Progress toward Noninvasive Prenatal Diagnosis

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For decades, noninvasive analysis of the fetal genotype has been the holy grail of the field of prenatal diagnosis. Noninvasive prenatal diagnosis (NIPD) would use a sample source other than amniocentesis or chorionic villus sampling. The focus has been primarily on the use of maternal blood samples, with less attention given to the possibility of recovering fetal cells from maternal cervical samples (as would be collected for cervical cancer screening). The initial efforts focused on the presence of fetal cells in the maternal circulation, and evidence indicated as early as 1979 that fetal cells could be recovered via fluorescence-activated cell sorting. The elegant strategy of using antibodies to paternal HLA antigens to identify fetal cells was demonstrated. Attempts to recover fetal cells focused for a time on fetal nucleated red blood cells via analysis with fluorescence in situ hybridization and the PCR, but the disappointing results led to skepticism that NIPD would ever become a reality.

By 1996 there were reports of tumor DNA detected in the plasma of cancer patients, and Lo et al. first reported in 1997 the presence of fetal DNA in maternal plasma. The use of fetal DNA in the plasma has important advantages over the use of fetal cells in that fetal DNA can be obtained consistently in virtually 100% of pregnancies, whereas fetal cells are recovered much less consistently. In recent years, the use of fetal DNA in maternal plasma to detect fetal sex has become a well-established clinical test [see references in Lo et al. (1) for background on this test and other details]. In fact, determining whether any genotype present in the father but not the mother has been transmitted to the fetus is relatively straightforward. This approach has been used to determine whether a fetus is Rh negative or positive in the context of the risk for Rh incompatibility. Similarly, a fetus at risk of inheriting a dominant mutation (e.g., polyposis coli) from the father can be tested for the presence of the mutation. In the case of recessive disorders (e.g., cystic fibrosis) in which the parents have different mutations, one can determine whether the fetus has inherited the paternal mutation.

Two newer technologies have greatly altered the prospects for NIPD. One technology is chromosomal microarray analysis, which has opened the possibility of detecting a wide range of disease-causing copy number variants (CNVs), such as those causing DiGeorge, Williams, and Smith–Magenis syndromes. This technology expands the emphasis on NIPD from detection of trisomy 21 alone to broader detection of tens and even hundreds of serious disabilities caused by microdeletions or microduplications. Array comparative genomic hybridization (CGH) enables the detection of CNVs and aneuploidy in single blastomeres. Thus, if single fetal cells could be recovered reliably from maternal blood or the cervix, NIPD could potentially be used to detect all forms of aneuploidy and most disease-causing CNVs. If such cells could be obtained, testing could be implemented with current technology and at costs similar to those currently for invasive prenatal diagnosis.

The second new technology is next-generation (Next-Gen) sequencing. This term refers to the many instruments and platforms that permit the collection of enormous amounts of DNA sequence data at lower costs. A recent report by Lo et al. (1) applied Next-Gen sequencing to the analysis of fetal DNA in maternal plasma and provided evidence that NIPD is closer at hand than ever. Although the report successfully analyzed a single pregnancy at risk for β-thalassemia, the findings have much broader implications. The investigators sequenced plasma DNA to provide 65-fold coverage, meaning that the amount of sequencing was equivalent to that of 65 haploid genomes. They used single-nucleotide polymorphism data from both parents in an elegant computational way to distinguish fetal sequence reads from maternal reads to the extent possible. Importantly, they demonstrated for the first time that the entire fetal genome was represented in a uniform manner, with close to 11% fetal sequence and 89% maternal sequence for each chromosome. This result is equivalent to about 7-fold coverage of the fetal haploid genome or about 3.5-fold coverage of each diploid allele. The authors demonstrated the feasibility of deciphering the entire genome of the fetus from a...
maternal blood sample. Fetal DNA is apparently in a nucleosomal configuration with a mononucleosomal DNA length of 143 bp, compared with 166 bp for maternal DNA. The authors suggest that this difference is likely due to cleavage of the approximately 20-bp linker fragment—and perhaps the histone H1 bound to it—from fetal nucleosomes. The fact that fetal DNA fragments are smaller than maternal fragments had been observed previously, and this difference had been exploited to enrich for fetal DNA relative to maternal DNA. Early reports indicated that fetal DNA is 3%–5% of the total DNA in maternal plasma, but data from Next-Gen sequencing has indicated that as much 8%–20% of plasma DNA is fetal during the first trimester. This percentage trends higher during the second trimester (2). The 11% value reported by Lo et al. (1) is comparable with the 8%–20% range and may mean that earlier numbers were in error or that certain Next-Gen sequencing protocols somehow enrich for fetal sequences.

For prenatal diagnosis, 2 types of information are generally of interest. One is inherited mutations, such as when both parents are carriers for cystic fibrosis or when a parent has a deleterious dominant mutation, as in neurofibromatosis. The second type is a concern for de novo mutations. The majority of de novo mutations are chromosomal or CNV in nature, although de novo point mutations, such as achondroplasia, can also occur. One important question is whether Next-Gen sequencing data can detect copy number changes down to 0.5–1 Mb in size. The detection of CNVs depends substantially on the read density across key regions. The level of coverage of the fetal genome necessary to diagnose CNVs by sequencing DNA from maternal plasma may be relatively low, but further data are needed.

Against this background, what are the prospects for NIPD in the near future? Array CGH would be feasible if individual fetal cells could be recovered regularly from maternal blood samples. Array CGH is very effective for detecting CNV and cytogenetic abnormalities; however, no one has yet demonstrated an ability to recover such cells reliably. If one turns to Next-Gen sequencing of plasma DNA, the feasibility is more favorable, although the costs of obtaining high-density reads from fetal DNA would be quite high given the expense of current sequencing equipment and other costs. Next-Gen sequencing has the potential to diagnose inherited mutations, which are usually point mutations, but preventing affected individuals would require the termination of pregnancies. For situations in which disease-causing dominant or recessive mutations are known to be present in the parents, preimplantation genetic diagnosis may be more attractive than NIPD, because the termination of pregnancy can be avoided. The Next-Gen sequencing approach would be greatly improved if an effective method were available to enrich fetal DNA from 3%–20% of the total DNA to 50%–90%. Such a capability could facilitate array CGH without the need to recover individual fetal cells. It would also greatly reduce costs and increase the reliability of diagnosis with Next-Gen sequencing. Perhaps the advance that would totally change the feasibility of NIPD using either array CGH or Next-Gen sequencing would be a simple 1-step purification of fetal DNA from maternal plasma DNA.

The use of Next-Gen sequencing for genetic diagnosis related to reproduction is moving very rapidly. A recent publication has described universal carrier testing for 437 target genes (3). The Lo group has recently extended the sequencing of fetal DNA to include target enrichment (4). Another report (5) claims highly reliable detection of trisomy 21 in plasma DNA by means of a methylated DNA immunoprecipitation methodology combined with real-time quantitative PCR.

If NIPD becomes a routine test offered to all pregnant women during the first trimester of pregnancy, as seems likely to this observer, it will have an enormous impact on populations across the globe. The ethical, legal, and social implications would be as great as any other medical technology has brought forward in recent decades.

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References


