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Editorials

Sizing Up Cell-Free DNA

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In 1947, Mandel and Métais discovered the unusual phenomenon of circulating cell-free DNA *(1)*. Although one might naively think that DNA is found only in the nuclei of cells, it turns out that a substantial amount of small DNA fragments circulates within the blood. These molecules are not part of intact cells but are the detritus of dead and apoptosed cells that have spilled their guts into the blood. Their genomes are chewed up into little pieces, the presence of which can be measured like any other blood analyte. Although great effort has been expended to try to understand how the relative concentrations of these DNA species may correlate with health, their greatest value has been to measure the proportion of foreign genomes within an individual. In certain cases that is quite straightforward: Cancer-related mutations can be used to some extent to determine the progress of disease *(2)*, male chromosomal markers can be used for prenatal sex determination *(3)*, and, in a similar fashion, women who have received organ transplants from males can be monitored for the amount of organ-specific DNA *(4, 5)*.

The cell-free DNA phenomenon might have remained a curiosity but for the development of powerful genomic-analysis techniques such as digital PCR, high-throughput sequencing, and other methods of molecular counting. In 2008, my group showed how molecular counting by high-throughput sequencing could be used to create a universal noninvasive prenatal diagnostic for aneuploidies such as Down syndrome *(6)*. This work was quickly reproduced by Dennis Lo and collaborators *(7)*, and by now thousands of women have been tested with this approach in blinded prospective clinical trials and other studies *(8–11)*. There is a very good chance that this approach will ultimately replace such invasive tests as amniocentesis and chorionic villus sampling. At least one company has launched a commercial test based on this approach, and others are in the offing. Similarly, organ transplants can be thought of as genome transplants, and one can use high-density genotype information from both the donor and the recipient to create a very sensitive universal test for rejection in heart transplant recipients *(12)*. Because this principle does not involve the use of any specific physiological data about the heart, it should be valid for virtually any organ transplant.

In the case of fetal DNA, there has been much discussion in the literature about the relative sizes of maternal vs fetal fragments. The majority of the literature has concluded that fetal fragments are relatively shorter than maternal fragments, but there has been substantial disagreement about the size of the effect. This issue is more than a purely academic argument, because if one could size-select for shorter fragments and thereby increase the fetal fraction of the DNA fragments, many of the diagnostic approaches would have increased sensitivity. Such measurements, however, are often confounded by the challenge of sample handling: Any lysis of maternal lymphocytes after the blood is drawn would contribute disproportionately longer fragments than the typical circulating cell-free DNA, thereby artificially enhancing the apparent size difference between maternal and fetal DNA. Our own results have shown a slight enrichment of fetal DNA among the smaller fragments, but unfortunately not enough to be practically useful at this point *(13)*.

There are many interesting questions about the origin and nature of circulating cell-free DNA; many have noted that the fragment size is roughly that of what can be wrapped around a nucleosome. The sequencing of cell-free DNA and mapping it back to the chromosome of origin have revealed a significant periodic enhancement near transcription start sites *(6)*. In fact, this periodicity has exactly the same size as a nucleosome and serves as evidence that nucleosome-protected DNA fragments are enriched in the circulation.

In this issue of *Clinical Chemistry*, Lo and collaborators *(14)* report on the size and periodicity of circulating cell-free DNA in organ transplant recipients. These authors make the intriguing observation that recipient-specific fragments have an enhanced periodicity in size that exactly matches the pitch of the double helix: 10 bp. Several of the peaks can be interpreted in terms of the known biochemistry of the nucleosome, and owing to the genetic differences between donor and recipient, Lo's group is able to report that circulating cell-free DNA is primarily hematopoietic in origin. They also observe a slight shift in length distributions, indicating that the mean length of donor fragments is a

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bit shorter than that of recipient fragments. The authors suggest that this observation may have some value in enhancing the sensitivity of noninvasive diagnostics, but to what extent one will be able to practically exploit it remains to be seen. The history of the study of length effects in noninvasive fetal diagnostics remains a cautionary tale. The practical challenges notwithstanding, this report presents another interesting twist in efforts to understand the biological origin of circulating cell-free DNA and provides several useful hints and challenges as the research community tries to understand the origin and general phenomena of this incredibly useful property of human biology.

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References

1. Mandel P, Métais P. Les acides nucleiques du plasma sanguin chez l'homme. C R Seances Soc Biol Fil 1948;143:241–3.
2. Fleischhacker M, Schmidt B. Circulating nucleic acids (CNAs) and cancer—a survey. Biochim Biophys Acta 2007;1775:181–232.
3. Devaney SA, Palomaki GE, Scott JA, Bianchi WB. Noninvasive fetal sex determination using cell-free fetal DNA: a systematic review and meta-analysis. JAMA 2011;306:627–36.
4. Lo YMD, Tein MS, Pang CC, Yeung CK, Tong KL, Hjelm NM. Presence of donor-specific DNA in plasma of kidney and liver-transplant recipients. Lancet 1998;351:1329–30.
5. García Moreira V, Prieto García P, Baltar Martín JM, Ortega Suárez F, Alvarez FV. Cell-free DNA as a noninvasive acute rejection marker in renal transplantation. Clin Chem 2009;55:1958–66.
6. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins UL, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci U S A 2008;105:16266–71.
7. Chiu RWK, Chan KCA, Gao Y, Lau VYM, Zheng W, Leung TY, et al. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci U S A 2008;105:20458–63.
8. Ehrich M, Deciu C, Zwiefelhofer T, Tynan JA, Cagasan L, Tim R, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. Am J Obstet Gynecol 2011;204:205.e1–11.
9. Chiu RWK, Akolekar R, Zheng YWL, Leung TY, Sun H, Chan KCA, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ 2011;342:c7401.
10. Sehnert AJ, Rhees B, Comstock D, de Feo E, Heilek G, Burke J, Rava RP. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. Clin Chem 2011;57:1042–9.
11. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med 2011;13:913–20.
12. Snyder TM, Khush KK, Calantine HA, Quake SR. Universal noninvasive detection of solid organ transplant rejection. Proc Natl Acad Sci U S A, 2011;108:6229–34.
13. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Analysis of the size distributions of fetal and maternal cell-free DNA by paired-end sequencing. Clin Chem 2010;56:1279–86.
14. Zheng YWL, Chan KCA, Sun H, Jiang P, Su X, Chen EZ, et al. Nonhematopoietically derived DNA is shorter than hematopoietically derived DNA in plasma: a transplantation model. Clin Chem 2012;58:549–58.