Will Epigenetic Allelic Ratio Analysis Turn Prenatal Diagnosis of Trisomy 18 on Its EAR?

In a proof of principle report elsewhere in this issue of Clinical Chemistry, Tong et al. (1) describe a novel approach to the noninvasive prenatal diagnosis of a human chromosomal abnormality by analyzing the allelic ratio of a polymorphism present within the methylated promoter of a DNA sequence on chromosome 18q21.3, maspin (SERPINB5). This method differs from other approaches to noninvasive diagnosis of aneuploidy in that it is truly diagnostic, as opposed to being a screen.

The technique described in this paper relies first on the isolation of cell-free fetal DNA from maternal plasma, and second, on the fact that there is differential methylation of maspin. In a previous report from the same laboratory group (2) maspin was shown to be hypomethylated (i.e., actively expressed) in placenta and hypermethylated (i.e., silenced) in maternal leukocytes, which are the source of most circulating cell-free DNA in plasma (3). By the method of bisulfite modification, unmethylated cytosine residues in the DNA sequence are converted to uracil, but methylated cytosine residues remain unchanged. This sequence difference is then exploited via methylation-specific PCR amplification, followed by allele-specific primer extension. A single-base variation [or single nucleotide polymorphism (SNP)] within the promoter sequence is then used as a focus for mass spectrometric analysis, with subsequent analysis of the ratio of copy numbers of the particular DNA sequence variation.

In the present study, the U-maspin-156 SNP was used. A normal euploid fetus might be genotyped as AC at this locus. Fetuses with trisomy 18, if polymorphic, would be genotyped as having AAC or ACC. By measuring the ratio of A to C, a euploid fetus would have 1, and a trisomy 18 fetus would theoretically have 2 or 0.5. It is important to recognize that the methylated maternal cell-free DNA, which derives mainly from blood cells, does not amplify in the methylation-specific PCR. The amplified product derives exclusively from the placental DNA and is therefore a reflection of the fetal genotype.

After analysis of placental tissue and maternal DNA, as well as artificial mixtures, the investigators proved the feasibility of this approach by analyzing cell-free fetal DNA in maternal plasma from euploid and aneuploid pregnancies.

While the concept is original and innovative, several technical, biological, and practical issues must be addressed before epigenetic allele ratio (EAR) can be translated into clinical practice. First, the authors readily acknowledge that the sensitivity and specificity depend upon the amount of fetal DNA extracted from maternal plasma. It is well documented that the amount of fetal DNA in maternal plasma is a function of gestational age (4). Fetal DNA levels are low in the first trimester but rise ~21% per week until the second trimester, where they plateau until the beginning of the third trimester (4–6). Using serial dilution experiments, Tong et al. (1) demonstrate that their assay becomes progressively less precise with smaller starting amounts of DNA. Furthermore, the bisulfite conversion technique degrades 84%–96% of the DNA present in the reaction, leaving precious little remaining for analysis of allele ratios. In the EAR analysis of actual maternal plasma samples, these authors used 8 “predelivery” (presumably third trimester) samples but needed to pool 2 second trimester plasma samples to determine reference EAR values for euploid pregnancies. Two plasma samples were studied from pregnancies with fetuses with trisomy 18 at 15 and 18 weeks of gestation. Additional work needs to be done to evaluate the clinical performance of EAR in samples obtained from women in the late first and early second trimesters of pregnancy.

Another issue is the requirement to identify highly polymorphic areas of the genome in which there exists tissue-specific methylation. In this report 173 euploid placenta were genotyped for polymorphisms at the U-maspin 156 site. Of these only 31/173 (17.9%) were informative. Not surprisingly, significant ethnic variation was observed. The investigators did not find the C allele in 129 placentas obtained from Caucasians, but did observe it in Chinese and Africans. This suggests that extensive SNP analysis and a thorough characterization of population-specific variation must precede clinical application of EAR analysis in maternal plasma.

Maspin, also known as mammary serine protease inhibitor B5, is a tumor suppressor gene that has inhibitory effects on cell motility, invasion, metastasis, and angiogenesis in human breast and prostate cancer cell lines. Hypermethylation of maspin correlates with tumor invasiveness and recurrence. In 2002, Dokras et al. (7) showed that maspin is expressed in the cytotrophoblast and synctiotrophoblast layers of the placenta, and that this expression is affected by gestational age. During the first trimester, low levels of maspin are expressed, which correlates with maximal trophoblast proliferation and invasion into maternal decidual tissue. As the pregnancy advances, maspin expression levels increase, to the point of being maximally detectable in term placentas. This presumably results from maximal tumor suppressive/anti-invasive effects and has been speculated to be the signal that stops the trophoblast from continued proliferation at the end of pregnancy.

Given the gestationally-age dependent differential gene expression pattern of maspin, it was initially somewhat surprising that unmethylated maspin would be considered as a target sequence for the detection of fetal aneuploidy, especially in the first trimester, when expression is low. Fortunately, however, very recent data from placentas obtained at a variety of gestational ages demonstrate that there is no significant change in the methylation of the promoter region of maspin throughout gestation (8). Regulation of maspin gene expression apparently occurs by changes in histone tail modifications. The biology of
maspin expression should therefore not affect the ability to accurately detect the hypomethylated sequence in maternal plasma nor its epigenetic allele ratios.

Perhaps the greatest obstacle to the clinical incorporation of the EAR approach is the fact that a relatively simple, accurate, and reproducible noninvasive screening test for trisomy 18 already exists. The current maternal serum screening algorithm is extremely cost-effective, as serum markers useful for the diagnosis of trisomy 18 are already assayed as part of protocols to screen for trisomy 21. Screening for trisomy 18 adds no (or very minimal) increased cost. Screening for trisomy 18 has been in practice since the 1980s, when unconjugated estriol (uE3) and human chorionic gonadotropin (hCG) were added to the measurement of alphafetoprotein (AFP) as part of second trimester serum maternal screening protocols for Down syndrome. uE3 levels are low in fetuses affected by both trisomies 21 and 18, but hCG levels are high in trisomy 21 and low in trisomy 18. This information facilitated the development of software programs that can simultaneously screen for trisomies 21 and 18. More recently, the incorporation of measurement of first trimester pregnancy-associated plasma protein A (PAPP-A) levels, along with algorithms that use a combination of first- and second-trimester markers (so-called “serum integrated” testing) have continued to improve screening performance. In a metaanalysis of 6 existing data sets, Palomaki et al. (9) stated that using 4 serum markers (first-trimester PAPP-A, second-trimester AFP, uE3, and hCG) and software parameters that identify all fetuses with at least a 1:100 risk of trisomy 18 as “screen positive”, 90% of fetuses with trisomy 18 would be detected with a very low false positive rate of 0.1%. With this approach, the odds of an affected fetus with trisomy 18 given a positive screen are 1 in 4. These performance numbers do not even take into account the nuchal translucency (NT) measurement, a first trimester sonographic study that has also been incorporated into routine screening for Down syndrome. Some fetuses affected with trisomy 18 have an increased NT measurement (10). Thus, performance of the full integrated test, incorporating both serum and sonographic measurements, can only get better, improving the already existing high detection rate and very low false positive rate for trisomy 18.

Thus, will epigenetic ratio analysis (EAR) turn prenatal diagnosis of trisomy 18 on its ear? Not yet. However, the innovative approach demonstrated in this study certainly encourages us to turn our heads and more closely examine the realistic possibility of noninvasive direct diagnosis of aneuploidy using maternal plasma DNA.

References


Diana W. Bianchi

Division of Genetics
Departments of Pediatrics and Obstetrics and Gynecology
Tufts-New England Medical Center
750 Washington St, Box 394
Boston, MA 02111
Fax: 617-636-1469
E-mail DBianchi@tufts-nemc.org

DOI: 10.1373/clinchem.2006.079129