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Citation Classic

The Quest for Accurate Measurement of Fetal DNA in Maternal Plasma

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Featured Article: Lo YMD, Tein MSC, Lau TK, Haines CJ, Leung TN, Poon PMK, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet 1998;62:768–75.[3]

There has been a multidecade search for noninvasive methods of prenatal diagnosis. In 1997, my group reported the presence of cell-free fetal DNA in maternal plasma and serum *(1)*, opening up exciting possibilities that this new noninvasive source of fetal genetic materials might be used for prenatal testing. Much remained unknown, however, regarding this newly discovered phenomenon. For example, it was unclear when fetal DNA first entered into the maternal circulation and whether its concentration would be sufficient for robust diagnostic assays to be developed.

The 1998 report featured here represented the first attempt to address these and other issues. I decided to use the then-new real-time quantitative PCR *(2)* as a tool for measuring fetal and total DNA in maternal plasma and serum. I was fortunate that my department head at the time, Professor Magnus Hjelm, had kindly provided me with the funding to purchase a real-time PCR instrument, one of the first in Asia. Armed with this new equipment, I was able to rapidly develop a set of protocols that would allow the robust detection and measurement of fetal DNA and total DNA in maternal plasma and serum. These protocols and their variants have since become the standard techniques in the field of noninvasive prenatal diagnosis and are used in other fields with a need to measure nucleic acids in plasma *(3)*.

With this technique, I was able to document that fetal DNA was detectable in maternal serum from the seventh week of gestation, with the absolute concentration of fetal DNA increasing as the gestational age progressed. These data also provided a set of normative values to compare with the concentrations occurring in pathologic conditions, such as preeclampsia, preterm labor, fetal trisomy 21, and so forth. Indeed, subsequent work has demonstrated that these pathologic conditions are associated with an increase in the concentrations of circulating fetal DNA.

Perhaps the most surprising aspect of this first quantitative study was the demonstration that the mean fractional concentration of fetal DNA in maternal plasma was approximately 3%. This high fractional concentration suggested the possibility of using maternal plasma for relatively robust prenatal diagnostic applications. This prediction has subsequently been shown to be correct, as evidenced by the accuracy of this approach for determining the fetal sex for sex-linked disorders and for fetal RhD genotyping. Another important finding that came out of this first study was the fact that maternal serum had a much lower fractional fetal DNA concentration than plasma, possibly due to the liberation of DNA during the clotting process, suggesting that maternal plasma might be preferred over maternal serum for noninvasive prenatal diagnosis.

Another important consequence of having reference values for fractional fetal DNA concentrations is that it has provided a foundation for investigators to develop prenatal tests that aim at detecting quantitative aberrations in the fetal genome, e.g., trisomy 21. Indeed, the fractional concentration of fetal DNA is a crucial parameter in the latest generation of "molecular counting" assays, such as those involving digital PCR *(4)* and next-generation sequencing *(5)*, for detecting fetal DNA in maternal plasma. In general, the lower the fractional concentration of fetal DNA is, the larger is the number of molecules that would need to be counted to detect a particular quantitative aberration in the fetal genome.

The last 13 years have been an exciting time for building the foundation for noninvasive prenatal diagnosis using circulating nucleic acids. It is hoped that the next 13 years might see this foundation transformed into clinical applications that will make prenatal testing safer for pregnant women and their fetuses.

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[3] This article has been cited more than 530 times since publication.

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