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First trimester phthalate exposure and male newborn genital anomalies

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ABSTRACT

Background: Anti-androgenic phthalates are environmental chemicals that affect male genital development in rodents leading to genitourinary birth defects. We examined whether first trimester phthalate exposure may exert similar effects in humans leading to an increased incidence of newborn male genital anomalies in a multi-center cohort study.

Methods: We recruited first trimester pregnant women within The Infant Development and the Environment Study (TIDES) from 2010 to 2012 from four study centers and limited analyses to all mother/male infant dyads who had complete urinary phthalate and birth exam data (N=371). We used multivariate logistic regression to determine the odds of having a genital anomaly in relation to phthalate exposure.

Results: Hydrocele was the primary abnormality observed in the cohort (N=30) followed by undescended testes (N=5) and hypospadias (N=3). We observed a statistically significant 2.5 fold increased risk (95% CI 1.1, 5.9) of having any anomaly and 3.0 fold increased risk (95% CI 1.2, 7.6) of isolated hydrocele in relation to a one log unit increase in the sum of di-ethylhexyl phthalate (DEHP) metabolites.

Conclusions: First trimester urinary DEHP metabolite concentrations were associated with increased odds of any newborn genital anomaly, and this association was primarily driven by isolated hydrocele which made up the majority of anomalies in newborn males. The association with hydrocele has not been previously reported and suggests that it may be an endpoint affected by prenatal phthalate exposures in the first trimester of development. Future human studies should include hydrocele assessment in order to confirm findings.

1. Introduction

Male genitourinary development during fetal life is sensitive to hormone and non-hormone input via signaling pathways, and endocrine disrupting chemicals (EDCs) can affect these processes (Gore et al., 2015; Speroff, 2005). The primary programming window occurs during weeks 6 through 15 of pregnancy when fetal testicular development occurs resulting in testosterone and insulin like 3 (INSL3) production that further stimulates genitourinary structure and growth (Chacko and Barthold, 2009; Rey and Picard, 1998; Speroff, 2005; Strauss and Barbieri, 2009). Perturbations in development during this critical programming window can lead to a spectrum of male genital defects from severe conditions such as ambiguous genitalia to more minor birth defects such as hypospadias, undescended testis (UDT), hydrocele, and spermatic cord dysfunction (Rey and Picard, 1998). The incidence of hypospadias and undescended testis are 0.5–3% (Lao et al., 2012; Osifo and Osaigbovo, 2008; van der Zanden et al., 2012). Hydrocele is a human abnormality in which fluid surrounds the testis in the scrotal sac at birth and occurs in 5–25% of all births depending on the population studied (Osifo and Osaigbovo, 2008).

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It develops due to delayed closure of the processus vaginalis in utero or at birth, mechanisms driving this closure are unknown (Lao et al., 2012). The etiology of male developmental defects include genetic syndromes that alter endocrine function as well as environmental factors that perturb in utero genital development via both androgen mediated and androgen independent mechanisms (Amann and Veeramachaneni, 2007; Bay and Anand-Ivell, 2014; George et al., 2015; Kalfa et al., 2009).

Phthalates are a family of ubiquitous synthetic endocrine disrupting chemicals; in rat models, di-ethyl-hexyl phthalate (DEHP), di-butyl phthalate (DBP), and butyl benzyl phthalate (BBzP) adversely affect male genital development via an anti-androgenic effect on testicular development (Howdeshell et al., 2008; Mylchreest et al., 1998; Wolf et al., 1999). Some phthalates may also exert non-androgenic dependent effects such as oxidative stress affecting Sertoli cell function and effects on other hormones such as INSL3, integral for testicular descent (Amann and Veeramachaneni, 2007; Bay and Anand-Ivell, 2014; Chang et al., 2015; Sedha et al., 2015; Sobarzo et al., 2015). Prenatal DEHP and DBP exposures have been associated with a reduced anogenital distance, a potential marker of future fertility and reproductive health in humans (Bornehag et al., 2015; Swan, 2006). DEHP has also been associated with reduced INSL3 activity (Chang et al., 2015). One study reported a positive relationship between phthalate exposures and male genital anomalies, but this was a case control study in which the exposure was measured after case status had been determined (Choi et al., 2012). A study of the endocrine disrupting chemical, polybrominated biphenyl (PBB), found that prenatal exposure to this polybrominated flame retardant was associated with an increased risk of hydrocele (Small et al., 2009). No human studies have examined whether hydrocele development may be associated with phthalate exposures and a part of the associated genital developmental spectrum of defects.

We previously reported that first trimester maternal concentrations of DEHP metabolites (and their molar sum) were associated with reduced anogenital distance in male newborns within The Infant Development and Environment Study (TIDES) (Swan et al., 2015). In the current analysis, we examine associations between first trimester phthalate metabolite concentrations and male genital abnormalities at birth in TIDES infants. Secondarily, we investigate associations between male genital anomalies and AGD measurements.

2. Methods

2.1. Study population

TIDES is a pregnancy cohort study designed to examine prenatal phthalate exposures in relation to sexually dimorphic development, including genital morphology. Pregnant women who could read and write English (or Spanish at the California site), were less than 13 gestational weeks, and were planning on delivering at a study hospital were recruited between 2010 and 2012 from university based prenatal clinics in San Francisco, CA, Rochester, NY, Seattle, WA, and Minneapolis, MN. Women gave urine and serum samples and completed questionnaires in each trimester. They consented to have infants examined after birth. All participating institutions received IRB approval and informed consent was obtained from all participating women, who also provided consent for their child. Further details on the study design and goals can be found elsewhere (Barrett et al., 2014). In the current analysis, we report on women who provided a first trimester urine sample and gave birth to a male infant who participated in a physical examination at birth (N=371).

2.2. Phthalate measurements

Urine samples were collected in phthalate-free cups, and specific gravity was measured within 30 min of collection. The Centers for Disease Control and Prevention's (CDC) Division of Laboratory Sciences within the National Center for Environmental Health analyzed specimens for phthalate metabolites using enzymatic deconjugation and automated online solid-phase extraction, separation with high performance liquid chromatography and detection by mass spectrometry; further details are provided elsewhere (Swan et al., 2015). We measured the following metabolites of DEHP: mono-2-ethylhexyl (MEHP), mono-2-ethyl-5-oxo-hexyl (MEOHP), mono-2-ethyl-5-hydroxyhexyl (MEHHP), mono-2-ethyl-5carboxy pentyl (MECPP), as well as the BBzP metabolite, monobenzyl phthalate (MBzP), and the DBP metabolite, monobutyl phthalate (MBP). Concentrations below the limit of detection (LOD) for each metabolite were assigned a value equal to the LOD value divided by the square root of 2 (Hornung RW, 1990). We examined associations between the molar sum of the four measured DEHP metabolites ($\sum$DEHP=(MEHP*(1/278))+(MEHHP*(1/294))+(MEOHP*(1/292))+(MECPP*(1/308)) nmol/ml) as well as the individual metabolites. All metabolite concentrations were log transformed to normalize distributions.

2.3. Genital measurements and assessment

All examiners completed a 2 day training before beginning the study. Training in genital inspection and the assessment of genital anomalies was led by Dr. Richard Grady, a pediatric urologist. Examiners were trained to inspect and identify genital anatomic abnormalities such as phimosis, chordee, hypospadias, epispadias, hydrocele, and UDT. If there was a question about an anatomic finding, a study physician at each center was consulted. A thorough exam including visual inspection, palpation, and measurement were performed during birth exam by study examiners. We used results from the TIDES birth exam to identify all male genital abnormalities in the cohort, and we compared these to medical record findings. Two measures of AGD, (anoscrotal distance, AGD_{AS}, and anopenile distance, AGD_{AP}) were taken from each male newborn infant. Further details on AGD measurement methods and the rigorous quality control program used for all measurements and exams are described elsewhere (Sathyanarayana et al., 2015).

2.4. Statistical methods

We examined genital anomalies in relation to demographic variables that were chosen *a priori* based on the scientific literature and included maternal age, study center, race/ethnicity, education, gestational age at birth, birthweight, and infant age at exam in bivariate analyses. We then tested the significance of potential covariates in the full multivariate models. Race/ethnicity and maternal education did not reach statistical significance and were excluded in final analyses. We classified anomalies into groups: any anomaly, isolated hydrocele (because this was the most prevalent condition), and combined hypospadias and UDT. We first used a Student's *t*-test to compare prenatal phthalate concentrations in infants with anomalies compared to those without anomalies. We then used multi-variate logistic regression analyses to obtain adjusted odds ratios for each anomaly classification group compared to those without anomalies. For all metabolites that were significant in the main analysis, we examined anomalies by quartile of phthalate exposures in order to assess for any dose response relationships. In addition, we calculated the adjusted odds ratios for having any anomaly in relation to continuous AGD measurement using multivariate logistic regression.

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