We are deeply grateful to Dr. Burton D. Cohen for valuable criticism during the preparation of this manuscript.

References


12. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 1990;87:1620–4.


DOI: 10.1373/clinchem.2005.058669

Investigation of the Genomic Representation of Plasma DNA in Pregnant Women by Comparative Genomic Hybridization Analysis: A Feasibility Study, K.C. Allen Chan,∗ Angela B.Y. Hui,ii Nathalie Wong,iii Tze K. Lau,iv Tse N. Leung,iv Kwok-Wai Lo,v and Y.M. Dennis Lo∗ (Departments of 1Chemical Pathology, 2Anatomical and Cellular Pathology, 3Clinical Oncology, and 4Obstetrics and Gynecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong Special Administrative Region, China; * address correspondence to this author at: Department of Chemical Pathology, Room 38023, 1/F, Clinical Sciences Building, Prince of Wales Hospital, 30-32 Ngan Shing Street, Shatin, New Territories, Hong Kong SAR; fax 852-2194-6171, e-mail loym@cuhk.edu.hk)

Prenatal diagnosis is part of the established obstetric care in most developed countries. However, current methods for obtaining fetal tissues for prenatal diagnosis, including chorionic villus sampling and amniocentesis, are invasive and carry a risk of fetal loss. Analysis of circulating cell-free DNA from pregnant women provides new possibilities for noninvasive prenatal diagnosis. Clinical applications of plasma fetal DNA analysis in maternal plasma include fetal RhD genotyping (1, 2), fetal aneuploidy detection (3, 4), and the prenatal diagnosis of several genetic diseases, including myotonic dystrophy (5), congenital adrenal hyperplasia (6), and β-thalassemia (7, 8). In addition to exploring the clinical applications of circulating DNA analysis, our group has investigated the molecular characteristics of cell-free DNA in maternal plasma and has shown that plasma DNA fragments in pregnant women are longer than those in nonpregnant.
DNA was extracted from 1.6 mL of plasma by means of a precipitation at 16,000 g for 10 min and microcentrifugation at 16,000 g for 10 min, as described previously (14). DNA was extracted from 1.6 mL of plasma by means of a QIAamp Blood Mini Kit (Qiagen) and eluted with 50 μL of H2O, according to the manufacturer’s recommendations. Blood samples from 5 healthy male control individuals were used to obtain pooled genomic DNA from theuffy coat, which was used as reference DNA. Plasma DNA samples from 10 pregnant women, 10 nonpregnant women, and 4 healthy men, as well as 2 ng of reference DNA, were amplified by degenerate oligonucleotide-primed (DOP) PCR with the DOP Master Kit (Roche).

The principle of DOP-PCR has been described previously (15). The DOP-PCR reaction volume was 100 μL. Each PCR reaction contained 2 μM DOP primer 5′-CCGACTCGAGNNNNNATGTTG-3′ and 50 μL of DOP-PCR master mixture, with 40 μL of plasma DNA or 2 ng of reference DNA as the template. Initial denaturation at 95 °C for 5 min was followed by 5 cycles of 94 °C for 1 min, 30 °C for 90 s, ramping to 72 °C over a 3-min period (3.5 °C/s), and 72 °C for 3 min, then 35 cycles of 94 °C for 1 min, 62 °C for 1 min, and 72 °C for 2 min (and increasing by 14 s for each subsequent cycle), with a final extension step of 72 °C for 7 min.

The DNA products after DOP-PCR were analyzed by agarose gel electrophoresis. A smeared pattern was observed when buffy coat reference DNA and plasma DNA from pregnant and nonpregnant women were used as the template for DOP-PCR. However, the DOP-PCR products obtained with plasma DNA of pregnant women as template were generally shorter than those with buffy coat DNA as template. Furthermore, the DOP-PCR products obtained with plasma DNA from nonpregnant women as template were even shorter, and the resulting smears appearing on agarose gel electrophoresis were less intense than those obtained with plasma DNA from pregnant women used as template.

Amplified plasma DNA and reference DNA were labeled with biotin-16-dUTP and digoxigenin (dig)-11-dUTP (Boehringer-Mannheim), respectively, by nick translation. Biotin-labeled maternal plasma DNA and dig-labeled reference DNA were precipitated together with 40 μg of Cot-1 DNA (BRL). The mixed probe was then dissolved in 10 μL of hybridization buffer and applied to slides with metaphase cells prepared from the blood of a healthy male donor. Hybridization was carried out for 3 days in a 37 °C incubator. Biotin-labeled DNA was detected by use of avidin-conjugated fluorescein isothiocyanate (FITC; Vector) and biotin-conjugated avidin (Vector). Dig-labeled DNA was detected with a mouse monoclonal anti-dig antibody (Sigma), rabbit anti-mouse IgG-conjugated tetramethylrhodamine isothiocyanate (TRITC; Sigma), and goat anti-rabbit IgG-conjugated TRITC (Sigma). Chromosomes were counterstained with an antifade solution containing 4,6-diamino-2-phenylindole (DAPI; Vector). Digital images of FITC, TRITC, and DAPI fluorescence were captured separately by 3 bandpass filters (TRITC, FITC, and DAPI) set in a cooled charge-coupled device camera connected to a Zeiss (Jena) fluorescence microscope. In each case, at least 12 metaphases were acquired. Averaged fluorescence ratios along each chromosome were calculated with a digital imaging system (ISIS3; Metasystems). Threshold amounts for the identification of chromosomal imbalances were set at 0.75 and 1.25. Any ratio profiles <0.75 were scored as underrepresented regions, and those >1.25 were scored as overrepresented regions. Heterochromatic regions in the centromeric and paracentromeric parts of some chromosomes (1, 9, and 16), and the short arms of the acrocentric chromosomes were not included in the analysis because of suppression of Cot-1 DNA in these regions.

CGH images for 10 pregnant and 10 nonpregnant women and 4 healthy men were analyzed. The hybridization of the DOP-PCR products of plasma DNA of pregnant women to metaphase slides was satisfactory, although the images were more granular than those of the products of buffy coat DNA. In contrast, the hybridization was poor when the DOP-PCR products of the plasma DNA of nonpregnant women or healthy men were used. None of the CGH images of the 10 nonpregnant women or the 4 men was satisfactory for analysis. A representative plasma CGH profile for pregnant women is shown on Fig. 1. CGH analysis of the plasma DNA obtained from each of the 10 pregnant women showed an even genomic representation. After delivery, 5 women were confirmed to have been carrying male fetuses. Their CGH profiles were identical to those of women carrying female fetuses and showed underrepresentation of the Y chromosome.
compared with the reference DNA from the male participants.

Because plasma DNA concentrations are higher in pregnant than nonpregnant women, we investigated whether the difference in hybridization efficiencies between pregnant and nonpregnant women was the result of the quantitative difference between DNA templates used for DOP-PCR. We extracted DNA from 1.6-mL plasma samples from 2 pregnant women and 3.2-mL plasma samples from 2 nonpregnant women. The DNA was eluted with 50 μL of H2O. The DNA concentrations in the eluates were quantified by real-time PCR targeting the β-globin gene, as described previously (11). The DNA concentrations of the eluates for the 2 pregnant women were 78 and 94 copies per 5 μL, respectively, and those for the 2 nonpregnant women were 73 and 103 copies per 5 μL. DOP-PCR amplification and hybridization of the amplified products to the metaphase chromosomes were poor for the DNA from nonpregnant women and satisfactory for the DNA from pregnant women. The CGH analyses of the DNA from the 2 pregnant women showed even genomic representation. Our findings suggest that the difference in the efficiencies of the DOP-PCR amplification and the hybridization of amplified products to metaphase chromosome is unlikely to be merely a result of the difference in the concentrations of plasma DNA in pregnant and nonpregnant women. Because the plasma DNA molecules in pregnant women are longer than those in nonpregnant women (9), the difference in the integrity of plasma DNA may also be a factor leading to the difference in the efficiencies of DOP-PCR amplification and subsequent hybridization.

Our finding that the whole human genome is equally represented in the plasma DNA of pregnant women

Fig. 1. Representative CGH profile of plasma DNA from a pregnant woman, with reference to buffy coat DNA from healthy male controls. Lines on the left and right sides of the ideograms indicate under- and overrepresentation of the genomic region, respectively. The genome encoded by the autosomes is evenly represented by the plasma DNA. Overrepresentation of the X chromosome and underrepresentation of the Y chromosome is caused by the sex mismatch between the pregnant woman and the male controls, which served as an internal positive control. The numbers below the ideograms represent the chromosome number and the number of the metaphase analyzed (in parentheses).
suggests that different targets should be equally effective for quantification of the total DNA in the plasma of pregnant women. Diagnostically, this would simplify the choice of maternal targets for circulating DNA analysis in pregnant women. This finding is particularly relevant to pregnancy-associated disorders in which quantitative aberrations of the total plasma DNA have been found [e.g., in preeclampsia (12, 16–18)]. It is important to emphasize, however, that the CGH analysis provided data only for the predominant DNA species in maternal plasma, which are of maternal origin. Methods for analyzing the genomic representation of fetal DNA in maternal plasma will be interesting but much more difficult to achieve. It would also be interesting to analyze the genomic representation of plasma DNA in other conditions, including cancer (19, 20) and trauma (21).

This work was supported by a Central Allocation Grant (CUHK 01/03C) from the Research Grants Council of the Hong Kong Special Administrative Region (China).

References

Blood Catalase Activity in Gestational Diabetes Is Decreased but Not Associated with Pregnancy Complications, Laszlo Geth,1,5 Zoltán Tóth,2 Ilidikó Tarnai,1 Maria Bérces,3 Peter Török,2 and William N. Bigler4 (1 Department of Clinical Biochemistry, Molecular Pathology, and Clinical Analytical Chemistry, 2 Department of Obstetrics and Gynecology, and 3 Neonatal Intensive Care Unit, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary; 4 Center for Biomedical Laboratory Science, San Francisco State University, San Francisco, CA; 5 Department of Laboratory, Municipal Hospital, Sümeg, Hungary; * address correspondence to this author at: Department of Clinical Biochemistry Molecular Pathology, Medical and Health Science Center, University of Debrecen, PO Box 70, Debrecen H-4012, Hungary; fax 36-52-417-631, e-mail goth@jaguar.dote.hu)

Gestational diabetes occurs with variable severity in 3%–5% of all pregnancies and may be related to oxidative stress and impaired antioxidant defenses (1). Antioxidant enzymes include superoxide dismutase, which produces hydrogen peroxide, and catalase, which consumes hydrogen peroxide. Catalase is the main regulator of hydrogen peroxide metabolism (2), which is associated with diabetes mechanisms such as Glut 4 expression, insulin secretion, insulin signaling, protein tyrosine phosphatase regulation, and glucose transport stimulation (3). Hydrogen peroxide has novel insulin-like effects, e.g., inhibition of lipolysis and reactivation of phosphoenolpyruvate carboxy kinase (4, 5), and insulin moderates hydrogen peroxide generation (6, 7) and catalase synthesis (8). High concentrations of hydrogen peroxide may damage heme proteins, cause cell death, and together with redox active metal ions, produce highly toxic hydroxyl radicals.

High catalase activity in erythrocytes seems to provide antioxidant defense for tissues with low catalase activity, particularly pancreatic beta cells. Catalase is important in antioxidant defense against hydrogen peroxide (9, 10), but there are conflicting reports of decreases (11, 12),