Noninvasive Prenatal Diagnosis: From Dream to Reality

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I became interested in the area of prenatal diagnosis when I was a medical student at Oxford. I had been following the impressive advances occurring in the prenatal diagnosis of the hemoglobinopathies through the pioneering work of Prof. Yuet Wai Kan at the University of California San Francisco (1) and Prof. David Weatherall at Oxford (2). I also had the good fortune of getting firsthand experience in a research laboratory by working under a pathologist, Dr. Kenneth Fleming (Fig. 1), in the after-hours of the medical school curriculum. One day, I heard a lecture from Prof. John Bell, currently Regius Professor of Medicine at Oxford, who at that time had just returned to Oxford from Stanford. Prof. Bell talked about a then-new method, polymerase chain reaction (PCR), that captured my attention. After his lecture, I was able to persuade Prof. Bell to teach me the method. I was soon able to appreciate the versatility (3) as well as the potential shortcomings (e.g., susceptibility to contamination) (4) of this powerful method.

I soon developed an interest in ways to further improve methods for prenatal diagnosis. One important disadvantage of the methods for DNA-based prenatal diagnosis was that invasive sampling of fetal tissues, e.g., by amniocentesis, was necessary to obtain fetal cells for further analysis. Such invasive sampling carries a small, but definite, risk to the fetus. To avoid such risks, numerous scientists had for many years dreamt about the possibility of performing noninvasive prenatal diagnosis. Toward this goal, there had been attempts since the late 1960s to isolate fetal nucleated cells that had entered into the maternal circulation. However, such early attempts were based on conventional cytogenetic (5) or staining (e.g., quinacrine staining for male cells) (6, 7) methods. I thought that one might be able to use the sensitivity of the PCR to detect such fetal cells that have entered into the maternal circulation. I further postulated that to maximize the chance of success, one could couple two sequential PCRs together. I referred to this as a dual-amplification system, which is now more commonly known as nested PCR. Using such a system, I was indeed able to demonstrate the detection of fetal DNA among maternal blood cells (8). Other workers soon also reported detecting circulating fetal cells using PCR (9, 10) and molecular cytogenetic methods (11), in combination with various fetal cell–enrichment techniques. However, all of these efforts, including my own, were hampered by problems such as false positivity and false negativity. The main reason for such difficulties is the extreme rarity of fetal nucleated cells in the maternal blood (12). Another reason is the persistence of certain fetal cell populations from one pregnancy into the next one (13). Indeed, I would spend the next 8 years working in this area without significant success. By 2002, it had become clear that the rarity of fetal nucleated cells in the maternal blood would make it very difficult to develop practical and robust methods for noninvasive prenatal testing using this approach (14). Hence, interest in such cell-based approaches has waned.

1997 was a very special year for Hong Kong, the city where I was born. It was the year that Hong Kong would return to Chinese rule after being a British colony since 1841. Although many had left Hong Kong before 1997 because of the uncertain future, my wife and I decided that it would be a good time to return home. Knowing that I would need to build a new career after I had moved back to Hong Kong, I thought that it would be a good time to try something new and even potentially risky. In September 1996, just 4 months before I would return to Hong Kong, 2 papers were published in Nature Medicine regarding the presence of tumor DNA in the cell-free fraction of blood obtained from cancer patients (15, 16). I thought that a cancer growing inside a cancer patient was somewhat analogous to a fetus developing inside its mother’s womb, and in my clinical practice, I had yet to see a cancer that was as big as an 8-pound baby. Hence, I thought that one should be able to detect fetal DNA in the cell-free portion, i.e., plasma or serum, of the mother’s blood. In collaboration with Prof. James Wainscoat, a hematologist in Oxford, we were able to show that fetal DNA was indeed present in maternal plasma and serum (17). The fact that in most cases when the mothers were pregnant with male fetuses we were able to obtain a robust fetal Y chromosome signal using just 10 μL boiled plasma or serum sug-
gested that the concentration of cell-free fetal DNA in such samples should be very high.

Eager to find out what were the concentrations at which fetal DNA could be found in maternal plasma and serum, I looked into methods for the quantitative analysis of DNA. At the end of 1996, a method called real-time PCR, in which one would monitor the progress of a DNA amplification reaction and hence would arrive at an accurate measurement of the input DNA concentration, had just been published (18). However, to perform such a technique, one would require an expensive instrument that was essentially a combined PCR thermal cycler and a fluorescence camera. On Boxing Day 1996, I was invited to the house, near London, of my future head of department in Hong Kong, Prof. Magnus Hjelm. I decided to ask him to install a real-time PCR machine in my new laboratory in Hong Kong. Rather surprisingly and almost immediately, Prof. Hjelm had agreed to my request and with it I had received the biggest Christmas present in my life!

I started working in Hong Kong in January 1997 and began the project for measuring the concentrations of fetal DNA in maternal plasma and serum. The fractional fetal DNA concentrations in maternal plasma were remarkably high, having a mean of some 3% in early pregnancy and 6% in late pregnancy (19). These concentrations were much higher than the corresponding figures for fetal nucleated cells that were present in maternal blood, which were typically at a level of 1 fetal cell per $10^5$ or $10^6$ maternal cells, or lower. Furthermore, a serial analysis on a cohort of pregnant women sampled at multiple times during pregnancy indicated that fetal DNA was present in maternal serum as early as the seventh week of gestation. All in all, these data indicated that fetal DNA in maternal plasma would be a valuable material for noninvasive prenatal diagnosis. Its very high concentrations would indicate that prenatal testing using this material would potentially be much more robust than using fetal cells in maternal blood. Furthermore, the ranges of fetal DNA concentrations at different gestational ages would become the foundation on which one could build diagnostic platforms based on this material.

As mentioned above, the use of fetal cells in maternal blood for noninvasive prenatal diagnosis is complicated by the persistence of certain fetal cell populations from one pregnancy to the next (13). I wanted to see if such a phenomenon would also complicate the diagnostic use of cell-free fetal DNA in maternal plasma. Hence, I recruited a number of women undergoing cesarean section and sampled their blood at multiple time points after delivery. The results showed that fetal DNA was cleared very rapidly from maternal plasma after delivery, with a half-life of some 16 min (20). These data thus showed that unlike fetal cells in maternal blood, the use of cell-
free fetal DNA in maternal plasma for prenatal diagnosis would not be complicated with persistence from a previous pregnancy.

Thus, with the above 3 studies laying the fundamental biological parameters of the phenomenon (17, 19, 20), I decided to apply cell-free fetal DNA testing to the noninvasive prenatal diagnosis of fetal rhesus D blood group status in cases where the mothers were rhesus D negative. This application is clinically useful because rhesus D–negative mothers might be immunologically sensitized if their fetuses were rhesus D positive and might produce antibodies to attack the fetal red blood cells in subsequent pregnancies involving rhesus D–positive fetuses. Hence, it would be useful to be able to identify which rhesus D–negative pregnant women were at risk and which were not. I was able to demonstrate that one could determine the fetal rhesus D blood group status with high accuracy using maternal plasma and real-time PCR (21). This test has now been available in Europe for clinical testing since around 2001 and in the US a few years later, and represents the first clinical application of DNA-based noninvasive prenatal diagnosis.

The prenatal diagnosis of fetal chromosomal aneuploidies such as Down syndrome represents one of the most common reasons that pregnant women go for prenatal testing. I have shown that the concentrations of cell-free fetal DNA in the plasma of pregnant women carrying Down syndrome fetuses are higher than those carrying chromosomally normal fetuses (22). Hence, maternal plasma DNA analysis can be used as a screening test for fetal Down syndrome. The challenge is that there is some overlap in the concentration ranges between the Down syndrome group and the normal group, and thus the diagnostic sensitivity and specificity of the test would need to be improved before it could become a valuable testing tool. I then spent the next 10 years exploring various approaches through which such testing can be enhanced.

Down syndrome is caused by an increased dosage of genetic material on chromosome 21, most commonly caused by the presence of an extra copy of the chromosome. Hence, a key to the noninvasive prenatal testing of Down syndrome is the development of methods that would allow one to accurately measure such a chromosome dosage increase. Because fetal DNA is normally present as a minor fraction of the total DNA that is found in maternal plasma (19), one approach that could enhance the performance of such chromosome dosage measurement is by targeting a subset of DNA molecules in maternal plasma that is specific to the fetus. I reasoned that DNA molecules from different tissues might have different biochemical modifications to their DNA and thus it might be possible to find modifications that would allow one to distinguish fetal from maternal DNA in plasma. Such modifications are generally referred to as epigenetic changes. The most studied type of epigenetic change is DNA methylation. In 2002, I described a method that was based on DNA methylation and demonstrated its use for detecting fetal DNA in maternal plasma (23). Subsequent experiments showed that this approach could indeed be used for detecting Down syndrome (24) and another chromosomal disorder called Edwards syndrome (25).

At the same time that I was exploring the use of DNA methylation, I also worked on another method in parallel. I thought that among cells in different tissues, the genes that would be switched on would be different. Hence, genes that were preferentially switched on in the fetal tissues, but switched off in the maternal tissues, would represent another class of fetal-specific markers that one could use for detection in maternal blood. I then turned to the detection of a product of gene expression, namely messenger RNA (mRNA), and showed in 2000 that fetal mRNA was indeed present in maternal plasma (26). The detection of fetal mRNA in maternal plasma was somewhat surprising, since the general belief at the time was that mRNA was so fragile that it would be highly unlikely to survive in plasma where various nucleases are present. Subsequent research has shown that such circulating mRNA molecules are protected by particles (27, 28). Further work has shown that plasma mRNA analysis can be used for the noninvasive prenatal testing of Down syndrome (29).

Whereas approaches based on DNA methylation and mRNA analysis represent feasible methods for detecting fetal chromosomal disorders, good markers based on such approaches require substantial optimization efforts and in many cases require the concomitant analysis of markers of DNA variations (such as single nucleotide polymorphisms) that may not work for all fetus-mother pairs. Hence, I had decided to explore an approach that did not have such a limitation. One way is to develop extremely precise methods of quantification that would allow one to measure a fetal chromosome dosage change (e.g., an increase in the case of Down syndrome), even when such a change is partially masked by the background of maternal DNA in maternal plasma.

I thought that one way whereby this could be achieved was to count DNA molecules in maternal plasma one at a time. Using this strategy, one could potentially reach any degree of precision simply by increasing the number of molecules that one would count. In collaboration with Rossa Chiu and Allen Chan from my department at the Chinese University of Hong Kong (Fig. 2), we reported in 2007 that this approach could indeed be done using single-molecule PCR (more commonly known as digital PCR) (30). We then showed in 2008 that massively parallel sequencing (also known as next generation sequencing) offered significant advan-
tages over digital PCR for implementing such a counting approach and would allow the detection of fetal Down syndrome using maternal plasma with very high sensitivity and specificity (31). We then initiated a large-scale, multicenter validation study of this technology and achieved a diagnostic sensitivity of 100% and a specificity of 98% (32). The results of this study have been replicated by other groups (33, 34). This technology was introduced into clinical service in the latter half of 2011 and in the subsequent 3 years had been performed on >700,000 maternal plasma samples in >50 countries. This development represents one of the most rapidly adopted genomic tests.

After the achievement of the robust noninvasive prenatal detection of Down syndrome, I wanted to explore how far one could push this technology. Hence, in 2010, my research team successfully developed an approach for sequencing the entire fetal genome from maternal plasma (35). This method is based on the performance of very deep sequencing of maternal plasma, covering the human genome several tens of times. This is then followed by deduction of the fetal genome from the sequencing data while taking reference to the genomic sequences of the father and mother of the fetus. This approach has been confirmed by other workers (36, 37). Moving beyond the genome, in 2013, my group further showed that one could determine a fetal epigenome from maternal plasma (38). This approach would be a potentially valuable tool for exploring the intricate relationship between biochemical modifications of the fetal genome and physiological or pathological development.

As my work in plasma DNA was initially inspired by work in the cancer area (15, 16), I decided to pursue the use of plasma DNA for cancer detection in parallel with my fetal work. Thus, I have developed cancer tests that are analogous to the corresponding fetal DNA test, using real-time PCR (39), DNA methylation markers (40), plasma mRNA markers (41), etc. Recently, because of the success of plasma DNA sequencing for the detection of fetal chromosomal abnormalities (31, 42), my group has also explored an analogous approach in an attempt to develop a universal test for cancer. Our recent data in this area are very promising (43, 44) and have shown that this approach can be used to detect multiple types of cancer using a blood sample. Our results have been supported by those from other groups (45, 46).

In summary, the past 17 years have been a very exciting time for me to be able to demonstrate the presence of fetal DNA in maternal plasma and develop the technologies that would allow us to study it and use it for molecular diagnosis. It is also very encouraging to me and my research team to see that this technology has been adopted on a worldwide scale, making prenatal testing less stressful for pregnant women and safer for their babies. It is also an opportune time for us to discuss the various social, legal, and ethical issues related to this new technology (47) and to explore the best way to integrate it into our healthcare systems.
References


