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Paraoxonase 2 gene polymorphisms and prenatal phthalates' exposure in Chinese newborns

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ABSTRACT

Background: Phthalates were reported to be associated with increased risk of LBW in newborns, but the mechanism and potential influencing factor was still unclear. The objectives of this study were to investigate whether paraoxonase-2 (PON2) Ala148Gly (A148G) polymorphisms have impacts on fetal growth, and evaluate potential modifying effect on the association between phthalate exposures and LBW and short birth length.

Methods: In the current case-control study, 185 mother–newborn pairs including 74 low birth weight (LBW) cases and 111 controls were enrolled. Newborns' meconium specimens were collected and detected for mono-n-butyl phthalate (MBP) and mono-2-ethyhexyl phthalate (MEHP) by the method of high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS). Umbilical vein blood samples were used to identify PON2 A148G polymorphisms by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: Newborns prenatally exposed to higher level of phthalates had lower birth weight ($\beta=-0.92$. $p=0.045$ for MBP, $\beta=-0.62$, $p=0.013$ for MEHP) and short birth length (SBL) ($\beta=-0.024$. $p=0.049$ for MBP, $\beta=-0.023$, $p=0.007$ for MEHP). Comparing with low-phthalate-exposed subjects with PON2 148AA genotype, newborns with PON2 148AG/GG genotype exposed to high concentrations of MBP and MEHP had higher risks of LBW and short birth length (LBW: OR: 5.0, $p=0.017$ for MEHP; OR: 2.6, $p=0.023$ for MBP; SBL: OR: 6.6, $p=0.005$ for MEHP; OR: 6.4, $p=0.017$ for MBP). Effects of MBP and MEHP on LBW were significantly modified by PON2 A148G ($p=0.044$ and 0.034, respectively), while the modifying effect of PON2 A148G polymorphisms on the association of two phthalate metabolites with SBL was not significant.

Conclusion: Prenatal exposure to phthalates affected birth weight and length in newborns. PON2 A148G polymorphisms modified the effects of prenatal phthalates' exposure on fetal development. Newborns with PON2 148AG/GG genotype and exposed to high concentrations of MBP and MEHP had higher risks of LBW and SBL.

1. Introduction

The escalating influence of fetal growth restriction (FGR) is a public health concern worldwide. FGR is a multifaceted condition which results in low birth weight (LBW). It is also pointed out that LBW is associated with long run effects on adulthood health in neurological, cardiovascular system and may cause psychological disorders (Norman, 2008; Datta Gupta et al., 2013; Elgen et al., 2013). Most studies of risk factors for LBW have focused on demographic, clinical and environmental factors, such as maternal condition, endocrine disruptor exposures (Chen et al., 2004; Rauch et al., 2012). Recently, gene–environment interaction studies have shown that genetic factors and environmental factors may have combined effect on fetal developmental disorder (Wolff et al., 2007; Moreno-Banda et al., 2009; Morahan et al., 2007).

Phthalates are plasticizers widely used in cosmetic products, polyvinyl chloride plastics, personal care products and medical

Abbreviations: BMI, body weight index; DAP, dialkyl phosphate; DBP, dibutyl phthalate; DEHP, di-2-ethylhexylphosphate; HPLC-MS/MS, high-performance liquid chromatography with tandem mass spectrometry; LBW, low birth weight; LOD, Limit of detection; MBP, di-n-butyl phthalate; MEHP, di-2-ethylhexyl phthalate; OR, odd ratio; PCR-RFLP, Polymerase chain reaction restriction fragment length polymorphism; PON, paraoxonase; SBL, short birth length

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devices (Foster et al., 2012). They are regarded as developmental and reproductive toxicants in rodents and endocrine disruptors for human beings (Foster, 2005). It is reported that phthalates' exposure is associated with increased risk of LBW in newborns (Zhang et al., 2009; Wolff et al., 2008), but the mechanism and potential influencing factors in body are unclear. Animal experiments showed that phthalate metabolites might increase reactive oxygen species (ROS) generation (Zhao et al., 2012; Tseng et al., 2013), and oxidative stress was reported as a likely promoter of several pregnancy-related disorders (Al-Gubory et al., 2010). Herein, we speculated that phthalate-inducing ROS generation, had likelihood of causing abnormal birth outcome. If it was correct, fetal phthalates' exposure would be accompanied by elevated oxidative stress, which could be affected by antioxidants in vivo.

Genes associated with both ROS production and fetal development include GST (glutathione S-transferase) family (such as GSTT1, GSTM1), CYP (cytochrome P-450) family (such as CYP1A1, CYP2E1), and PON (paraoxonase) gene family (such as PON1, PON2). Among them, GST gene family (Lee et al., 2010; Wang et al., 2000; Raijmakers et al., 2001; Sram et al., 2006; Danileviciute et al., 2012) and CYP gene family (Sasaki et al., 2008; Infante-Rivard, 2007; Kishi et al., 2008; Delpisheh et al., 2009; Wang et al., 2002) were found to affect fetal development in lots of human epidemiological studies. However, epidemiological evidence for PON genetic effect on fetal development is rare to date. Paraoxonase-2 (PON2) deficiency can increase ROS production (Yang et al., 2012; Ng et al., 2006). PON gene is a multigenic family (at least consists of 3 genes PON1, PON2 and PON3) located in the long arm of the human chromosome 7 (q21.3–22.1) (Primo-Parmo et al., 1996). Data from literature indicated that PON gene polymorphisms, comprising PON1 C108T, PON1 R192Q and PON2 A148G, had impacts on fetal development (Rauch et al., 2012;, Harley et al., 2011; Costa et al., 2003; Deakin et al., 2003; Busch et al., 1999; Liang et al., 2002). Compared with its isoform, PON2 gene is expressed in placenta and embryo (Mackness et al., 2010; Zhou et al., 2011; Ono et al., 2003), and has intracellular antioxidant proprieties (Horke et al., 2007; Giordano et al., 2013). So we conducted a case-control study and chose PON2 gene to explore the effect of PON2 polymorphisms on fetal growth, and to investigate whether PON2 polymorphisms modify phthalates' effects on fetal development.

2. Methods

2.1. Cases and controls enrollment

Between January 2011 and December 2011, over 100 newborns at Shanghai Medical Center for Maternal and Child Health were diagnosed as LBW (birth weight < 2500 g, gestational age > 37 weeks). All of these newborns together with their mothers were initially recruited as cases. Controls were normal birth weight infants (birth weight > 2500 g) recruited from the same hospital and paired by maternal age. After excluding pregnant mothers who were administrated to intravenous drip, multiple-birth pregnancies or premature deliveries, totally 74 cases and 111 controls were enrolled eventually. No gender difference existed between cases and controls during participants' enrollment.

2.2. Ethic statement

This study was conducted in accordance with protocols approved by Institutional Review Board of Fudan University. Informed written consent including the nature of this study and the procedure of sample collection was well-explained and signed by each mother voluntarily.

2.3. Clinical information and specimen collection

A questionnaire was sent to participants, in which include information about phthalates and other environmental exposures, socio-demographic characteristics, medical history, nutrition and health conditions. Information on delivery characteristics and birth outcomes, including birth weight, birth length, gestational age, and infant gender were obtained from hospital records. Measurements of weight, length at birth were based on standardized clinical techniques. For each infant, meconium was collected directly from every diaper during the first 48 h after delivery. All specimens were collected with glass devices to avoid contamination by phthalates during handling and then transferred on dry ice to the Key Lab of Public Health Safety of the Ministry of Education of China for analysis.

2.4. DNA extraction and genotype analysis

Five ml umbilical vein blood was collected from all recruited 74 LBW and 111 control infants immediately after delivery by a syringe, and the same volume of maternal blood samples were collected soon afterwards. Genomic DNA was extracted from blood cells using the QIAamp blood kit (Qiagen Corporation, Hilden Germany).

PON2 A148G polymorphisms were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Biometra GmbH, Gottingen, Germany). Primers were: forward 5′CAACCCACCATAGGGATTGTTTG 3′; reverse: 5′TATATACAGTGGAAATTTTTAAATTTGAAGCAG 3′. PCR products were digested by Fnu4HI restriction enzyme (Sigma, USA). The digested DNA products were detected using agarose gel electrophoresis for PON2 A148G identification. The initial three-category (AA, AG and GG) genotype variable was dichotomized into AG or GG genotype and AA genotype, since we observed no differences in AG and GG genotypes between cases and controls.

2.5. Phthalates measurement

MBP (primary metabolite of DBP) and MEHP (primary metabolite of DEHP) were analyzed using the method modified by Kato et al.(2006). Briefly, the determination of phthalates and metabolites in meconium (1 g) involved enzymatic deconjugation of the metabolites, solid-phase extraction, separation with high-performance liquid chromatography (HPLC), and detection by tandem mass spectrometry(MS/MS). In more detail, samples were enzymatically hydrolyzed and purified by solid-phase extraction. Phthalate and its metabolites extracted from samples were resolved by an Agilent 1100 Series high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA) and detected by an API 2000 electrospray triple quadrupole mass spectrometer (ESI-MS/MS; Applied Biosystems, Foster City, CA) (Zhao et al., 2012). C_4-labeled internal standards and conjugated internal standards were used to increase the precision of the measurements. One method blank, two quality control samples, and two sets of standards were analyzed along with unknown meconium samples. The limit of detection (LOD) was 1.0 ng/g for MBP and MEHP in meconium. The values below LOD were substituted for LOD divided by 2.

2.6. Statistical analysis

Data analysis was carried out using the statistical package SAS (version 9.2). We evaluated the Hardy–Weinberg equilibrium of this population and compare genotypic frequencies between the groups using the Chi-squared test (Hardy–Weinberg equilibrium: $p > 0.1$). Socio-demographic and lifestyle characteristics of