Comprehensive Noninvasive Fetal Screening by Deep Trio-Exome Sequencing

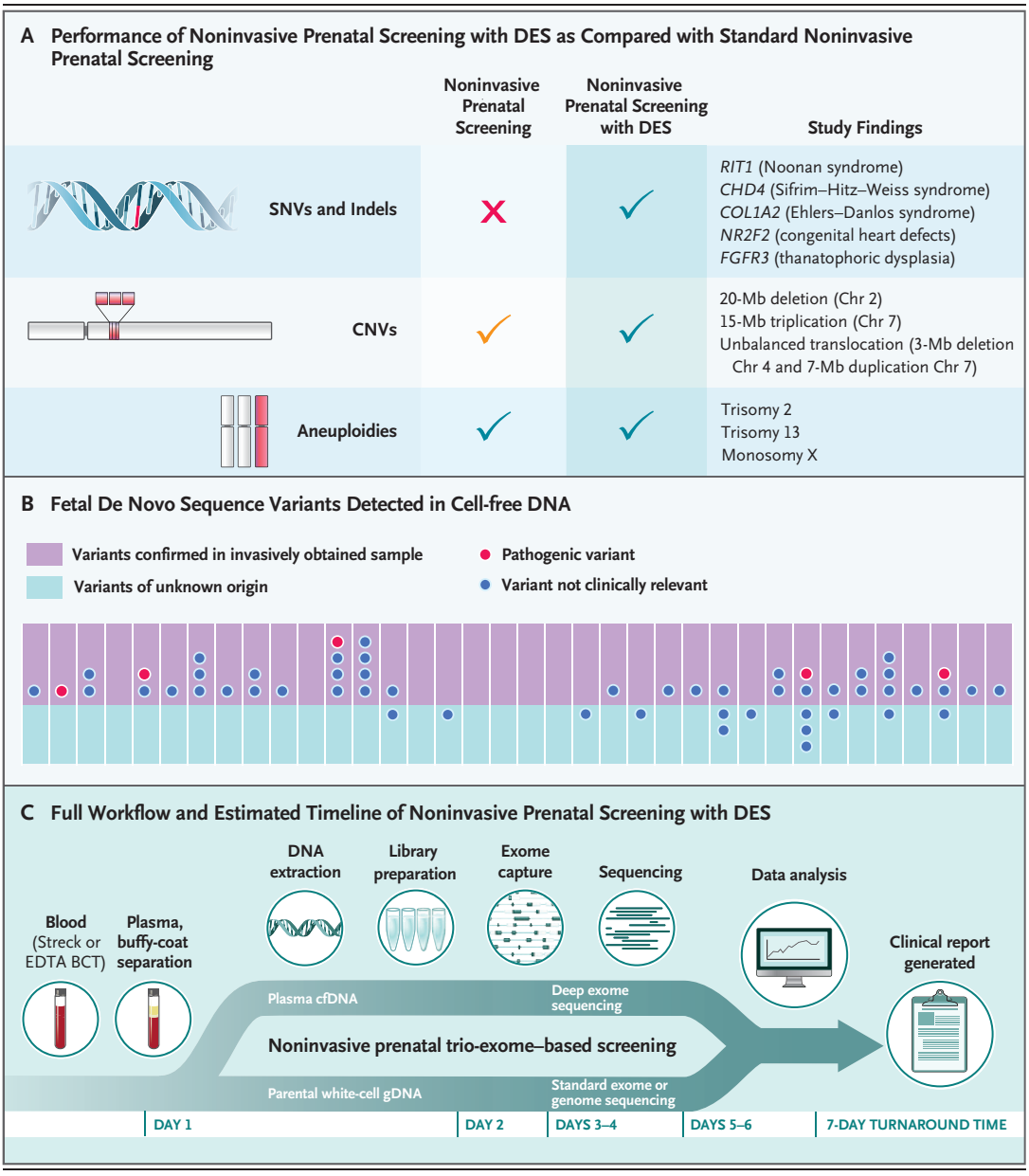
TO THE EDITOR: Fetal genetic diagnosis is pivotal in prenatal care, and recent advancements in prenatal trio-exome sequencing have shown diagnostic gains.[1,2] However, owing to the invasive nature of fetal sampling, its use is limited to fetuses with identifiable structural anomalies. This limitation 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 translocation (Fig. S4). All the pathogenic variants that were detected by noninvasive prenatal screening using deep trio-exome sequencing were confirmed in the matched invasively obtained fetal samples, which aligned with the fetal phenotype and provided a genetic diagnosis in 11 of the 36 pregnancies. No additional diagnostic de novo variants were identified by means of invasive analysis in the 36 pregnancies. Thus, we observed full concordance between noninvasive prenatal screening using deep trio-exome sequencing and invasive prenatal analysis, with 100% detection for pathogenic de novo variants.

To assess the representation of the fetal exome in cell-free DNA, we broadened our analysis to encompass all the paternally inherited fetal sequence variants that were located within the coding region (Fig. S5A). The mean sensitivity of our new method for the detection of fetal sequence variants across all protein-coding genes was 95.12% (Fig. S5B). Sensitivity depended on the fetal fraction and sequencing coverage (Fig. S6). In addition, noninvasive prenatal screening using deep trio-exome sequencing detected all the fetal de novo sequence variants that were identified by invasive prenatal analysis. In a subset of plasma samples, noninvasive prenatal screening using deep trio-exome



sequencing revealed a small number of sequence variants of unknown origin that were not identified in the corresponding invasively obtained samples (Fig. 1B). These variants may arise from polymerase-chain-reaction or capture artifacts, low-grade maternal somatic mosaicism, or placental-confined variants; further investigation is needed to determine their source. None of these variants were deemed to be clinically relevant or to necessitate further testing.

Noninvasive prenatal screening using deep trio-exome sequencing analysis includes parental samples, which enables the option of carrier screening in both biologic parents to identify pregnancies that are at high risk for recessive disorders. Our study showed that this approach to testing can accurately determine the fetal inheritance of paternal variants in the event that the mother does not carry the same variant (Fig. S7). Confirmation of the presence of a maternally inherited variant in the fetus would necessitate invasive testing.

Figure 1 (facing page). Performance and Workflow Timeline of Noninvasive Prenatal Screening with Deep Trio-Exome Sequencing (DES).

A performance comparison between standard noninvasive prenatal screening and noninvasive prenatal screening using DES is shown in Panel A. The orange checkmark denotes that although detection of large copy-number variants (CNVs) is technically possible with standard noninvasive prenatal screening, it is usually not included in screening. Additional information regarding the fetal variants detected is provided in the Supplementary Appendix. Panel B shows fetal de novo sequence variants that were identified with the use of noninvasive prenatal screening with DES from maternal plasma cell-free DNA (cfDNA). Red circles indicate five pathogenic de novo sequence variants that were identified. Variants that were deemed to be not clinically relevant were de novo sequence variants found in the coding region that were not associated with a fetal phenotype or any known developmental disorder and would not be reported in prenatal analysis. Variants that are shown on a purple background were confirmed in the corresponding prenatal invasive samples, and variants shown on a blue background were not observed in the invasive prenatal or parental sample analysis, a finding that suggests an unknown origin of those variants. Panel C shows the full workflow of noninvasive prenatal screening with DES with an estimated timeline, with a turnaround time of approximately 7 days. BCT denotes blood-collection tube, gDNA genomic DNA, indel insertion–deletion, and SNV single-nucleotide variant.

Noninvasive prenatal screening using deep trio-exome sequencing provides a means to screen, noninvasively and comprehensively, for a wide range of genetic disorders without posing a risk to the fetus or the mother. The integration of noninvasive prenatal screening with deep trio-exome sequencing into routine prenatal care in conjunction with fetal ultrasonographic screening would provide an opportunity to improve early detection rates, reduce the number of invasive procedures, and facilitate prompt interventions. Although large studies are needed to test this hypothesis, results from our study (Fig. 1B) would predict a low false-positive rate. All the positive findings should be confirmed through prenatal invasive diagnostic testing. With a turnaround time of approximately 7 days (Fig. 1C), noninvasive prenatal screening using deep trio-exome sequencing provides timely results.

Findings from our study provide support for further investigation of deep-exome sequencing of cell-free DNA to screen for pathogenic variants in fetuses other than those with structural anomalies.

Ieva Miceikaitė, Ph.D.

University of Southern Denmark
Odense, Denmark
ieva.miceikaite@gmail.com

Qin Hao, Ph.D.
Charlotte Brasch-Andersen, Ph.D.
Christina R. Fagerberg, M.D.
Pernille M. Torring, M.D., Ph.D.
Britta S. Kristiansen, M.D.

Odense University Hospital
Odense, Denmark

Lilian B. Ousager, M.D., Ph.D.

University of Southern Denmark
Odense, Denmark

Lene Sperling, M.D., Ph.D.

Odense University Hospital
Odense, Denmark

Mette H. Ibsen, M.D.

University Hospital of Southwestern Jutland
Esbjerg, Denmark

Katrin Löser, M.D.

Hospital of Southern Jutland
Aabenraa, Denmark

Martin J. Larsen, Ph.D.

University of Southern Denmark
Odense, Denmark

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