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Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances affects leukocyte telomere length in female newborns☆

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ABSTRACT

Evidence has shown that leukocyte telomere length (LTL) at birth is related to the susceptibility to various diseases in later life and the setting of newborn LTL is influenced by the intrauterine environment. Perfluoroalkyl and polyfluoroalkyl substances (PFASs), as a kind of persistent organic pollutants, are commonly used in commercial and domestic applications and are capable of crossing the maternal-fetal barrier during pregnancy. We hypothesized that intrauterine exposure to PFASs may affect fetal LTL by increasing oxidative stress. To verify this hypothesis, LTL, concentrations of PFASs and reactive oxygen species (ROS) were measured in umbilical cord blood of 581 newborns from a prospective cohort. Our results showed that there were interactions between PFOS/PFDA and sex on LTL and ROS. The LTL was significantly shorter (0.926 ± 0.053 vs 0.945 ± 0.054, $P = .023$ for PFOS; 0.919 ± 0.063 vs 0.940 ± 0.059, $P = .011$ for PFDA) and the ROS levels were extremely higher (252.9 ± 60.5 [M] vs 233.5 ± 53.6 [M], $P = .031$ for PFOS; 255.2 ± 62.9 [M] vs 232.9 ± 58.3 [M], $P = .011$ for PFDA) in the female newborns whose PFOS or PFDA concentrations fell in the upmost quartile compared with those in the lowest quartile after adjusting for potential confounders. ROS levels were inversely associated with LTL in female newborns ($\beta = -1.42 \times 10^{-4}$, $P = .022$). 13% of the effect of PFOS on female LTL was mediated through ROS approximately by the mediation analyses. However, in male newborns, no relationships among PFASs, ROS and LTL were observed. Our findings suggest a "programming" role of PFASs on fetal telomere biology system in females in intrauterine stage.

1. Introduction

Telomeres are protein-bound DNA with tandem TTAGGG repeat structures at the end of chromosomes (Moyzis et al., 1988). It is of significant importance in maintaining genetic stability and integrity. Telomere acts as a mitotic clock as it shortens with cell division and aging. A reverse transcriptase enzyme called telomerase which carries its own RNA molecule is responsible for extending telomeres via adding new telomere repeat (5′-GGTTAG-3′) sequences to the end of chromosomes. However, this repair process is strictly regulated as telomerase activity is normally absent from most human somatic cells (Weng, 2012). Population studies on telomeres predominantly focus on the adults especially the elderly. It has been reported that accelerated telomere shortening might increase the risk of various chronic diseases such as cardiovascular disease (Fitzpatrick et al., 2007) and diabetes (Zhao et al., 2014). Recently, growing studies considered that the associations between leukocyte telomere length (LTL) and diseases were established in early life (Benetos et al., 2013b). Several investigations have been

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conducted to assess the impacts of intrauterine environmental exposures on offspring LTL at birth. Results indicated that adverse intrauterine conditions including tobacco exposure (Salihu et al., 2015) and maternal psychosocial stress (Entringer et al., 2013) might play a role in the process of newborn LTL attrition. In concordance with the "fetal programming" concept developed by Barker (1998), telomere may serve as a potential mechanism which links developmental programming with health consequences in later life (Entringer et al., 2012). Since telomere homeostasis is plastic and susceptible to the intrauterine conditions, it is important to clarify the effects of those influence factors on telomere biology system, thus providing evidence for interventions.

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a kind of persistent organic pollutants (POPs) and are widely used in various industrial and commercial products since the 1950s because of their water and oil repellent, heat stable and nonflammable properties. Among the variety of PFASs, perfluorooctanoic acid (PFOA) and perfluorooctyl sulfonate (PFOS) are the most ubiquitous chemicals. Elimination half-lives of PFASs vary depending on the sex, the population and the background exposure. Half-life of PFOA ranges from 2.3 to 3.5 years (Bartell et al., 2010; Brede et al., 2010; Zhang et al., 2013b). The estimated PFOS elimination half-life is 6.2 years in younger females, while 27 years in males and older females (Zhang et al., 2013b). Although PFOS has been added to the POPs list in the Stockholm Convention in 2009 (Ahrens, 2011) for the restriction of production, manufacturing of PFOS and its homologues is still on the rise in China. Drinking water, dietary, food package materials and house dust are the primary pathways for human exposure to PFASs (Fromme et al., 2009). A variety of endocrine targets such as thyroid, ovaries and mammary gland can be affected by the toxicological characteristics of PFASs. Furthermore, PFASs are capable of crossing the maternal-fetal barrier during pregnancy (Glynn et al., 2012). Individuals in the fetal period are highly sensitive to the environmental toxins because of their rapid growth rates. Intrauterine exposure to PFASs in early life may exert a long term effect on health in later life by conferring a greater susceptibility to chronic diseases (Landrigan et al., 2002).

It has been reported that PFOS-induced damage to humans at the cellular level resulted from excessive generation of reactive oxygen species (ROS) (Qian et al., 2010). The overwhelming production of ROS indicates a higher level of oxidative stress, which plays a role in various biological and pathological processes. The body's oxidant/antioxidant imbalance further leads to cell damage and strand breaks in DNA. As telomere length shortens faster under the circumstance of high oxidative stress levels, we hypothesized that PFASs exposure during pregnancy may accelerate fetal telomere shortening via excess ROS. Hence, we detected PFASs exposure levels and newborn LTL in umbilical cord blood specimens in our study. Concentrations of ROS in cord serum of all the newborns have also been measured.

2. Materials and methods

2.1. Study participants and procedures

Sub-sample of this study consisted of 581 subjects, in which measurements of both newborn PFASs concentrations and LTL were available. This sub-sample of pregnant women and newborns was from a larger cohort-the Shanghai Allergy Cohort which was performed at two large hospitals in Shanghai between 2012 and 2013. Comparisons of parental and newborn characteristics between the current study population ($n = 581$) and the larger cohort ($n = 1245$) were presented in Table S1. All participants were permanent residents of Shanghai city with decision to deliver in these two hospitals. At enrollment, informed consent was obtained from each subject and a structured questionnaire survey with information of sociodemographic characteristics and maternal behaviors during pregnancy was processed face-to-face by trained interviewers. At delivery, 10 mL umbilical cord blood specimens were collected by trained nurses. Medical information, such as infant sex, birth weight, gestational age at birth, mode of delivery and gestational complications of the mothers was extracted from the medical record system. Ethics approval was obtained by the Ethics Committees of both hospitals. The methods were carried out in accordance with the relevant guidelines, including any relevant details. There were no significant differences in paternal, maternal or newborn characteristics between the sub-sample and the whole cohort with the exception of obstetric risk conditions.

2.2. DNA extraction and LTL measurements

10 mL umbilical cord blood was collected in BD EDTA tubes and was centrifuged at 4 °C with a relative centrifugal force of 1500 g for 10 min immediately. Plasma (upper layer), buffy coat (middle, white colored layer) and erythrocyte (bottom layer) were stratified after the centrifugation. Genomic DNA was isolated from 200 μL buffy coat (DNeasy Blood and Tissue Kit; Qiagen). Quality of extracted DNA was checked by gel electrophoresis and Epoch Multi-Volume Spectrophotometer System (BioTek, USA). Qualified DNA samples were stored at −80 °C until subsequent assays. LTL was measured by the use of a validated established quantitative polymerase chain reaction (qPCR) technique (Cawthon, 2002). Details of the method have been described previously (Liu et al., 2017). In brief, for each experimental sample, the ratio of telomere to single copy gene (T/S ratio) was calculated to measure the difference between the unknown DNA sample and the reference DNA sample in its ratio of telomere repeat copy number to single copy gene copy number. We used β-globin as the reference gene. T and S PCR reactions had identical reagents except for oligonucleotide primers. Relative standard deviation values were 1.1% for intra-day assays and 2.3% for inter-day assays. Repeated measurement was conducted if the standard deviation between the duplicate wells of one sample exceeded 0.167. In addition, we chose 5% of the samples randomly for the reproducibility test of the assay.

2.3. Determinations of cord plasma PFASs concentrations

Cord plasma concentrations of 10 PFASs including PFOA, PFOS, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUA), perfluorododecanoic acid (PFDoA), perfluorooctane sulfonamide (PFOSA), perfluoroheptanoic acid (PFHpA), perfluorohexane sulfonic acid (PFHxS) and perfluorobutane sulfonic acid (PFBS) were measured using previous published methods (Wang et al., 2016). Briefly, plasma samples were primarily prepared by the method of protein precipitation developed by Lien (Lien et al., 2011) et al. with a few modifications. Then liquid chromatography system coupled with tandem mass spectrometry (HPLC-MS/MS, Agilent1290–6490, Agilent Technologies Inc., USA) was applied to analyze PFASs using ZORBAX Eclipse Plus C18 columns (2.1 × 100 mm, 1.8 μm; Agilent, USA). Identification and quantification of analytes were operated in electrospray ionization (ESI) negative mode with multiple reaction monitoring (MRM). For each analyte, a six serially diluted internal standard solution ranging from 0.5 to 100 ng/mL was performed to generate the standard curve for quantification with good linearity ($R^2 > 0.99$). Reliability and accuracy of the determinations were tested by replicate analysis of samples at a low concentration (1.6 ng/mL) and a high concentration (80 ng/mL). Recovery ranged between 80 and 110%. Intra- and inter coefficient of variations were