Placenta 59 (2017) 39–45

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Lipidomic analysis reveals the significant increase in diacylglycerophosphocholines in umbilical cord blood from pregnant women with gestational hypercholesterolemia

Ran Zhang [a, 1], Qi Zhou [b, 1], Xueping Cai [a], Shan Dong [a], Zhiyin Le [a], Xiaxia Cai [a], Rong Xiao [a], Huanling Yu [a, *]

[a] School of Public Health, Beijing Key Laboratory of Environmental Toxicology, Capital Medical University, Beijing 100069, China
[b] Xuanwu Hospital, Capital Medical University, Beijing 100023, China

ARTICLE INFO

Article history:
Received 11 March 2017
Received in revised form
22 July 2017
Accepted 7 August 2017

Keywords:
Lipidomic
Cord blood
Gestational hypercholesterolemia
Glycerophosphocholines

ABSTRACT

Gestational hypercholesterolemia has been recognized as a risk factor of some pregnancy complications. We supposed that maternal hypercholesterolemia modified the lipid profile of the fetus. Thirty pregnant women with hypercholesterolemia and matched controls were recruited and cord blood was sampled. Lipidomic analysis was used to evaluate the lipid profile change between the two groups. The results showed that the content of diacylglycerophosphocholines (PC) was significantly high in cord blood from hypercholesterolemic pregnant women. PC (16:0/20:4) and PC (18:0/20:4) were selected as the most important lipid species in cord plasma and their contents were positively related to the total cholesterol and high-density lipoprotein cholesterol levels in cord blood. The contents of these two PCs were significantly higher in the hypercholesterolemic group than in the control group. These results suggested that gestational hypercholesterolemia might program the phospholipid metabolism in offspring.

1. Introduction

Cholesterol, an important molecule vital for fetus development, is believed to transport from mother to fetus via the placenta, although the fetus has the ability to synthesis it [1]. During the gestation, serum lipids, including total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels were significantly elevated from the second trimester and reached a maximum in the third trimester [2]. Thus, gestational dyslipidemia was thought to be a common physiological phenomenon during pregnancy characterized by a significant increase in circulating lipid levels [3].

The developmental origins of health and disease (DOHaD) theory, which originated from Barker's hypothesis, suggests that a wide range of environmental conditions during gestation, including under-nutrition, gestational diabetes mellitus (GDM), preeclampsia, pre-pregnancy obesity, might now be recognized as risk factors for metabolic diseases in later life, such as cardiovascular disease [4], obesity and diabetes [5], some types of mental disorders [6], and even breast cancer [7]. In animal models, researchers found that diet-induced gestational hypercholesterolemia resulted in atherogenic programming [8] or reduced vascular reactivity and increased insulin resistance in offspring [9]. In observational studies in human, fatty streaks in arteries were found in premature fetuses from mothers with gestational hypercholesterolemia [10], although in a cohort study, the cholesterol levels in children with familial hypercholesterolemia were not predicted by maternal hypercholesterolemia during gestation [11]. The notion that maternal hypercholesterolemia programmed the cholesterol metabolism of offspring seems to be controversial [11]. Most importantly, the underlying mechanism is still unclear.

Abbreviations: PC, diacylglycerophosphocholines; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; GDM, gestational diabetes mellitus; FA, fatty acyls; GL, glycerolipids; GP, glycerophospholipids; PK, polyketides; PR, prenol lipids; SL, saccharolipids; SP, sphingolipids; ST, sterol lipids; PCA, principal component analysis; OPLS-DA, orthogonality partial least squares-discriminant analysis; PC, diacylglycerophosphocholines; SM, ceramide phosphocholines; ROC, receiver-operating characteristics; AUC, area under the curve; PEMT, phosphatidylethanolamine *N*-methyltransferase.

* Corresponding author. Department of Nutrition and Food Hygiene School of Public Health, Beijing Key Laboratory of Environmental Toxicology, Capital Medical University, Beijing 100069, China.
E-mail address: yuhlzjl@ccmu.edu.cn (H. Yu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.placenta.2017.08.004
0143-4004/

Lipidomics, a brunch of metabolomics, focuses on the global analysis of molecular lipids in a cell, tissue, and biofluids. Lipidomics technology analyzes both the lipid species and their abundance. Rapid progress of lipidomics makes it possible to study the lipid metabolism specifically [12]. In the present study, we hypothesized that lipid profiles in fetal cord blood from hypercholesterolemic mothers might change and which of them might program the disease of offspring.

2. Materials and methods

2.1. Subjects and recruitment

The pregnant women were recruited at their first antenatal visit, before the 12th gestational week at Bei Jing Xuanwu hospital, from January to December 2014. This study received ethical approval from the ethics committee of Capital Medical University (2012SY29). Written informed consent was obtained from all participants before their enrollment in the study. After signing a consent form, each woman was interviewed and the information containing general sociodemographic data, medical history, and drinking and smoking habits was obtained via a questionnaire.

There is no definition of gestational hyperlipidemia or hypercholesterolemia and no cutoff value for normal serum cholesterol level for pregnant women. According to Pietiläinen et al. [13], hypercholesterolemic pregnant women were selected if their serum TC concentration were higher than 7.25 mM ($n = 30$) at the third trimester (36th to 37th week of pregnancy); pregnant women whose serum TC levels were lower than 7.25 mM were grouped into the control group ($n = 30$). All the subjects were no-drinking, non-smokers, and had no gestational disease. Pregnant women with endocrine or metabolic disorders (such as diabetes, hypertension, and hypercholesterolemia), severe infectious diseases, and those with multiple pregnancies, premature birth, and asphyxia in the offspring were excluded. Pregnant women who conceived the fetus using artificial methods including in-vitro fertilization were also excluded. The pregnant women in the control group were matched with the hypercholesterolemia group with age, pre-gestational BMI, and the fetal gender. A follow-up was made to assess the body weight and length of the neonates.

2.2. Blood samples

Fasting venous blood samples were collected at the first and third trimesters from the pregnant women. At delivery, the venous cord blood was collected in a gel Vacutainer tube (BD, Oakville, ON, Canada). The serum levels of TC, LDL-C, HDL-C, TG, insulin, hsCRP, and glucose were measured individually using the Unicel 36 DX600 Synchron Clinical System (Beckman-Coulter, Mississauga, ON, Canada).

2.3. Lipidomic profiling using LC-MS

An ultrahigh-performance liquid chromatography (UPLC) system (Waters, Manchester, UK) interfaced with an ESI-QTOF/MS (Xevo G2-S Q-TOF) mass spectrometer (Waters, Manchester, UK) was utilized for lipidomic analysis. The protocol was in accordance with the reported method established by Sarafian [14]. Before each analytical batch, instrument maintenance (source cleaning and mass calibration) was performed. A reversed phase LC column (Acquity UPLC CSH C18 Column, 130 Å, 1.7 μm, 1 mm × 50 mm, 1/pkg) was used for separation with two solvents: 'A' comprising acetonitrile and water (3:2) with 10 mM ammonium formate and 0.1% formic acid, and 'B' comprising isopropanol and acetonitrile (9:1) with 10 mM ammonium formate and 0.1% formic acid. The UPLC autosampler temperature was set at 10 °C and the injection volume for each sample was 2 μL. Column temperature was maintained at 55 °C.

Data were collected in both positive and negative ESI QTOF mode operated in full-scan mode at 100–2000 Da, scan time 0.25 s, and a collision energy ramp 35–60 V were employed. Source temperature was set at 150 °C with a cone gas flow of 50 L/h, and the desolvation temperature was set at 500 °C with a desolvation gas flow of 800 L/h. Leucine–enkephalin (Waters, Manchester, UK) was used as the lock mass generating a reference ion at m/z 556.2771 in positive mode and m/z 554.2615 in negative mode, which was introduced by a lockspray at 10 μL/min for data calibration. The MSE data were acquired in centroid mode using ramp collision energy in two scan functions.

The QC samples were analyzed at regular intervals throughout each batch analysis to monitor the reproducibility of the LC-MS. The extracted samples were re-randomized for LC-MS analysis such that the injection order was independent from the order of sample preparation to minimize systematic bias. To determine the number of conditions necessary for testing, ≥3 technical replicates should be performed.

MassLynx 4.1 Software (Waters, Massachusetts, USA) was employed to record results, and obtained the molecular features of the samples for nontargeted lipidomic analysis of the extracted features.

2.4. Lipidomics analysis

The screened data were taken into account after correcting for individual bias using QC and blank data. EZinfo 3.0 controlled by Progenesis QI was used to perform principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), variable importance in projection (VIP) and coefficients vs. VIP spots. Besides, the heatmap and cluster were performed by HEMI (http://hemi.biocuckoo.org/down.php), and the correlation matrix was carried out via the R project (https://www.r-project.org/).

2.5. Statistical analyses

Data were expressed as the means ± SD, and analyzed using paired Student's t-test at a level of significance of $p < 0.05$, to evaluate the difference between groups. For the relationship between two variables of the same population, results are expressed as Pearson's correlation and the curve represents Pearson's linear correlation. All statistical analyses were performed using the SPSS software 13.0. The ROC is plotted using Stata/MP 14.0 statistical package (Stata Corp, LP) based on the predicted real value of each sample from the trained support vector machine model.

3. Results

3.1. Biochemical characteristics of the subjects

An ultra-performance liquid chromatography tandem mass spectrometer (UPLC-MS) was used for lipidomic analysis of 60 umbilical cord blood samples from mothers with normal or significantly high plasma TC levels at the third trimester. All the subjects (Table 1) were of Han nationality and there were no statistically significant differences between the two groups in terms of age (p value = 0.810), neonate gender (p value = 0.15), gestational age (p value = 0.634), and gestational body weight gain (p value = 0.084).