Rapid Prenatal Testing Using Semiconductor Sequencing?

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Recent developments in sequencing technology have had a large impact in several areas of human genetics. One area in which the implementation of this new technology is closest to clinical use is prenatal testing. One tube of blood drawn from a pregnant woman is sufficient for testing the unborn child for Down syndrome with high diagnostic sensitivity and specificity. Approximately 10% of the DNA in maternal plasma is of fetal origin (1). Next-generation sequencing (NGS)2 allows the parallel determination of the chromosomal origin for millions of plasma DNA molecules. This capability allows the detection of an overrepresentation of chromosome 21 in the case of a fetus with Down syndrome, although the majority of the sequenced molecules are derived from the healthy mother. This noninvasive testing will replace invasive tests, which come with a small risk of miscarriage.

Several NGS platforms have proved the feasibility of the detection of fetal trisomies (2–5); however, these platforms come with relatively high costs and a sequencing time of 2–3 days. In this issue of Clinical Chemistry, the groups of Yu Liang and Caixia Liu describe the first feasibility study of noninvasive trisomy detection in maternal plasma with a semiconductor-based sequencing platform (6). Semiconductor-based sequencing detects pH differences due to hydrogen release after DNA polymerase-mediated nucleotide incorporation. Most competing NGS platforms rely on fluorescence-based measurements and therefore require an imaging step during every sequencing cycle, which necessitates longer run times.

The authors used the Ion Torrent Personal Genome Machine (PGM) with the recently developed 318 chip to generate approximately $5 \times 10^6$ reads $>100$ bp in a single run of less than several hours. Previous studies that used Illumina NGS platforms have shown sufficient diagnostic sensitivity and specificity with a minimum of $2.3 \times 10^6$ mapped reads (7–10). Therefore, the throughput of the Ion Torrent PGM seems to have achieved the appropriate range of the number of reads. The Ion Torrent PGM requires approximately 24 h for library preparation and sequencing, thus allowing, in principle, improved turnaround times compared with other NGS platforms, which require a minimum run time of 2–3 days.

In their study, the Liang and Liu groups analyzed plasma DNA from 13 pregnant women and showed that the Ion Torrent PGM platform was able to detect trisomies 13, 18, and 21. As the authors state in their conclusions, a larger-scale study is needed to determine the imprecision of the test. Several issues remain to be addressed in follow-up studies. The plasma samples were collected in the second trimester, from 13 + 6 to 29 weeks of gestation, with half the samples having been obtained after week 20 of gestation. The fraction of fetal DNA in plasma is known to increase with time of gestation; therefore, detection later in pregnancy is easier than at the beginning of the second trimester. For clinical applicability, demonstrating reliable performance during gestation weeks 12–15 is essential. Another issue is the effect of interassay imprecision. Other sequencing platforms produce sufficient reads to allow the pooling of bar-coded libraries from several plasma samples into a single run, where interassay imprecision is easily noticed. The current number of reads on the Ion Torrent PGM is just sufficient for a single plasma sample. Yet another issue is the number of reads obtained. The Liang and Liu groups report a mean of $3.5 \times 10^6$ mappable reads, with an SD of $0.7 \times 10^6$ reads. This result means that for approximately 10% of the runs, the number of reads was less than the above-mentioned minimum of $2.3 \times 10^6$ mapped reads.

The Ion Proton platform (Life Technologies) is expected to yield a higher throughput at a run duration similar to that of the Ion Torrent instrument. The sequencing technology of these platforms is based on semiconductor detection technology. It will be interesting to see if noninvasive prenatal testing with semiconductor-based sequencing is possible.

In conclusion, this study (6) provides a clear step toward semiconductor-based noninvasive testing for trisomy; however, several steps remain to be taken before actual clinical application.

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