The effect of phthalate exposure and filaggrin gene variants on atopic dermatitis

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ABSTRACT

Background: Phthalate exposure may increase the risk of atopic disorders. However, little is known about the joint effects of phthalate exposure and filaggrin (FLG) gene variants on atopic dermatitis (AD). We want to investigate whether FLG variants are related to a higher urine concentration of phthalates and whether an interaction of FLG and phthalates increases the risk of AD.
Methods: We conducted a case-control study comprised of 106 AD children and 347 controls, all of whom were selected from CEAS cohort. Urine phthalate metabolite levels (MEP, MBP, MBzP, and 5OH-MEHP) were measured by UPLC–MS/MS. FLG variants were analyzed by TaqMan assay. At 3 years of age, information about the development of AD and environmental exposures were collected. Logistic regressions were performed to estimate the association of genotypes and phthalate metabolite levels with AD.
Results: Urine MBP and MBzP levels were higher in children with AD than in controls ($p < 0.001$). Children with the FLG P478S TT genotype had higher urine phthalate metabolite levels as compared with CC carriers, with MBP and MBzP having a statistically significant difference (geometric mean(s.e.) 5.51 (3.77) vs. 3.03(3.48), $p = 0.015$ and 0.76(3.01) vs. 0.53(2.56), $p = 0.018$). After stratifying by phthalate metabolite levels, FLG P478S TT genotype was related to a higher odds of AD in children with high MBP levels (aOR=4.74, 95% CI 1.45–15.5) and in children with high MBzP levels (aOR=3.46, 95%CI 1.03–11.58).
Conclusions: FLG variants may increase skin permeability leading to higher skin absorption of phthalate and thus confer a higher susceptibility for AD. Or alternatively, the internal burden of phthalates metabolites is increased in children with AD who also have risky variant of the FLG gene.

1. Introduction

Phthalates are an environmental hazard. They are commonly used in a variety of cosmetic and personal care products, such as lotions, shampoos, diaper creams, skin cleansers, hair sprays, nail polishes, and fragrances, where they stabilize the fragrance, increase spreadability, and enhance absorption (Koniecki et al., 2011). Most commonly, the phthalate intake also results via diet

and inhalation. Dermal absorption from personal care products may contribute to the overall phthalates body burden (Janjua et al., 2008). Although phthalates are not highly sensitizing, they may promote the development of allergic contact dermatitis (Howdhury and Statham, 2002). There is also evidence that atopic dermatitis (AD)-like lesions in mice are exacerbated by phthalate exposure at low levels and also by exposure in utero, suggesting that exposure to phthalates may be responsible for the increasing prevalence of AD (Takano et al., 2006; Yanagisawa et al., 2008). Consistent human findings were reported from Bulgaria, Sweden, and Taiwan, where researchers found that the risk of AD was related to the house-dust BBzP concentrations (Kolarik et al., 2008; Huang et al., 2009; Hsu et al., 2012). Furthermore, it has been reported for humans that prenatal exposure to BBzP may increase the risk of developing eczema in early childhood (Just et al., 2012). In a prior study, we also found that urine phthalate metabolite levels are significantly associated with AD (Wang et al., 2014). The

above findings indicate that phthalate exposure early in life plays an important role in developing susceptibility to atopic disorders.

Filaggrin (*FLG*) is a key epidermal protein in facilitating the terminal differentiation of keratinocytes and thereby in maintaining normal skin barrier function and hydration (Cork et al., 2009). Loss-of-function mutations of *FLG* R501X and 2282del4, frequent in European populations, have related to AD (Weidinger et al., 2006). In Chinese and Korean AD children, another specific *FLG* P478S variant has been identified (Wang et al., 2011; Kim et al., 2013). Therefore, *FLG* variants is a significant predisposing factors for AD in many populations. The impact of *FLG* gene variation on AD risk exceeds that of any other investigated candidate gene thus far (Baurecht et al., 2007). However, 40% carriers of *FLG* variants do not develop AD (Henderson et al., 2008), suggesting that other conditions, such as environmental factors, may interact with a *FLG* variants in the pathogenesis of AD.

Young children are particularly susceptible to environmental exposure. Children with AD often suffer from considerable school absences, family stress, and health care expenditures (O'Connell, 2004). As such, searching for early environmental risk factors for AD among genetically susceptible children is imperative. Since impaired epidermal skin barriers play a crucial role in the pathogenesis of AD, understanding the interplay of gene and environment in AD will provide the rationale for targeted measures capable of preventing disruption of the barrier function. However, whether the skin barrier function in individuals with *FLG* variants may modulate the effect of environmental exposure, thus modifying the expression of AD remains to be elucidated. This gap in understanding motivated us to evaluate whether *FLG* variants may increase internal burden of phthalate, and if so, whether this would alter the risk of AD in susceptible children.

2. Methods

2.1. Study population

We conducted a nested case-control study that was comprised of 106 AD children and 347 controls with urine specimens and oral scrapes collected in 2010 in Taipei, all of whom were selected from the Childhood Environment and Allergic diseases Study (CEAS) cohort. Full enrollment into the study required the completion of phthalate exposure monitoring of urine phthalate metabolite levels and *FLG* genotyping. Parents were interviewed using a standardized questionnaire at clinics regarding child birth history, parental age and education levels, family income, parental history of atopy, duration of breast feeding, child environmental exposures, etc. At the age of 3, information about development of AD was collected. Written informed consent was obtained from all parents. The hospital's Institutional Review Board approved the study protocol, which complied with the principles of the Helsinki Declaration.

2.2. Case definition

The validated International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was administered to parents. Parents were asked the following questions: "Have you ever had AD diagnosed by a physician?" and "Have you ever had recurrent itchy rash for at least 6 consecutive half-months periods over elbows, knees, face, wrists, neck, periauricular and eyebrow areas?" Experienced pediatric allergists performed a standardized history and clinical examination on participants with a maternal report of child's AD on questionnaires at the clinic. Finally, AD cases were defined according to the diagnostic criteria developed by Hanifin

and Rajka (Hanifin and Rajka, 1980). Children not included in these categories were classified as control subjects.

2.3. Laboratory methods

2.3.1. Exposure monitoring

First mid-stream urine in the morning were collected and stored at $-20\,°C$ until analysis. Four phthalate metabolites (monoethyl phthalate (MEP), monobutyl phthalate (MBP), monobenzyl phthalate (MBzP), and mono(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP)) representing the exposure to four commonly used phthalates (diethylphthalate (DEP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBzP), and di(2-ethylhexyl) phthalate (DEHP)) were measured by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS), as described previously (Silva et al., 2004). One blank and one QC standard sample with the mixture of phthalate metabolites (100 ng/ml) are included in each batch of samples analyzed. Recovery of $^{13}C_{12}$-labeled internal-standard and native-standard of each phthalate metabolite in samples are higher than 50%, respectively. The phthalate metabolite levels of the blank samples are lower than twice the minimum detectable limit in each batch. The calibration range of all phthalate metabolites are 1–1000 ppb. Only R^2 values higher than 0.99 and relative percent difference of calibration curves less than 40% are accepted. For concentrations below the detection limits, a value of half the lower limit of quantitation was assigned. All results involved duplicate analysis. Urine creatinine levels were analyzed by enzymatic assay according to the manufacturers' instructions (Cayman Chemical, Ann Arbor, MI) (Cayman Chemical Company, 2012). All statistical models were adjusted for urine creatinine levels.

2.3.2. Genotyping

Genomic DNA was extracted from oral epithelial cells by Gentra Purgene DNA Buccal Cell Kit (QIAGEN Sciences, Maryland, USA). The *FLG* variant of rs11584340 (P478S) polymorphisms, selected from hot spots of Asian populations on NCBI website, were analyzed by TaqMan SNP Genotyping Assay (ABI, Foster City, CA, USA) as described elsewhere (Yu et al., 2013). The samples of all children were measured in the same assay in duplicate for each subject to reduce the influence of unavoidable inter-assay variances. A subgroup was reconfirmed by DNA sequencing. To control for errors and technical problems in genotyping, derived genotype frequencies were compared with the expected allelic population equilibrium based on the Hardy–Weinberg equilibrium test.

2.4. Statistical analysis

Baseline characteristics were compared between case and control subjects with two-sample *t*-test for continuous variables or Chi-square tests for categorical variables. Differences of geometric means of phthalate metabolite levels among different *FLG* P478S genotypes (TT, TC, and CC) were analyzed using ANCOVA test. To study the modification effect of phthalate exposure, we divided phthalate metabolite levels into two categories (lower and higher level groups) by median levels. For the association of phthalate levels and *FLG* variants with AD, odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated using logistic regression. Next, we tested for gene–environment interaction by adding a product term in the regression model. Potential confounders from the literature reviews, such as gender, premature birth, maternal age and education, maternal history of atopy, family income, duration of breast feeding, number of older siblings, pet raising, environmental tobacco exposure (ETS), usage of carpets at home, and fungi on house walls were taken into consideration. We adjusted for those confounders that resulted in a 10% change in point