Gene-Dioxin Interactions and Birthweight in the Seveso Second Generation Health Study

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Introduction

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is proposed to interfere with fetal growth via altered activity of the aryl hydrocarbon receptor (AhR) pathway which regulates diverse biological and developmental processes including xenobiotic metabolism [1]. Animal and *in vitro* research has documented the elevated expression of AHR and its regulatory protein network in the placenta, a tissue regulated by both maternal and child genetics [2-5]. Genetic variation in the AhR pathway has been implicated as an important driver of susceptibility to low birthweight in children exposed to prenatal smoking [6,7]. Little is known, however, about fetal susceptibility in regards to gene-dioxin interactions. Genetics could resolve the conflicting body of epidemiological evidence examining the relationship between prenatal dioxin exposure and reduced birthweight [8-11].

Materials and methods

The Seveso Women's Health Study (SWHS), initiated in 1996, is a cohort of 981 Italian women exposed to TCDD from an industrial explosion in July 1976 [12]. In 2014-2016, we enrolled post-accident offspring of the SWHS in the Seveso Second Generation Health Study. Genomic DNA was extracted from blood specimens collected from mothers (n=567) and their children (n=582). Participants were genotyped using the Sequenom iPLEX platform [13] at 87 common single nucleotide polymorphisms (SNPs) from 7 candidate genes in the AhR-pathway known to be associated with the metabolic response to xenobiotic exposure and/or human health.

In utero TCDD exposure was defined as initial TCDD concentration in maternal serum collected soon after the explosion. Measurements were performed at the Centers for Disease Control and Prevention (CDC) using high-resolution mass spectrometry. Maternal TCDD level, analyzed as a continuous variable, was $\log_{10}$-transformed prior to modeling to approximate a normal distribution.

Birthweight (grams) was obtained from maternal report and confirmed with birth records in a subsample. We used multivariate regression with generalized estimating equations to account for sibling clustering to model the associations between individual SNPs and child birthweight. For preliminary analyses, SNPs were modeled assuming a dominant model of inheritance of the minor allele. Covariates considered in the models included maternal age at pregnancy (continuous, years), maternal smoking during pregnancy (cigarettes/day), total parity, maternal height (cm), pre-explosion history of low birthweight, year of pregnancy, socioeconomic status (as defined by highest education of the household), child sex, and gestational age (maternal report in weeks).

Results and discussion

Geometric mean maternal serum TCDD near the time of the accident was 69.1 ppt, lipid-adjusted (range: 2.5 – 56,000 ppt). In preliminary analyses, we found 9 child SNPs across the aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor repressor (AHRR) genes to be significantly associated with birthweight and 5 maternal SNPs across Cytochrome P450 1B1 (CYP1B1) and AHRR with suggestive associations. One of these child SNPs (rs3757824, minor allele frequency=20%), located in the promoter region of AHR, was associated with a -76.4g reduction in birthweight (95% CI: -173.9, -13.4) and has previously been linked to cryptorchidism in a separate study of Italian boys [14]. In interaction with maternal TCDD levels, the maternal genotype at this SNP was significantly associated with birthweight while the child's genotype was not (p_{int}=0.02 and p_{int}=0.85, respectively). For a 10-fold increase in maternal TCDD levels, children of mothers with the AG/GG genotype had birthweights that were on average 99.6g higher (95%CI: -0.37, 199.6) than children of mothers with the AA genotype. In models that examined the child's genotype at this SNP, children with the AG/GG genotype had birthweights that were on average -53.7g lower (95%CI: -170.3, 63.0) than children with the AA genotype with a 10-fold increase in maternal TCDD levels.

Significant interaction with maternal TCDD levels was observed with over 30 other SNPs across 6 genes (AHR, ARNT, AHRR, CYP1A1, CYP1A2, and CYP1B1) including the child genotypes of rs162562, a Cytochrome P450 1B1 (CYP1B1) variant associated with fetal development (p_{int}<0.2). In agreement with other studies [6,7], the maternal SNP of rs2066853, a functional missense mutation in AHR, was linked to reduced birthweight in interaction with maternal smoking in the present sample of the Seveso Second Generation (p_{int}=0.07). However, the maternal SNP was not found to be associated with birthweight in interaction with maternal TCDD levels (p_{int}=0.31), a finding that is consistent with a recent study of maternal AHR genetics and prenatal dioxins in a Japanese mother-child cohort [15]. We will present results from polygenic models as well as analyses jointly examining maternal and child genotypes. In addition, we will present models of gene-dioxin interaction on birthweight using TCDD exposure estimated at the time of pregnancy.

This is the first study of how both maternal and child genetics shape fetal susceptibilities to dioxin exposure.

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