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Associations of urinary phthalate and phenol biomarkers with menarche in a multiethnic cohort of young girls

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ABSTRACT

To study potential environmental influences on puberty in girls, we investigated urinary biomarkers in relation to age at menarche. Phenols and phthalates were measured at baseline (6–8 years of age). Menarche was ascertained over 11 years for 1051 girls with menarche and biomarkers. Hazards ratios were estimated from Cox models adjusted for race/ethnicity and caregiver education (aHR, 95% confidence intervals [CI] for 5th vs 1st quintile urinary biomarker concentrations). 2,5-Dichlorophenol was associated with earlier menarche (aHR 1.34 [1.06–1.71]); enterolactone was associated with later menarche (aHR 0.82 [0.66–1.03]), as was mono-3-carboxypropyl phthalate (MCPP) (aHR 0.73 [0.59–0.91]); the three p-trends were <0.05. Menarche differed by 4–7 months across this range. Enterolactone and MCPP associations were stronger in girls with below-median body mass index. These analytes were also associated with age at breast development in this cohort. Findings from this prospective study suggest that some childhood exposures are associated with pubertal timing.

1. Introduction

Menarche, or the first menstrual period, marks the beginning of a woman's reproductive life. Earlier or later onset has been linked with risk of chronic disease in adulthood. Its decline from about 18–12 years of age over 300 years is explained by lifestyle changes that accompanied industrialization. Primary factors are a girl's age, adiposity, and race. Individual factors such as race and body mass index (BMI) account for 6–12 months difference in menarche [1,2]. Racial differences may reflect both genetic and environmental factors. Much but not all of variation in menarche is explained by genetics, perhaps 70% [3]. These observations, in combination with strong experimental evidence that some chemical exposures alter pubertal timing, have triggered research regarding whether environment influences menarche [4]. Most research has come from cross-sectional investigations that assess environmental exposures among girls over a range of ages. Cross-sectional designs may not be able to address adequately the question of temporality needed to evaluate exposures in relation to menarche.

We are interested in highly prevalent hormonally active environmental exposures that have been identified in the past 20

Abbreviations: aHR, adjusted hazards ratio; B2, breast stage 2; BMI%, body mass index percentile age and sex specific.

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[1] http://www.bcerp.org/index.htm.

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years [5]. Common sources are diet and the physical environment, including household and personal care products. Among these, a number of phthalates and phenols, including phytoestrogens, have exhibited agonist and antagonistic hormone activity as well as possible obesogenic and anti-obesogenic effects. Estrogen, androgen, thyroid, and peroxisome proliferator-activated receptor (PPAR) activity has been observed [6].

To examine relationships between such exposures and pubertal onset, the NCI and NIEHS established the Breast Cancer and Environment Research Program (BCERP) cohort of girls to enable longitudinal study during childhood [7]. Here we report associations of 19 urinary biomarkers of environmental exposures with menarche in the BCERP cohort.

2. Materials and methods

2.1. Study design and data collection

The BCERP Puberty Study is a cohort that enrolled girls between 2004 and 2007, concluding in 2015 (up to eleven years' follow-up or 9 annual visits). Study sites included Icahn School of Medicine at Mount Sinai (NYC) that recruited black or Hispanic girls mainly from East Harlem in New York City; Cincinnati Children's Hospital (Cincinnati) that recruited from the greater Cincinnati metropolitan area; and Kaiser Permanente Northern California (California) that recruited members of the KPNC Health Plan in the San Francisco Bay Area. Eligible girls were 6–8 years of age without serious endocrine medical conditions. Informed consent was obtained from parent or guardian and assent from the girl, administered by the institutional IRBs. The IRB of the Centers for Disease Control and Prevention (CDC) also approved the analysis of urine specimens, but CDC had no access to identifiers. A study timeline is provided in Supplemental Table 1. We obtained demographics, anthropometry, pubertal assessment, and urine at enrolment (baseline) and annually or semiannually thereafter. Race/ethnicity of the girl was self-reported and coded as black, non-black Hispanic, non-Hispanic white, or Asian. Anthropometry and breast stages were determined by trained examiners to ascertain age at the first appearance of breast development (stage B2 vs B1, no development) as well as age- and sex-specific BMI percentiles (BMI%) calculated using the CDC growth charts [8]. Complete exam protocols and laboratory methods have been described previously along with quality control measures [7,9]. At the second annual visit after enrolment (NYC and California) or the 5th year (Cincinnati), the guardian was asked for the age and date of the girl's menarche; the girl also reported menarche from 2012 to 2015. The first guardian-reported date was used to determine age at menarche (82%) unless it was not provided in which case the first girl-reported date was used (11%). Reported ages were used if dates were not reported. Sensitivity analyses comparing guardian vs daughter reports found that 78% of reported dates agreed within 6 months.

Urine specimens collected within a year of enrolment were analyzed at the CDC National Center for Environmental Health laboratory. Results and analytic procedures including quality control measures have been reported in previous papers investigating pubertal onset [9]. Analytes included phenols (benzophenone-3, enterolactone, bisphenol A, methyl-, ethyl-, propyl-parabens, 2,5-dichlorophenol, triclosan, genistein, and daidzein) and phthalate metabolites (monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), mono-isobutyl phthalate, monobenzyl phthalate (MBzP), mono-3-carboxypropyl phthalate (MCPP), mono(2-ethyl-5-carboxypentyl) phthalate, mono(2-ethyl-5-hydroxylhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethylhexyl) phthalate (MEHP)). Phenols were detected in >80% of samples except for butyl paraben (48%). As described in previous papers, we summed the paraben metabolites based on molecular weight (micromoles/L), expressing the paraben sum as propyl paraben (molecular weight 180.2 g/mol). We also combined the phthalate metabolites into groups based on similar sources and biologic activity, low- (<250 Da, or "low-MWP") and high-molecular weight (>250 Da, "high-MWP") [9]. Low-MWP include MEP, MBP and MIBP. High-MWP include the four di(2-ethylhexyl)phthalate (DEHP) metabolites (ΣDEHP: MECPP, MEHHP, MEOHP and MEHP), MBzP, and MCPP. We expressed low-MWP molar sum as MEP (molecular weight 194) and high-MWP as MEHP (molecular weight 278) so that units were the same as the single analytes (μg/L). Phthalate metabolites were detected in >98% of samples, except for MEP (>80%). Concentrations below the limit of detection (LOD) were assigned the value LOD/$\sqrt{2}$. Concentrations (ln-μg/L) were normalized for urine dilution in linear models using ln-creatinine as a covariate or, in models with quantiles, using cut-points based on creatinine-corrected biomarkers (μg/g-creatinine or ug/gCr). LOD imputation and creatinine adjustment have been described earlier in more detail [10]. There were 1051 girls with at least one exposure biomarker and menarche information including censored values for 139 girls (no menarche reported at last followup). To assess intraindividual reliability of biomarkers over time, assays were repeated in additional samples taken approximately one and three years after enrolment (ca. 100 per site, except for phytoestrogens which were done for two sites; total 927 serial samples except for phytoestrogens, n = 465).

2.2. Statistical analyses

Analyses were performed with SAS (version 9.4; SAS Institute, Inc). Using Cox Proportional Hazards models to compute hazard ratios (HR) and 95% confidence intervals (CI), we modeled relative risk for menarche associated with urinary biomarker concentrations as both continuous and quantiles (quintiles or tertiles) using PROC PHREG. The Cox model allows for censored observations; therefore the 139 girls with no menarche report were included in all analyses. We computed median age at menarche for quantiles of urinary concentrations using the baseline survivor function of multivariable adjusted Cox models. From these models we graphed adjusted Kaplan-Meier survival curves for menarche by quantiles of biomarkers using output produced with the STRATA statement. We tested proportionality assumptions by including quintiles as a time-dependent variable as well as using the *Assess* function in PROC PHREG which analyzes martingale residuals. To resolve violations of proportionality, the covariate race/ethnicity was included in the STRATA statement of PROC PHREG. For quintile models with non-linear associations, such as U-shaped, we verified fit using splines to verify the effect patterns (PROC PHREG and PROC PLM). To obtain p-trend for quintiles, we substituted the natural log of the medians of quintile urinary biomarker concentrations as a continuous variable. Potential confounders were identified by examining the exposure-menarche pathway for biological plausibility and correlations among the variables, i.e. those in Table 1 as well as season at urine donation. We retained the two covariates that altered the estimates by more than 10% or materially improved precision of models (girl's race/ethnicity and her caregiver's education). Study site was not considered a confounder as it is not causally related to menarche nor biomarker concentrations, and as it was collinear with the main covariates (race/ethnicity and caregiver education). In addition, our multi-site study was designed to include geographic variation in order to provide a range of exposures [7].

In order to explore possible multiple exposure effects, we computed Cox models for mutually adjusted tertiles of 2,5-dichlorophenol, enterolactone, and MCPP. The risk estimates were almost identical to models for single biomarkers, though slightly attenuated, and significance was unaffected, with the CIs shifting by