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Metabolic targets of endocrine disrupting chemicals assessed by cord blood transcriptome profiling

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ABSTRACT

Early life exposure to endocrine disrupting chemicals (EDCs) has been frequently associated with impaired perinatal growth, an important risk factor for later onset of metabolic disorders.

We analyzed whether the cord blood transcriptome showed early indications of alterations in metabolic processes in 195 human samples in relation to cord blood levels of dichlorodiphenyldichloroethylene (p,p′-DDE), polychlorinated biphenyl-153 (PCB-153), perfluorooctanoic acid (PFOA), and perfluorooctane sulfonate (PFOS).

Overall, 39 metabolically relevant transcription factors were significantly enriched (31 by p,p′-DDE, 10 by PCB-153, 8 by PFOA, and 2 by PFOS). These included the glucocorticoid receptor (p,p′-DDE and PCB-153) and the progesterone receptor (PFOA and PFOS). The 'insulin receptor signaling', 'acute phase response signaling', 'Interleukin(IL)-6 signaling', and 'prolactin signaling' pathways were significantly enriched in relation to p,p′-DDE.

Transcriptional changes at birth suggest a role for specific metabolic targets as a link between prenatal EDC exposure and metabolic disorders later in life.

Abbreviations: DDT, dichlorodiphenyltrichloroethane; EDC, endocrine disrupting chemicals; FLEHS(I/II/III), Flemish Environment and Health Survey (I/II/III); HNF1A, hepatocyte nuclear factor 1 homeobox A; HPA, hypothalamic-pituitary-adrenal; ICA1, islet cell autoantigen 1; IL, interleukin; IPA, ingenuity pathway analysis (Ingenuity Systemsâ); IQR, interquartile range; IRS2, insulin receptor substrate; NR3C1, glucocorticoid receptor; OBELIX, FP7 project:obesogenic Endocrine disrupting chemicals linking prenatal exposure to the development of obesity later in life; p,P′-DDE, dichlorodiphenyldichloroethylene; PCB-153, polychlorinated biphenyl-153; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PGR, progesterone receptor; PPARA, peroxisome proliferator-activated receptor alpha; PRL, prolactin; TF, transcription factor; VIF, variance inflation factor.

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1. Introduction

Early development is a critical period of rapid growth and imprinting and of high susceptibility to exogenous influences such as stress and hormonal signals [1]. The European FP7 project OBELIX (OBesogenic Endocrine disrupting chemicals: Linking prenatal eXposure to the development of obesity later in life) examined the hypothesis that prenatal exposure to endocrine disrupting chemicals (EDCs) in food plays a role in the development of obesity later in life [2]. Based on a meta-analyses of European birth cohorts, prenatal polychlorinated biphenyl-153 (PCB-153) and dichlorodiphenyldichloroethylene (p,p'-DDE, a common breakdown product of dichlorodiphenyltrichloroethane (DDT)) exposure have been linked to respectively decreased birth weight [3] and increased incidence of rapid infant weight gain during the first 24 months after birth [4]. Rapid infant weight gain has been associated to increased risk on childhood obesity at the age of 10 [5]. Based on the risk estimates of these associations, Legler et al. calculated that 0.26% of all cases of childhood obesity can be attributed to prenatal p,p'-DDE exposure in Europe [6]. Changes in birth weight have also been linked with later development of obesity [7]. The association between PCB exposure and birth weight has been mentioned above [3]. Metabolites of di-2-ethylhexyl phthalate (DEHP) have been related to both high (MEHHP) and low birth weights (MECPP) [8]. Current literature on the associations between perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) and human fetal growth has been recently reviewed [9]. Based on 14 publications, it was concluded that PFOA and PFOS exposures in pregnancy were associated with lower average birth weights in human newborns in most studies, although not all results were statistically significant. Prenatal exposure to persistent organochlorine pollutants (PCBs, DDE, and hexachlorobenzene (HCB)) has been associated with high insulin levels in 5-year-old girls [10]. Despite advancements in knowledge on the endocrine and neuronal regulation of energy balance and food intake, the underlying mode of action of EDCs concerning their impact on obesity and other metabolic disorders is not well understood at the molecular level.

The field of molecular epidemiology to identify targets of prenatal environmental exposure is gaining interest. –Omics wide analyses enable the study of the whole cascade of molecular processes influenced by exposure. In studying the effects of maternal cigarette smoking during pregnancy, positive associations have been reported between cotinine levels in placenta and mRNA levels of CYP1A1 in fetal lung and liver tissue; and with CYP1B1 in lung tissue [11]. Increases in metabolic enzyme transcripts in fetal liver in relation to maternal smoking have also been demonstrated by O'Shaughnessy et al. (2011) with more marked responses in male fetuses [12]. Activation of inflammation/NF-kappaB signaling has been demonstrated in infants born to arsenic-exposed mothers [13].

Three levels of functional analysis are of specific interest to derive biological processes that link prenatal EDC exposure to increased risk on later development of metabolic diseases. The first level consists of identification of altered transcription factor (TF) regulation, especially of TFs that are known to play a role in metabolic disorders. Many of the TFs involved in metabolic disorders (such as glucose metabolism disorders, insulin resistance, weight gain, and obesity) are activated upon hormone binding. Mimicking endogenous hormone binding is a well-known mechanism of action of EDCs [14]. Second, it has been documented that chronic inflammation plays an important role in various metabolic diseases [15]. It has been hypothesized that in type 2 diabetes, there is an ongoing cytokine-mediated acute-phase response (part of a wide-ranging activation of innate immunity), and this is closely involved in the pathogenesis of the disease (1,2). Studies providing evidence of this theory have been reviewed and may be summarized as follows: 1) Markers of inflammation are associated with type 2 diabetes and features of the metabolic syndrome in cross-sectional studies; 2) Markers of inflammation predict the risk for type 2 diabetes; 3) Inflammation is involved in the pathogenesis of atherosclerosis, a common feature of type 2 diabetes; 4) Anti-inflammatory agents decrease the acute-phase response, may reduce the risk of developing type 2 diabetes, and improve control; and 5) Gestational diabetes, a risk factor for type 2 diabetes, is associated with an inflammatory response [16]. When profiling immune competent cells, it is hence relevant to include alterations in immunologic pathways as a potential target of EDC exposure. Third, significant influences of EDC exposure on the expression of separate key genes that play a well-known role in disease development/onset should be documented. This is necessary to overcome a limitation of focusing on 'significance of pathway enrichment' in –omics studies, which is solely based on 'number of molecules' (Fisher Exact Test) and does not take into account the importance of individual molecules in the biological process.

In 195 cord blood samples collected immediately after birth of newborns from the general population of Flanders, we examined global transcriptome responses in cord blood cells in relation to birth parameters (sex, gestational age at birth, season of birth, being delivered by caesarian section, etc.), maternal parameters (age, pre-pregnancy body mass index, etc.), environmental exposure parameters (heavy metals, endocrine disruptors, air pollutants, etc.). Cord blood can be easily obtained. The exposure biomarkers in cord blood reflect exposure at birth, and are considered as a proxy for exposure during the fetal period and as a proxy for maternal concentrations during pregnancy. The relation between maternal levels and cord levels is documented in literature in our study population with respect to metals [18] and in other studies for persistent organic pollutants [19,20]. Transcriptome wide, the association between cord blood gene expression and tissue expression has not been documented. However, for specific molecules, it has been well described that cord blood gene expression reflects transcriptional changes in other tissues, such as for the expression of sFLT1–a key molecule in regulating infant growth – in the placenta [21,22].

One of the objectives of the OBELIX project was to relate early life exposure to EDCs with effect biomarkers and health outcome data which are related to risk of obesity later in life [2]. In this context, we focused on molecular signatures that are specific for prenatal exposure to EDCs that can be associated to obesity and metabolic pathways. The statistical approach allowed to adjust the associations for influential factors (covariates) at the gene specific level. Potential influential factors that were considered included co-exposures, maternal body mass index before pregnancy, newborn sex, parity, maternal smoking during pregnancy, maternal alcohol consumption, season of birth, maternal age, and being delivered by caesarean section, gestational age at birth, and cord blood lipid content.

This paper proposes potential targets of EDCs that underlie metabolic disorders later in life by functional analysis of the gene expression changes that correlate with exposure.

2. Methods

2.1. Ethics statement

A written informed consent was provided by all participating mothers. The study protocol was approved by the ethical committee of the University of Antwerp (Reference UA A08 09).

2.2. Study population

The study made use of samples obtained from a birth cohort that was initiated as part of the human biomonitoring program