Evidence for Feasibility of Fetal Trophoblastic Cell-Based Noninvasive Prenatal Testing

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ABSTRACT

The potential use of fetal cells in maternal blood for prenatal diagnosis has been a promising technology for decades. Several strategies have been tried and tested for recovering fetal cells, although they are inconsistent and not reproducible. The present study aimed to develop methods for the recognition of chromosomal abnormalities in fetal trophoblast cells circulating in maternal blood between 10 and 16 weeks of gestation, based on array comparative genomic hybridization (CGH) and/or next-generation sequencing (NGS) analyses. Blood was collected from participants, and fetal gender was determined by polymerase chain reaction (PCR). Fetal trophoblasts were enriched by RareCyte’s trophoblast enrichment and staining method, subsequent to which the fetal cells were recovered and subjected to automated image capture and analysis. Cells identified to be cytokeratin positive and CD45 negative were manually confirmed as potential fetal trophoblasts. Whole-genome amplification (WGA) was performed, and WGA products were analyzed by array CGH and whole-shotgun NGS to determine copy number. The potential fetal cells that underwent WGA were characterized by Y chromosome–specific PCR and genotyping with short tandem repeats to evaluate gender and allelic inheritance patterns, respectively. Blood samples from 30 male pregnancies (including one 47,XXY) from several collection sites were established to have cells that were genotypically male by Y-PCR. Array CGH and/or NGS was performed on 40 single cells from 16 pregnancies to illustrate the sex chromosome copy number differences, 1 47,XXY, 14 trisomy 21, 1 trisomy 18, 1 trisomy 13, 2 cells with a 2.7 Mb deletion on 15q, and 4 cells with a 1.2 Mb deletion on 1q. The gain of copy number for the X chromosome was demonstrated for a 47,XXY case sampled at 13 weeks of gestation subsequent to a positive cell-free fetal DNA result for sex chromosome abnormality. Fetal cells from 5 pregnancies were later shown to have a chromosomal abnormality and 1 pregnancy with probable confined placental mosaicism. An incidence of a 2.7 Mb deletion of chromosome 15q25.2q25.3 was also observed, from which 2 fetal cells were discovered, and both had deletions. This showed the potential for detection of minor deletions from individual fetal cells. The study concluded that circulating trophoblasts could be successfully isolated and used for noninvasive genome analysis by array CGH and NGS to reveal chromosomal aneuploidy and confirm fetal gender.

EDITORIAL COMMENT

(The Holy Grail! Identifying and separating fetal/placental cells from the maternal circulation have been an unachievable goal in prenatal diagnosis for more than 2 decades. Whereas early prenatal diagnosis using amniocentesis obtained fetal cells, chorionic villus sampling obtained trophoblast cells. Both versions of prenatal diagnosis were associated with an increased risk of pregnancy loss and were not widely utilized except in patients who were at increased risk of aneuploidy either by increased maternal age or positive screening tests. The idea of undertaking a test that put the pregnancy at increased risk of loss was not something that many women or providers had much tolerance for, although it is unclear whether more widespread adoption would have occurred had the testing been more routinely offered.)
It appeared that potential risks to the pregnancy were a sticking point, so noninvasive screening approaches were adopted, evolving from maternal serum α-fetoprotein screening to triple screen, to quad screen, to combined screening. Yet, these screening tests all had at least a 5% false-positive rate and imperfect sensitivities. When cell-free DNA (cfDNA) screening demonstrated a better than 99% sensitivity and specificity, the promise was that this was almost diagnostic. Subsequently, this method of screening for chromosomal abnormalities has become rapidly accepted and adopted. The testing had such high sensitivity and specificity that it appeared cfDNA might replace standard serum screening. The problem with cfDNA screening is that while its sensitivity and specificity are greater than 99% for most individual aneuploidies, the rates of false positives and false negatives are cumulative. That means that screening for all of the potential trisomies, the specificity would likely fall to less than 95% and potentially worse, so that there would be a greater than 5% false-positive rate. In parallel, the number of any chromosomal abnormalities that would be missed on screening increases with the number of items screened for. This has been demonstrated in recent studies that find that when one considers the wide range of chromosomal abnormalities, that combined screening has a higher sensitivity than cfDNA (*Obstet Gynecol* 2014;124:979–986).

So, cfDNA appears to fall short of the solution we have been seeking. However, isolation of a fetal cell from the maternal circulation on which a whole-genome microarray CGH could be conducted may be a dramatic improvement. This would allow the identification not only of common aneuploidies, but also of clinically important copy number variants. In the article abstracted above, the authors identified not only the Y chromosome, but also trisomy 18, trisomy 21, and even a 2.7 Mb microdeletion case using single trophoblastic cells isolated from the maternal blood.

Why not start such testing today? This kind of a study merely shows that such science is possible. Similar to the early findings that cfDNA could identify trisomy 21 in the maternal circulation, we hold hope that these findings might be expanded into a test that utilizes reasonable resources to reliably diagnose the wide range of clinically relevant, fetal chromosomal abnormalities for most pregnant women. The importance of clinical testing in this case isn’t so much the safety issue that concerned providers of amniocentesis 4 decades ago, but the accuracy of this testing. Showing that it can be performed does not give us any measure of certainty. It may be that there will be both diagnoses missed and diagnoses overcalled. We won’t know until at least the first large cohort study is conducted. Hopefully, such a study is already underway.—ABC)