Environment International 106 (2017) 60–68

Contents lists available at ScienceDirect

Environment International

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

Neonatal exposure to environmental pollutants and placental mitochondrial DNA content: A multi-pollutant approach

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ARTICLE INFO

Keywords:
Placenta
Mitochondrial DNA content
Environmental pollution
Newborns

ABSTRACT

Background: Placental mitochondrial DNA (mtDNA) content can be indicative of oxidative damage to the placenta during fetal development and is responsive to external stressors. *In utero* exposure to environmental pollutants that may influence placental mtDNA needs further exploration.

Objectives: We evaluated if placental mtDNA content is altered by environmental pollution in newborns and identified pollutants independently associated to alterations in placental mtDNA content.

Methods: mtDNA content was measured in placental tissue of 233 newborns. Four perfluoroalkyl compounds and nine organochlorine compounds were quantified in cord blood plasma samples and six toxic metals in whole cord blood. We first applied a LASSO (least absolute shrinkage and selection operator) penalized regression model to identify independent associations between environmental pollutants and placental mtDNA content, without penalization of several covariates. Then adjusted estimates were obtained using an ordinary least squares (OLS) regression model evaluating the pollutants' association with placental mtDNA content, adjusted for several covariates.

Results: Based on LASSO penalized regression, oxychlordane, p,p′-dichlorodiphenyldichloroethylene, β-hexachlorocyclohexane, perfluorononanoic acid, arsenic, cadmium and thallium were identified to be independently associated with placental mtDNA content. The OLS model showed a higher placental mtDNA content of 2.71% (95% CI: 0.3 to 5.2%; $p = 0.03$) and 1.41% (0.1 to 2.8%, $p = 0.04$) for a 25% concentration increase of respectively cord blood β-hexachlorocyclohexane and arsenic. For a 25% concentration increase of cord blood thallium, a 4.88% lower placental mtDNA content (95% CI: -9.1 to −0.5%, $p = 0.03$) was observed.

Conclusion: In a multi-pollutant approach, low fetal exposure levels of environmental organic and inorganic pollutants might compromise placental mitochondrial function as exemplified in this study by alterations in mtDNA content.

1. Introduction

Fetuses and newborns are particularly vulnerable to exogenous compounds. Since their detoxification system is not fully developed, exposures can interfere with birth outcomes (Al-Gubory, 2014), but can also modify an individual's susceptibility to adult disease in later life (Barker and Thornburg, 2013). The placenta is a transient organ during gestation involved in the implantation of the early embryo, produces hormones to facilitate the fetal growth and exchanges nutrients, gases and waste products between the developing fetus and the mother.

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http://dx.doi.org/10.1016/j.envint.2017.05.022
Received 10 February 2017; Received in revised form 29 May 2017; Accepted 30 May 2017

Nevertheless, the placenta does not embody a perfect barrier to harmful substances and transplacental migration of for example toxic metals (Needham et al., 2011; Gundacker and Hengstschlager, 2012), organochlorine compounds (Needham et al., 2011; Rogan et al., 1986; Vizcaino et al., 2014) and perfluoroalkyl compounds (Needham et al., 2011; Midasch et al., 2007; Fromme et al., 2010) has been described.

An important limitation in the current understanding of early life environmental exposures is that exposure to multiple pollutants is rarely taken into account simultaneously and mechanisms activated at low levels of exposure are still largely undefined. Mitochondria are prone to damage induced by reactive oxygen species (ROS) and can compensate genomic insults by altering their abundance, as such they can react very quickly to exposures (Lee et al., 2000). Mitochondrial physiological states determined by mitochondrial DNA (mtDNA) copy number can sense environmental perturbations, and might be indicative of disease mechanisms (Taylor and Turnbull, 2005; Herrera et al., 2015; Wallace, 2016). Environmental stressors, such as particulate matter in air pollution, can alter placental mtDNA content (Janssen et al., 2012), and its potential role to mediate birth weight has been suggested (Clemente et al., 2016). Oxidative stress is not only involved in metal toxicity (Valko et al., 2005), but is also proposed as a possible mechanism of toxicity of organochlorines (Sharma et al., 2010; Abdollahi et al., 2004) and perfluoroalkyl compounds (Liu et al., 2007; Wielsoe et al., 2015).

The Flemish Environment and Health Study (FLEHS) was initiated to establish updated reference values of pollutants and to study the impact of exposure to these pollutants on human health. In this framework, we examined the association between environmental exposure to toxic metals, organochlorines and perfluoroalkyl compounds and placental mitochondrial DNA content. Environmental exposure was represented by the biomarkers quantified in cord blood (i.e. arsenic (As), cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb) and thallium (Tl), polychlorinated biphenyl (PCB) − 138, − 153, − 180, hexachlorobenzene (HCB), oxychlordane (OXC), β-hexachlorocyclohexane (β-HCH), transnonachlor (TN), p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE), p,p′-dichlorodiphenyltrichloroethane (p,p′-DDT) and perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA) and perfluorobutanesulfonic acid (PFBS)). We independently selected exposures using shrinkage-based regression to identify independent effects of the most predictive exposures for placental mtDNA content.

2. Methods

2.1. Study population and recruitment

This study was a part of the third cycle of FLEHS, which recruited mother-newborn pairs in six hospitals in Flanders, Belgium to obtain a representative sample of the population. All women giving birth in one of the participating hospitals could enter the study, if they lived at least five years in Flanders and were able to fill out an extensive Dutch questionnaire. On a subset of the population we determined the recruitment rate, which was 18.7% of the deliveries. Respectively 8.3% and 1.6% of the mothers did not meet the language requirements and 5-year residency.

In total, 281 mother-newborn pairs were recruited from November 2013 to November 2014. Of these 281 pairs, 250 had placental biopsies available of which five were excluded because of caesarean section, six did not have a quantification of the organochlorine compounds and an additional three had no cord blood perfluoroalkyl compounds measurement available. Three newborns lacked information on socio-economic status. The remaining 233 subjects were included in the current study. The medical ethical committee of University of Antwerp and University Hospital of Antwerp as well as the local ethical committee of each participating hospital approved the study. All subjects in the study gave informed consent to participate and filled out a questionnaire during their stay in the hospital maternity. The questionnaire addressed the general health status of the mother (e.g. weight management, infections, illness or complications during the pregnancy, allergy), lifestyle (e.g. smoking and alcohol use during pregnancy), socio-economic status (e.g. occupation, education), household composition and housing conditions and dietary patterns.

2.2. Sample collection

Umbilical cord blood was collected immediately after delivery using polypropylene Na-EDTA tubes, which were previously tested for metal contamination. Until the biopsies were taken placentas were kept in the delivery room and stored at 4 °C. Villous tissue, which is protected by the chorioamniotic membrane, was sampled at the fetal side of the fresh placenta. One biopsy was taken 4 cm from the umbilical cord, via a standardized protocol. Afterwards, the biopsies were stored at − 80 °C until DNA isolation.

2.3. Measurement of biomarkers of exposure

The selection of pollutants was based on (i) health impact, (ii) current policy relevance, (iii) potential for remediation and (iv) feasibility. As such, chemicals with known historical pollution in Flanders, Belgium (metals, organochlorines) as well as more recent emerging chemicals (perfluoroalkyl compounds) were included.

The toxic metal pollutants As, Cd, Cu, Pb, Mn and Tl were determined in whole cord blood samples after acid digestion. The measurements were carried out using high resolution inductively coupled plasma – mass spectrometry (HR-ICP-MS) as described by (Schroijen et al., 2008). The limit of detection (LOD) in the blood samples was 0.09 μg/L for As, 0.0097 μg/L for Cd, 0.49 μg/L for Cu, 0.22 μg/L for Pb, 0.15 μg/L for Mn and 0.48 ng/L for Tl.

The following organochlorine compounds were quantified in cord blood plasma samples: PCB congeners 138, 153, and 180, p,p′-DDE, p,p-DDT, HCB, OXC, TN and β-HCH. The quantification was carried out following earlier described protocols by (Dirtu et al., 2010). The limit of quantification (LOQ, which corresponds to LOD*3.33) is 2 ng/L for each PCB congener, 20 ng/L for p,p′-DDE, 10 ng/L for p,p′-DDT and HCB, 2 ng/L for OXC and TN and 5 ng/L for β-HCH.

The perfluoroalkyl compounds PFOS, PFOA, PFHxS, PFNA and PFBS were determined in cord blood plasma. The measurements were carried out based on the protocol described by (Midasch et al., 2007; Midasch et al., 2006). The LOQ for PFOS, PFOA, PFHxS and PFBS was 0.2 μg/L and 0.1 μg/L for PFNA.

Samples with a concentration below the LOD or LOQ were assigned LOD/2 or LOQ/2 as concentration. At least 74% of the measurements were above the respective LOD or LOQ for all exposures except for p,p′-DDT, for TN and PFBS. For p,p′-DDT 18% of the measurements was above the LOQ, for TN 44% was above the LOQ and PFBS was for all samples below the LOQ. p,p′-DDT and TN were considered binary (above or below the LOD) in the analyses, while PFBS was omitted from the analyses.

2.4. Measurement of mtDNA content

Excess blood was removed from the placental biopsies, the samples were homogenized and DNA was isolated using the QIAamp DNA mini kit (Qiagen), which uses silica-membrane columns.

Mitochondrial DNA content (mtDNA) in placental tissue was measured by determining the ratio of two mitochondrial gene copy numbers (*MTF3212/R3319* and *MT-ND1*) to a single –copy nuclear control gene (*36b4*) using a real-time quantitative polymerase chain reaction (qPCR). qPCR reactions were carried out on a 384-well plate on the 7900HT Fast Real-Time PCR System (Applied Biosystems) in a 10 μL volume containing: 5 μL QuantiTect SYBR Green (Qiagen) mastermix,