to 84.6) for de novo and paternally inherited indels and 71.3% (range, 51.7 to 77.8) for inherited variants that were heterozygous in the mother. The sensitivity of high-resolution noninvasive prenatal screening before filtering against germline exome sequencing was 99.7% for SNVs and 93.9% for indels — a finding that suggests the potential for higher sensitivity with further development of these methods (Table S10). The low number of large CNVs in this cohort precluded benchmarking of this variant class. We also found that high-resolution noninvasive prenatal screening enabled maternal variants to be genotyped with high sensitivity across 582,839 variants that were confirmed by germline exome sequencing in 28 mothers (sensitivity, 98.3%) (Table S11), a result that highlights the potential for this method to be used for carrier screening.

We assessed the usefulness of high-resolution noninvasive prenatal screening across 14 pregnancies that had been referred for standard-care diagnostic procedures and detected 100% of the variants (6 of 6) that had been identified by invasive testing (Fig. 1 and Tables S12 and S13). These results included a de novo CNV (NC_000007.14:g.155368937-159327017del) in a fetus with multiple anomalies, a splice variant in *COL2A1* (NC_000012.12:g.47982610C→T) in a fetus with micrognathia, and a homozygous p.Phe508del variant (NC_000007.14:g.117559592_117559594del) associated with cystic fibrosis in *CFTR*. Carrier screening with this method yielded a reportable variant for 57.1% of the mothers — a finding similar to those from standard screening (Table S14).[4] Sex was accurately inferred for all the samples, including complex cases such as a female fetus with a vanishing male co-twin and a mother who had received a stem-cell transplant from a male donor (Fig. S3).

These results suggest that high-resolution noninvasive prenatal screening may provide a robust fetal screen at nucleotide resolution for de novo variants across gestational ages, with the potential to detect maternally inherited and recessive disease variants at fetal fractions of more than 15 to 20%. The method also provided accurate carrier screening and detected reportable incidental findings (e.g., a *BRCA2* risk variant) that will require analogous reporting guidelines to those that are applied to invasive prenatal testing.[5] Our proof-of-principle analyses suggest that a fetal exome screen is accessible from maternal plasma samples that are already routinely obtained for fetal aneuploidy testing.

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1. Bianchi DW, Parker RL, Wentworth J, et al. DNA sequencing versus standard prenatal aneuploidy screening. N Engl J Med 2014;370:799-808.
2. Zhang J, Li J, Saucier JB, et al. Non-invasive prenatal sequencing for multiple Mendelian monogenic disorders using circulating cell-free fetal DNA. Nat Med 2019;25:439-47.
3. Fang H, Wu Y, Narzisi G, et al. Reducing INDEL calling errors in whole genome and exome sequencing data. Genome Med 2014;6:89.
4. Guo MH, Gregg AR. Estimating yields of prenatal carrier screening and implications for design of expanded carrier screening panels. Genet Med 2019;21:1940-7.
5. Van den Veyver IB, Chandler N, Wilkins-Haug LE, Wapner RJ, Chitty LS, ISPD Board of Directors. International Society for Prenatal Diagnosis updated position statement on the use of genome-wide sequencing for prenatal diagnosis. Prenat Diagn 2022;42:796-803.

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Comprehensive Noninvasive Fetal Screening by Deep Trio-Exome Sequencing

TO THE EDITOR: Fetal genetic diagnosis is pivotal in prenatal care, and recent advancements in prenatal trio-exome sequencing have shown diagnostic gains.[1,2] However, owing to the invasive nature of fetal sampling, its use is limited to fetuses with identifiable structural anomalies. This limitation leaves many monogenic disorders undiagnosed because they do not manifest in the prenatal period, resulting in a large number of neonates with lethal and severely debilitating pediatric disorders.[3] The development of noninvasive fetal tests using cell-free DNA from a maternal blood sample has revolutionized prenatal screening,[4] but its application has mainly been limited to chromosomal disorders owing to the low resolution available with the existing screens.

To address these limitations, we developed noninvasive prenatal screening using deep trio-exome sequencing; this comprehensive noninvasive method has high sensitivity for detecting fetal single-nucleotide variants, small insertions and deletions, large copy-number variants, and chromosomal aneuploidies from cell-free DNA obtained from a maternal blood sample (Fig. 1). This proof-of-concept approach leverages ultra-deep, error-corrected, trio-exome sequencing that enables the detection of fetal de novo variants with high accuracy. De novo variants are recognized as the primary cause of severe pediatric developmental disorders.[5]

In our study, we recruited 36 pregnant women and their partners (Fig. S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). Key inclusion criteria were a nuchal translucency measurement of at least 5 mm, at least one fetal anomaly identified during the first or second gestational trimester, or both (Tables S1 and S6). We performed ultra-deep exome sequencing on cell-free DNA extracted from maternal plasma, achieving sequencing coverage per sample ranging from 2710× to 8075× (mean, 4548×). A customized pipeline was used for accurate variant calling and filtering, incorporating error correction by means of unique molecular identifiers, site-specific noise modeling, and estimation of the fetal fraction (i.e., the proportion of cell-free DNA in a maternal blood sample that is of fetal origin) (see the Methods section in the Supplementary Appendix). Fetal fraction ranged from 3.72 to 19% (Table S3). Trio-exome analysis, including maternal plasma and matched parental blood samples, was performed for identification of fetal de novo and inherited paternal variants. The performance of noninvasive prenatal screening using deep trio-exome sequencing was evaluated by assessing the results obtained with this new method as compared with those from invasive prenatal trio whole-exome sequencing and whole-genome sequencing and with chromosomal microarray analysis of chorionic villus or amniotic fluid sampling for all included cases.

We identified the following pathogenic fetal de novo variants, which we considered to be clinically relevant in pregnancy: four single-nucleotide variants, one small deletion (Table S2), two autosomal trisomies (Fig. S2), one sex-chromosome aneuploidy (Fig. S3), two large copy-number variants, and one unbalanced trans-