Several studies using quantitative real-time PCR to measure fetal DNA and mRNA in maternal plasma have suggested that their concentrations could indicate the presence of certain pregnancy-related disorders or predispositions to them (1). Significant increases in the median concentration of cell-free fetal DNA have been reported in pregnancies bearing aneuploid fetuses (2,3), in those affected by or at risk for preeclampsia (4–8), or those with preterm labor (9). Fetal-derived corticotropin-releasing hormone (CRH) mRNA is reportedly increased in pregnancies with aneuploid fetuses (11). In this study, we measured CRH mRNA by a real-time quantitative reverse transcription-PCR assay (10,12) in women with preterm labor. Preterm delivery remains a leading cause of perinatal mortality, has an unchanged incidence of almost 7% over the past decade (13,14), and has no known etiology. No test can identify those pregnant women with preterm contractions who will deliver prematurely and those who will respond to treatment (14).

We performed a retrospective analysis of circulatory CRH mRNA concentrations in samples we had collected for the assessment of fetal cell trafficking in pregnancies with preterm labor (15). The definition of preterm labor was made in accordance with those of the Canadian preterm labor investigations group (14). All samples were collected at a median of 26 weeks of pregnancy (range, 19–33 weeks). Tocolytic treatment was carried out with hexoprenalin, a β-sympathomimetic (15). In addition, all women with preterm contractions received an in utero glucocorticoid application at the time of blood sampling to assist with fetal lung maturation (15). Exclusion criteria included pregnancies with known fetal malformations and those women who had received previous glucocorticoid treatment (15). Of the original study cohort, three cases had premature rupture of membranes, six cases had vaginal bleeding, and four fetuses were intrauterine growth retarded (15). No invasive procedures were performed during the time of the study (15).

In our current analysis, of the 35 cases with preterm labor, 11 women delivered prematurely (median gestational age at delivery, 33 weeks), and 24 responded to tocolytic treatment and delivered at term (median gestational age at delivery, 38 weeks). Fifteen matched control cases who all delivered at term were included in our current analysis. mRNA was extracted from 800 μL of plasma stored frozen at −80 °C by use of TRIzol LS Reagent (Invitrogen) and commercial column technology (Qiagen). CRH mRNA concentrations were quantified by real-time reverse transcription-PCR as described previously; results are reported in copies per mL of maternal plasma (10,12). The data were analyzed by use of SPSS for Windows, and the Mann–Whitney U-test was used to determine significance of differences between the study and control groups (9). The results are presented in Fig. 1 as box plots.

Circulatory CRH mRNA was not significantly higher (P = 0.09) in the group with preterm contractions (n = 35; median, 81 copies/mL; range, 11–6285 copies/mL) than in the control cohort (n = 15; median, 38 copies/mL; range, 17–147 copies/mL). Circulatory CRH mRNA concentrations were, however, higher than in controls in the subgroup that delivered prematurely despite treatment (PTD group; n = 11; median, 100 copies/mL; range, 19–743 copies/mL; P = 0.003), but not in the treated group that delivered at term (TD group; n = 24; median, 66 copies/mL; range, 11–6285 copies/mL; P = 0.502; Fig. 1). This effect was apparent, although four cases with very high circulatory CRH mRNA were noted in the TD group (range, 1392–6265 copies/mL). Despite this apparent anomaly concerning the TD group, our overall analysis nevertheless suggests that the analysis of circulatory CRH mRNA concentrations may assist in distinguishing between pregnancies with true preterm delivery and those that respond to tocolysis. It should be noted, however, that such an assessment will not be 100% specific.

![Figure 1. Circulatory fetal CRH mRNA concentrations in normal pregnancies (CON) and in pregnant women with preterm contractions who either delivered prematurely (PTD) or responded to tocolysis and delivered at term (TD).](image-url)
In conclusion, the remarkably similar combined data from these two independent studies suggest that analysis of circulatory fetal nucleic acids may assist obstetricians in identifying pregnant women with an increased risk of preterm labor who do indeed deliver prematurely.

References


A variety of genetic alterations are associated with the initial steps of carcinogenesis in sporadic tumors. Inactivation of tumor suppressor genes frequently occurs in a sequential process of genomic deletion of one allele and missense or nonsense mutation of the other allele in somatic cells (1). Cytogenetically undetectable deletions can be identified at the molecular level as loss of heterozygosity (LOH). Comparative genotyping of polymorphic markers such as microsatellites or single-nucleotide polymorphisms (SNPs) in healthy and tumor tissue can detect the loss of one allele by demonstrating the conversion of a heterozygous marker to a hemizygous genotype. LOH analysis of solid tumors does not necessarily show 100% deletion of one allele, as blood and immune cells without LOH may contaminate the tumor. Therefore, LOH analysis often shows a reduction, rather than a complete disappearance, of one allele.

Genotyping of polymorphic markers has been performed predominantly by gel-based methods, using microsatellites or short tandem repeats. Because of the repetitive nature of these polymorphic structures, PCR amplification often leads to so-called “shadow bands” resulting from DNA polymerase slippage. Shadow bands may overlap with major polymorphic bands and are frequently sources of genotyping ambiguities, particularly in the case of apparent incomplete deletion of one allele (2).

Breast cancer is the most common malignancy of women, with up to 95% of cases sporadic (3). Cytogenetic investigations and microsatellite LOH analysis have revealed deletions in the terminal part of Xp in breast carcinoma (4, 5). On the basis of analysis of 13 SNPs within the genomic region of the potential tumor suppressor gene PPP2R3B, located in the terminal band Xp22.3 (6, 7), we established a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) approach to analyze LOH in nonmicrodissected tissue.

Comparative sequencing of PCR-amplified fragments in tumor and healthy tissue revealed reduced allelic signals in tumor tissue in 3 of 29 patients, whereas in healthy tissue, sequencing signals of both alleles were within 20% of each other (Fig. 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue3/). All three (patients 2, 5, and 20) showed loss of several heterozygous markers, indicating that true genomic deletion had occurred.

This study was approved by the Ethics Committee of