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COMMENTARY

Fetal DNA in Maternal Plasma/Serum: The First 5 Years

Commentary on the article by Jimenez and Tarantal on page 18

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Prenatal diagnosis is an established part of the obstetrical care in many countries. However, current methods for obtaining fetal tissues for prenatal diagnosis, such as amniocentesis and chorionic villus sampling, are invasive and constitute a finite risk to the fetus and mother. The development of noninvasive prenatal diagnostic methods that do not carry such a risk has been a long-sought goal of human genetic research. Initially, researchers have focused on the possibility of detecting and isolating fetal cells that have been passed into the maternal circulation during pregnancy (1, 2). However, the rarity of such cells in the maternal circulation (3) has made the routine implementation of such a testing strategy difficult, despite the many years of research and efforts that have been spent on this area (4).

In 1997, Lo *et al.* (5) demonstrated that readily detectable quantities of fetal DNA can be found in the noncellular fractions, *i.e.* plasma and serum, of maternal blood. The subsequent development of real-time quantitative assays for fetal DNA in maternal plasma and serum (6) has allowed a close to 100% sensitivity and specificity for fetal DNA detection in maternal plasma/serum (7). The reliability of this approach has allowed the diagnostic applications of fetal DNA in maternal plasma/serum to be developed very rapidly. Thus, the prenatal diagnosis/exclusion of sex-linked diseases (7), fetal rhesus D status (8), myotonic dystrophy (9), achondroplasia (10), and congenital adrenal hyperplasia (11) has been achieved. In addition, quantitative abnormalities involving fetal DNA in maternal plasma/serum have also been described for preeclampsia (12), preterm labor (13), and hyperemesis gravidarum (14).

In this issue of the *Pediatric Research*, Jimenez and Tarantal (15) describe an important new development in this field. These investigators have demonstrated that fetal DNA can be detected in the maternal serum of rhesus monkeys, with gestational changes and postnatal clearance characteristics that parallel the situation in humans. Thus, in this animal model (15), the concentration of fetal DNA in maternal serum has been found to increase with gestational age; with fractional concentrations that are very similar to those found in humans (6). Furthermore, after delivery, fetal DNA has been found to be cleared rapidly from the serum of rhesus monkeys (15), once again analogous to the situation in humans (16). These data thus suggest that this rhesus monkey system would represent a good animal model for studying the phenomenon of fetal DNA in maternal plasma/serum.

The availability of such an animal model would aid in the elucidation of several previously unanswered questions in the field. One area concerns the origin of fetal DNA in maternal plasma/serum, which could in theory come from the degradation of fetal nucleated cells that have entered into the maternal circulation; or be released from trophoblasts from the placenta (17). Such a model could also be used for studying the possible relationship between fetomaternal *cellular* transfer and *cell-free* DNA transfer between the mother and fetus (18). A related area is the mechanisms whereby pregnancy-associated disorders, such as preeclampsia (12), result in the elevation in fetal DNA in maternal plasma/serum. In this regard, it would be necessary to extend the rhesus monkey model to simulate such disorders. Another unsolved puzzle concerns the maternal organ systems that are involved in the efficient removal of fetal DNA from the maternal circulation (16). The availability of an animal model would potentially allow the design of experiments that will test the role of candidate organ systems, such as the liver and kidneys (19), in the removal of fetal DNA from maternal plasma/serum. These experiments would also help in the possible resolution of controversial areas, such as whether fetal DNA is also found in maternal urine (19, 20).

Two recent developments in the circulating fetal nucleic acid field include the detection of fetal RNA (21) and fetal epigenetic markers (22) from maternal plasma. The rhesus model

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Conflict of interest statement: The author and his collaborators have held or have applied for patents on aspects of the use of fetal nucleic acid in maternal plasma/serum for noninvasive prenatal diagnosis. The author is a consultant and shareholder of Plasmagene Limited, which holds licenses to the use of some of these technologies.

system developed by Jimenez and Tarantal could be used to further develop these areas. Thus, it would potentially be easier to obtain fetal tissues for gene expression and epigenetic analyses, so that RNA species that are preferentially expressed in fetal tissues and DNA sequences that are differentially methylated between the fetus and mother could be identified and possibly detected in maternal plasma/serum.

Apart from biologic issues, a detailed understanding of the technical parameters affecting the reliability of the detection of fetal DNA in maternal plasma/serum would also be essential for its use as a routine prenatal diagnostic approach. Technical parameters that have been studied to date include a comparison of plasma *versus* serum (6) and the effects of centrifugation (23). The availability of an animal model would allow many of these parameters to be tested in a highly controlled fashion. It is hence interesting to note that Jimenez and Tarantal have hinted in their article that their preliminary data have suggested that increasing the volume of maternal serum could increase the sensitivity of this type of analysis for the early gestational age (15).

The fetomaternal transfer of nucleated cells and cell-free DNA has now been recognized to be a bidirectional phenomenon (18, 24), with passage of cells and cell-free DNA from the fetus to the mother, and *vice vera*. Thus, it would be interesting to investigate whether the fetomaternal transfer of cells and cell-free DNA is also bidirectional in the rhesus monkey model. Furthermore, as a population of fetal cells have been found to persist in the mother's body after delivery (25), it would also be interesting to see if such fetal cell persistence would also be present in this animal model.

In conclusion, the last 5 years have seen a rapid development in our understanding and application of the phenomenon of fetal DNA in maternal plasma/serum. The timely availability of a primate model of this phenomenon (15) will further catalyze the development of this field. It is highly likely that by the 10th anniversary of this field, fetal DNA analysis in maternal plasma/serum will already be a routine part of many prenatal diagnostic protocols. Such a development will make prenatal testing less hazardous and much less psychologically stressful for many pregnant women.

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