Association of filaggrin gene mutations and childhood eczema and wheeze with phthalates and phosphorus flame retardants in house dust: The Hokkaido study on Environment and Children's Health

Yu Ait Bamai[1], Atsuko Araki[1], Toshifumi Nomura[2], Toshio Kawai[3], Tazuru Tsuboi[3], Sumitaka Kobayashi[1], Chihiro Miyashita[1], Masae Takeda[2], Hiroshi Shimizu[2], Reiko Kishi [1*]

[1] Hokkaido University Center for Environmental and Health Sciences, North 12, West 7, Kita-ku Sapporo 060-0812, Japan

[2] Department of Dermatology, Hokkaido University Graduate School of Medicine, North 15 West 7, Kita-ku, Sapporo 060-8638, Japan

[3] Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association, 2-3-8, Tosabori, Nishi-ku, Osaka 550-0001, Japan

*Corresponding author: Reiko Kishi, MD, PhD, MPH

Hokkaido University Center for Environmental and Health Sciences,

Kita 12, Nishi 7, Kita-ku, Sapporo 060-0812, Japan

Phone: +81-11- 706-4746

Fax: +81-11-706-4725

E-mail: rkishi@med.hokudai.ac.jp

Yu Ait Bamai: u-aitbamai@med.hokudai.ac.jp; Atsuko Araki: AAraki@cehs.hokudai.ac.jp; Toshifumi Nomura: nomura@huhp.hokudai.ac.jp; Toshio Kawai: kawaitoshio125@gmail.com; Tazuru Tsuboi: t-tsuboi@jisha.or.jp; Sumitaka Kobayashi: sukobayashi@cehs.hokudai.ac.jp; , Chihiro Miyashita: miyasita@med.hokudai.ac.jp; Hiroshi Shimizu: shimizu@med.hokudai.ac.jp

Abstract

Background and Aim Exposure to phthalates and phosphorus flame retardants (PFRs) is considered to be a risk factor for asthma and allergies. However, little is known about the contribution of loss-of-function mutations in the gene encoding filaggrin *(FLG*) gene, which are considered to be predisposing factors for eczema and asthma, to these associations. We investigated the associations between exposure to phthalates and PFRs in dust and eczema/wheeze among Japanese children, taking into consideration loss-of-function mutations in *FLG*.

Methods This study was part of the Hokkaido study on Environment and Children's Health. Seven phthalates and 11 PFRs in household dust were measured by gas chromatography-mass spectrometry. Eczema and wheeze were assessed in children aged 7 years using the International Study of Asthma and Allergies in Childhood questionnaire. Eight *FLG* mutations previously identified in the Japanese population were extracted from cord blood samples. Children with one or more *FLG* mutations were considered to be positive for *FLG* mutations. The study included 296 children who had complete data (birth records, *FLG* mutations, first trimester and 7 years questionnaires, and phthalate/PFR levels). Odds ratios (ORs) and 95% confidential intervals (CIs) of eczema and wheeze were calculated for log-transformed phthalate/PFR levels by logistic regression. We also performed stratified analyses based on *FLG* mutations.

Results The prevalence rates of eczema and wheeze were 20.6% and 13.9%, respectively. Among children without any *FLG* mutations, tris (1, 3-dichloro-2-propyl) phosphate (TDCIPP) increased the OR of wheeze, (OR: 1.22, CI: 1.00–1.48). Significant *p* values for trends were found between tris (2-butoxyethyl) phosphate (TBOEP) and eczema and di-iso-nonyl phthalate (DiNP) and eczema among children without any *FLG* mutations, respectively.

Conclusions Despite our limited sample size and cross-sectional study design, the effects of indoor environmental factors on childhood eczema and wheeze were clearer in children without loss-of-function mutations in *FLG* than in children with mutations. Children with *FLG* mutations might already be cared for differently in terms of medication or parental lifestyle. Further studies in larger populations are warranted so that severity of symptoms and combinations of *FLG* mutations can be investigated.

Keywords: Filaggrin, Eczema, Wheeze, Child, Phthalate, Flame retardants

Abbreviations: ; BBzP, butyl benzyl phthalate; DEHP, di-(2-ethylhexyl) phthalate; DEP, diethyl phthalate; Der p1, *Dermatophagoides pteronyssinus*; Der f1, *Dermatophagoides farinae*; DiBP, di-iso-butyl phthalate; DiNP, di-iso-nonyl phthalate; DMP, dimethyl phthalate; DnBP, di-n-butyl phthalate; *FLG*, filaggrin gene; GC-MS, gas chromatography–mass spectrometry; Ig, immunoglobulin; IL, interleukin; ISAAC, International Study of Asthma and Allergies in Childhood; LOQ, limit of quantification; MHCII, major histocompatibility complex class II; PFR, phosphorus flame retardant; SVOC, semi-volatile organic compound; TBOEP, tris (2-butoxyethyl) phosphate; TCEP, tris (2-chloroethyl) phosphate; TCiPP, tris (2-chloro-iso-propyl) phosphate; TDCiPP, tris (1, 3-dichloro-2-propyl) phosphate; TEP, triethyl phosphate; TEHP, tris (2-ethylhexyl) phosphate; TMP, trimethyl phosphate; TCP, tricresyl phosphate; TNBP, tri-(n-butyl) phosphate; TPhP, triphenyl phosphate

1. Introduction

Phthalates and phosphorus flame retardants (PFRs) are types of semi-volatile organic compounds (SVOC); phthalates are mainly used as plasticizers, personal care products, fragrances and PFRs are used as flame retardants and plasticizers, respectively. Because they are semi-volatile, phthalates and PFRs do not chemically bond when applied to a matrix; therefore, they accumulate in dust and air particles. It is believed that food intake is the main exposure route for phthalates (Kishi et al., 2017). However, house dust and dermal contact should also be considered when assessing exposure to phthalates and PFRs in the indoor environment. Recent experimental studies have demonstrated cutaneous exposure to indoor SVOCs (Cao et al., 2016; Cao et al., 2018; Pelletier et al., 2017). In particular, children are more highly exposed than adults from indoor environments because of their hand-to-mouth behavior, longer time spent in the home, a higher ratio of body surface area to volume than adults, and high level of dust ingestion (Ait Bamai et al., 2015; U.S.EPA 2002).

We previously reported that high levels of di-iso-butyl phthalate (DiBP) and butyl benzyl phthalate (BBzP) in dust increased the odds ratio (OR) of parental-reported eczema in Japanese children (Ait Bamai et al., 2014); we also found that tris (2-chloro-iso-propyl) phosphate (TCiPP) and tris (1, 3-dichloro-2-propyl) phosphate (TDCiPP) in dust increased the OR of self-reported eczema, and that tri-(n-butyl) phosphate (TNBP) in dust increased the OR of self-reported asthma in Japanese inhabitants living in newly built detached houses (Araki et al., 2014a). Previous studies in Swedish, Bulgarian, and Danish children reported that high levels of di-(2-ethylhexyl) phthalate (DEHP) and BBzP in dust were associated with asthma and eczema, respectively（Bornehag et al., 2004; Callesen et al., 2014; Kolarik et al., 2008）. A recent meta-analysis suggested that postnatal exposure to DEHP and BBzP from dust had strong positive associations with childhood asthma (Li et al., 2017). Animal and *in vitro* studies suggest that phthalates and PFRs may have immunocytotoxic effects in mice (Canbaz et al., 2017; Koike et al., 2010; Tanaka et al., 2013). Mice sensitized to DEHP and ovalbumin had increased levels of immunoglobulin E (IgE), IgG1, interleukin-21 (IL-21) and IL-4, suggesting that DEHP acts as an allergy adjuvant (Tanaka et al., 2013). Mice injected with di-iso-nonyl phthalate

(DiNP) had increased expression of histamine and eotaxin (Koike et al., 2010). Suppressive or repressive effects on major histocompatibility complex class II (MHCII) and cytokines such as CD80, CD86, CD40, IL-6, and IL-10 were observed in bone marrow-derived dendritic cells exposed to triphenyl phosphate (TPhP) and TDCiPP (Canbaz et al., 2017).

Previous findings are unclear and inconsistent regarding the effects of each specific phthalate or PFR on asthma and allergies. Some observed differences might be due to differences in genetic factors among study populations. Therefore, not only environmental chemical exposure, but also genetic factors should be considered in relation to allergic diseases. Filaggrin (filament aggregating protein) is a key molecule in skin barrier function, preventing transepidermal water loss and minimizing entry of allergens, toxic chemicals, and infectious microorganisms (Candi et al., 2005). Loss-of-function mutations in the gene encoding *FLG* impairs skin barrier function and hydration. Therefore, *FLG* null variants are considered to be risk factors for allergic disease development. Several studies have reported that loss-of-function mutations in *FLG* are the major predisposing factors for not only eczema (Palmer et al., 2006), but also allergic sensitization, asthma, and rhinitis (Chan et al., 2018; Debinska et al., 2017). Furthermore, parameters of barrier dysfunction such as stratum corneum dehydration and transepidermal water loss were significantly increased in *FLG*-related eczema patients (Nemoto-Hasebe et al., 2009a). To date, more than 50 *FLG* null mutations have been reported in the European, American, Asian, and African populations. In the Japanese population, six recurrent *FLG* mutations—*3321delA*, *Q1701X*, *S2554X*, *S2889X*, *S3296X*, and *K4022X*—have been identified, which are carried by more than 20% of Japanese atopic dermatitis patients (Nemoto-Hasebe et al., 2009b; Nomura et al., 2008; Nomura et al., 2009). Recent epidemiological studies have reported that *FLG* mutation carriers have significantly higher levels of environmental chemicals in urine such as mono-n-butyl phthalate, mono-iso-butyl phthalate, and methyl paraben compared to non-carriers and suggested that *FLG* loss-of-function mutation carriers may have higher internal exposure to phthalates because of increased trans epidermal absorption and/or higher exposure to topical medication (Joensen et al., 2014; Joensen et al., 2017). On the other hand, Overgaard et al. (2017) have reported

that there is no association between *FLG* mutations and urinary phthalate metabolite levels in Danish children. However, children with eczema had significantly higher mono-n-butyl phthalate levels than children without eczema due to frequent use of emollients (Overgaard et al., 2017). Moreover, two birth cohort studies have indicated that cat exposure enhances the effect of *FLG* mutations on the development of eczema and allergic sensitization (Bisgaard et al., 2008); (Schuttelaar et al., 2009). Cat ownership in early life substantially increases the risk of developing eczema and sensitization within the first year of life in children with *FLG* mutations (Bisgaard et al., 2008; Schuttelaar et al., 2009). However, no other epidemiological studies have assessed the contribution of *FLG* mutations to the effects of environmental chemicals on asthma and allergies. To help fill in the knowledge gaps regarding associations between exposure to phthalates and/or PFRs and allergies, we investigated whether children with *FLG* mutations are more vulnerable to cutaneous exposure to phthalates and PFRs in dust, and thus have higher risk of eczema and wheeze than those without such mutations.

2. Methods

2.1. Study population

Recruitment of children for the Hokkaido study on Environment and Children's Health (Hokkaido cohort) has been reported elsewhere (Kishi et al., 2017; Kishi et al., 2013; Kishi et al., 2011). For the present study, among 20,926 children who were enrolled from February 2003 to March 2012, 7,350 children who reached the age of 7 years by March 2013 were selected; mothers of these children received the follow-up questionnaire for 7-year-olds and 2,697 were returned. At the same time, mothers were asked to collect house dust samples, of which 2,087 mothers agreed, and finally, 888 house dust samples were obtained. *FLG* mutation carriers were screened from 1066 cord blood samples of the participants who had complete data sample at birth, birth record, maternal first-trimester questionnaire, and follow-up questionnaires for 7-year-olds. Among these, 2 participants were excluded due to missing data in the eczema category of the ISAAC questionnaire. Finally, we

selected 296 children who had both dust samples and cord blood *FLG* mutation assessments, in order of dust arrival for measurement of phthalates, PFRs, and mite allergens.

2.2. Assessment of allergic diseases

Self-administered questionnaires were given to mothers to collect information on the occurrence of eczema in the 7-year-old children. The questionnaire was the Japanese version of the validated International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaire (ISAAC Steering Committee, 1998). Eczema was defined as (a) "Having an itchy rash that comes and goes for at least 6 months," or (b) "Having the aforementioned itchy rash at any time during the last 12 months," or (c) "Having the aforementioned itchy rash affect one or several of the following areas: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes." Wheeze was determined by an affirmative answer to the following question: "Has your child had wheezing or whistling in the chest in the last 12 months?"

2.3. Phthalates, PFRs, and mite allergens in house dust

At the time of questionnaire survey, mothers of 7-year-old children were asked to collect house dust samples from living room floor surfaces. Each mother was sent a polyethylene dust bag (Nichinichi Pharmaceutical Co., Ltd., Mie, Japan), along with directions for use with the vacuum cleaner regularly used to clean the dwelling and instructions for return of the sample. To avoid non-uniform dust and unwanted substances such as human hair, animal hair, and food scraps, the dust was sieved with a 300-μm filter. Fractions of dust were weighed out into micro tubes for mite allergen analysis and stoppered glass tube for phthalate and PFR analysis, and stored at -20 °C.

The gas chromatography-mass spectrometry (GC-MS) analysis, quality control, and quality assurance methods used herein have been described in detail previously (Ait Bamai et al., 2013; Tajima et al., 2014). GC-MS in signal-to-ion mode was used to analyze phthalate and PFR levels from collected dust. Target phthalate compounds included dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), DiBP, BBzP, DEHP, and DiNP; target PFRs included tris (2-butoxyethyl) phosphate (TBOEP), tris (2-chloroethyl) phosphate (TCEP), triethyl phosphate

(TEP), tris (2-ethylhexyl) phosphate (TEHP), trimethyl phosphate (TMP), tricresyl phosphate (TCP), TNBP, TPhP, TCiPP, and TDCiPP. The analyses were conducted at Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association.

To determine dust mite-related allergen levels, we used the method described by (Saijo et al., 2011). Levels of *Dermatophagoides pteronyssinus* allergens (Der p1) and *Dermatophagoides farinae* allergens (Der f1) were determined by enzyme-linked immunosorbent assay (ELISA) using Der p1 and Der f1 ELISA kits (Nichinichi Pharmaceutical Co., Ltd.) The values for Der p1 and Der f1 were summed to create a single factor called Der 1.

2.4. FLG analysis

FLG mutation status was determined using the methods previously reported by (Kono et al., 2014; Nemoto-Hasebe et al., 2009b; Sandilands et al., 2007). After excluding rare *FLG* mutations from the 11 *FLG* mutations that had been identified in most Japanese *FLG* mutation carriers (Kono et al., 2014; Nemoto-Hasebe et al., 2009b; Nomura et al., 2008; Nomura et al., 2007), we screened for *3321delA*, *Q1701X*, *S2554X*, *S2889X*, *S3296X*, and *K4022X* by Sanger sequencing. Briefly, genomic DNA extracted from cord blood was amplified using the AmpliTaq Gold PCR Master Mix (Applied Biosystems, Foster City, CA). Primer sequences and PCR conditions are available upon request. The PCR amplicons were treated with ExoSAP-IT reagent (Affymetrix, Santa Clara, CA), and the sequencing reaction was performed using BigDye Terminator version 3.1 (Applied Biosystems). Sequence data were obtained using an ABI 3130xl genetic analyzer (Applied Biosystems). *FLG* mutation was defined as the presence of at least one specified mutation identified by sequence analysis.

2.5. Data analysis

Chi-squared tests were used to analyze the associations between eczema or wheeze and children's demographic characteristics. Detection rates <60% of compounds were excluded from statistical analyses. Values lower than the limit of quantification (LOQ) were calculated as LOQ/2. Because measured values of phthalates, PFRs, and mite allergens were skewed and not normally distributed

according to the Shapiro-Wilk W-test, these concentrations were natural log-transformed. Logistic regression was used to determine relationships between phthalates, PFRs, or mite allergen levels and eczema/wheeze. The results were presented as odd ratios (ORs) with 95% confidence intervals (CIs) for the risk of eczema/wheeze using natural log-transformed exposure levels. We further analyzed the models including the gene-environment interaction term and stratified them to assess potential effects of *FLG* mutations. Potential confounders were selected based both on literature review and more than 10% change of the estimate in the model. In cases where the relationship between phthalate or PFR levels and allergies showed significance at $p<0.1$, the levels of phthalates and PFRs were categorized into quartiles (for compounds with a detection rate >75%) and >LOQ vs. ≤LOQ (for compounds with a detection rate ≤75%). The lowest categories were used as the reference to estimate the linear trends of the ORs. For all statistical analyses, two-tailed tests and 5% level of significance were used. Analyses were performed using SPSS 19 for Macintosh (SPSS, Inc., Chicago, IL, USA) and JMP Pro 10 for Macintosh (SAS Institute, Inc., Cary, NC, USA).

2.6. Ethical considerations

This study was approved by the Institutional Ethical Review Board for Epidemiological Studies at Hokkaido University Graduate School of Medicine, Hokkaido University Center for Environmental and Health Sciences, in accordance with the principles of the Declaration of Helsinki. All adult participants provided written informed consent.

3. Results

Table 1 shows the characteristics of children with and without eczema and wheeze. 20.6% and 22.0% of children had eczema and wheeze, respectively. In this study, 9.1% (n= 27) of all children had an *FLG* mutation and the mutation was more common in girls than in boys (not shown). However, there were no significant differences in *FLG* mutation between children with (8.2%: n= 5) and without (9.4%: n= 22) eczema or between children with (7.3%: n= 3) and without (9.4%: n= 24) wheeze. Children with eczema had significantly greater comorbidity of allergic rhino-conjunctivitis ($p<0.05$)

and parents with history of eczema ($p<0.01$) than children without eczema. Wheeze was significantly more prevalent in boys than in girls ($p<0.05$). Children with eczema were more likely to have mothers who smoked tobacco, and boys had a higher prevalence of eczema than girls; however, these differences were not significant. Although several papers suggested that breast feeding might have protective effects on allergic disease developments in children (Oddy et al., 1999; Sears et al., 2002), in present study, neither the duration of breast feeding nor type of feeding (breastfed, powder milk fed, or mixed) were associated with prevalence of wheeze/eczema.

Table 2 shows the distributions and the comparisons of the levels of phthalates, PFRs, and mite allergens in house dust. DnBP, BBzP, DEHP, DiNP, TCiPP, and Der 1 were detected with more than 90% of house dust samples. DEHP and TBOEP were detected at the highest concentration among phthalates and PFRs, respectively. Levels of TBOEP in house dust were significantly higher for children with eczema compared to children without eczema; however, there were no significant differences in phthalate or PFR levels in house dust for children with and without wheeze (Supplementary Table 1).

Table 3 shows the associations between eczema and phthalates, PFRs, and mite allergens in house dust. TBOEP had a borderline association with decreased risk of eczema (OR, 95% CI: 0.91, 0.81–1.02; p=0.091). Other compounds did not have statistically significant associations with eczema. A significant gene-environment interaction was observed for DiNP (p=0.039). After stratification by *FLG* mutation, BBzP (2.19, 0.93–5.16; p=0.074) and DiNP (0.27, 0.06–1.28; p=0.099) had borderline associations with eczema among children with *FLG* mutation. DiNP had a borderline association with eczema among children without any *FLG* mutations (1.29, 0.98–1.69; p=0.068). Furthermore, in the categorical model, positive dose-response relationships were found between DiNP levels and eczema (Q1 vs. Q4 p for trend=0.060) overall, as well as among children without *FLG* mutation (Q1 vs. Q4 p for trend=0.011) and negative dose-response relationships with *FLG* mutation (Q1 vs. Q4 p for trend=0.087) (Figure 1). TBOEP also showed positive dose-response relationships with eczema (Q1 vs. Q4 p for trend=0.020) overall and negative dose-response relationships among children without *FLG*

mutation (Q1 vs. Q4 p for trend= 0.024) (Figure 2).

Table 4 shows the associations between wheeze and phthalates, PFRs, and mite allergens in house dust. No significant gene-environment interactions were observed for any compound. TDCiPP was significantly associated with wheeze (1.26, 1.04–1.52; p=0.021). Furthermore, TDCiPP increased risk of wheeze among children without *FLG* mutations (1.22, 1.00–1.48; p=0.047). In the categorical model, a positive dose-response relationship was found between TDCiPP levels and wheeze (>LOQ vs. ≤LOQ OR=2.01,1.02–4.02; p=0.044) overall, and among children without *FLG* mutations (>LOQ vs. ≤LOQ OR=1.94, 0.96–3.97; p=0.066) (Figure 3).

4. Discussion

To our knowledge, this is the first study to take *FLG* mutations into consideration when assessing the effects of exposure to phthalates, PFRs, and mite allergens in house dust on children's eczema and wheeze. The prevalences of eczema and wheeze in this study were similar to those among 7-year-old children in the overall Hokkaido study. The prevalence of *FLG* mutation among this study's participants was 9.2%, similar to the prevalence in the general population (approximately 10%) (Kono et al., 2014; Rodriguez et al., 2009; van den Oord and Sheikh 2009). Due to the role of filaggrin in facilitating terminal differentiation of the epidermis and formation of the skin barrier, other researchers have proposed that loss-of-function *FLG* variants are major predisposing factors for eczema (Debinska et al., 2017) and have reported that *FLG* mutation carriers have significantly higher internal exposure to environmental chemicals than non-carriers (Joensen et al., 2014; Joensen et al., 2017) due to increased transepidermal absorption and/or higher exposure to topical medication (Overgaard et al., 2017). In our study population, however, there was no significant association between *FLG* mutations and ISAAC-defined eczema and/or wheeze.–Levels of DiNP in house dust showed a tendency to decrease the risk of eczema among the children with one or more *FLG* mutations, but increased the risk of eczema among the children without *FLG* mutations. High levels of the PFRs TBOEP and TDCiPP increased the risk of wheeze, especially among children without any *FLG* mutations.

There were no significant differences between concentrations of DEHP, DnBP, and DiNP in this study and our previous studies in different populations (Ait Bamai et al., 2013; Ait Bamai et al., 2014). Levels of phthalates in the present study were similar to or slightly higher than in other previous studies reported from Europe and the USA (Abb et al., 2009; Fromme et al., 2004; Guo and Kannan 2011; Nagorka et al., 2005). Regarding PFRs, concentrations of TBOEP in house dust in this study were higher than in the dust collected from houses of elementary school children in Sapporo (Tajima et al., 2014) and considerably higher than observed in other countries (Ali et al., 2012a; Ali et al., 2012b; Brommer et al., 2012; Dirtu et al., 2012; Van den Eede et al., 2011). However, they were lower than in the dust collected from newly built detached houses in Sapporo and six other Japanese regions (Araki et al., 2014b).

Several studies have reported a potential relationship between phthalates and allergy symptoms in children, including wheeze, asthma, rhinitis, and eczema (Bertelsen et al., 2013; Bornehag et al., 2004; Smit et al., 2015; Whyatt et al., 2014; Callesen et al., 2014; Kolarik et al., 2008). An inverse association of prenatal exposure to DiNP with eczema was found in the INUENDO birth cohort of 1,024 mother-child pairs from Greenland and Ukraine (Smit et al., 2015). In our previous study focusing on inhabitants of newly built detached houses, we reported that higher levels of DiNP in dust were associated with eczema among adult inhabitants (Ait Bamai et al., 2014). However, to our knowledge, there are no other reports regarding the associations between DiNP exposure and eczema and/or wheeze in children. Murine models have shown that intraperitoneally exposed to DiNP aggravated eczema-like skin lesions induced by *Dermatophagoides pteronyssinus* on their ears of atopic-prone NC/Nga mice (Koike et al., 2010); increases in histamine level and decreases in eotaxin and eotaxin-2 expression in ear tissue are considered to be possible mechanisms underlying the aggravation of eczema-like skin lesions by DiNP. Recent murine models have reported that long-term oral exposure to DiNP aggravated allergic-dermatitis-like lesions, indicated by an increase in the number of mast cells, and in increased skin edema in fluorescein isothiocyanate (FITC)-induced contact hypersensitivity (Kang et al., 2016). A possible mechanism of these reactions induced by DiNP

is suggested that DiNP in combination with FITC triggers the activation of nuclear factor-κB, leading to the initiation of the sensitizing process and the exacerbation of allergic dermatitis. However, the mode of intake of DiNP in this study, which the exposure is through dust, may differ from murine models of the intraperitoneal and/or long-term oral exposures.

The previous studies of Bornehag et al. (2004), Kolarik et al. (2008), and Callesen et al. (2014) have reported significant associations between DEHP in dust and asthma (Bornehag et al., 2004; Callesen et al., 2014; Kolarik et al., 2008). However, in this study, despite the high levels of DEHP in dust, we did not observe any significant associations. Median levels of DEHP in children with wheeze were slightly higher than those without; however, the difference was not significant (Supplementary Table 2). In the previous studies, dust samples were collected from the bedroom of the children. Alternatively, in our study, dust samples were collected from the living room. Hence, the observed inconsistency between results of previous studies and our study may be caused by the differences in dust sampling site. Usually at the study age, children sleep for approximately 8-10 hours, while, the time spent in the living room might be less than 8 hours due to school and other activities. Thus, collecting dust from the bedroom seems to show clearer association with symptoms than dust from the living room. Hence, the results in this study may lead to weak associations.

In this study, we showed that high levels of TBOEP (4th quartile) decreased the ORs of eczema among children. TBOEP is commonly used in floor polishes and finishing products for compressed wooden floors (Dirtu et al., 2012). High levels (4th quartile) of TBOEP were associated with the use of compressed wooden flooring (chi-square, $p<0.001$). The use of compressed wooden flooring was higher in the children without eczema (chi-square, $p= 0.022$). Thus, higher TBOEP, wooden flooring type, and lower prevalence of eczema are associated. This could mean that TBOEP and the usage of compressed wooden flooring confers an apparent protective effect from eczema. In addition, parents whose children have allergies may wish to avoid the use of compressed wooden floor containing chemicals such as TBOEP. However, in this cross-sectional study, we could not tell the directions of causality between TBOEP and flooring use, and between eczema and flooring. High levels of TDCiPP

increased the OR of wheeze among children without *FLG* mutations. Our previous report (Araki et al., 2014b) also suggested that TDCiPP in house dust increased the OR of eczema. *In vitro* studies have indicated that TDCiPP and TPhP have an immunocytotoxic effect on the expression of MHCII, which induced a marker for oxidative stress, and suggested that these PFRs have an effect on the immunological health in the respiratory tract (Canbaz et al., 2017). Taken together, epidemiological and *in vitro* studies suggest potential immunotoxic effects of PFRs in humans; however, the mechanisms of PFRs in affecting allergies and immune function are still not well known. Further studies are needed to clarify these mechanisms.

The results of this study did not confirm our hypothesis that phthalates and PFRs in house dust increase the risk of eczema or wheeze among children who have *FLG* mutations carriers. On the contrary, an inverse (OR<1) association was observed between DiNP and eczema among children with *FLG* mutations ($p<0.1$). A possible explanation of the contradictory findings from our hypothesis is that the main exposure routes of DiNP, TDCiPP, and TBOEP may not be via dermal absorption. Relatively high molecular weight SVOCs, such as DHEP, DiNP, TDCiPP, TBOEP, and TEHP, distribute more particle phase than gas phase and they attach to dust (Kanazawa et al., 2010). However, the contributions of daily intake of these SVOCs from dermal exposure through dust adhered to skin are very small compared to gas phase (Beko et al., 2013). Another possible reason for our divergent findings is that because children with one or more *FLG* mutations lead to disruption of stratum corneum formation, they frequently use the emollients which contains low molecular weight phthalates (Overgaard et al., 2017) and resulted in reduce the symptom, but higher urinary metabolite level of low molecular weight phthalates than those without eczema (Overgaard et al., 2017). In fact, the prevalence of eczema among *FLG* mutation carriers in this study decrease from 2 years old (25.9%), 4 years old (25.9%) to 7 years old (18.5%). Furthermore, the ISAAC questionnaire does not allow assessment of the severity of children's eczema or wheeze, resulting in possible misclassification of outcomes. On a related note, severity of a child's allergic symptoms may affect parental attitudes about life style and house cleaning; consequently, this could affect exposure levels in

house dust. On the other hand, in children without any *FLG* mutations, positive (OR>1) associations were observed between DiNP and eczema, and TDCiPP and wheeze, respectively. The potential immunotoxic effects of DiNP have been suggested from an animal study (Koike et la., 2010). Similarly, potential immunotoxic effects of TDCiPP have been reported from an in vitro study (Canbaz et al., 2017). Generally, the children without *FLG* mutations are less likely to have skin problems, and this may warrant laxity in parental attitudes about lifestyle and house cleaning. Subsequently, the associations between DiNP and TDCiPP and eczema and wheeze on children may be clearly shown in the children without *FLG* mutations. However, because this is a cross-sectional study, further longitudinal studies focused on lifestyle and parental behavior are needed to clarify this relationship.

One strength of our study was that we screened for all of the recurrent *FLG* mutations (*3321delA*, *Q1701X*, *S2554X*, *S2889X*, *S3296X*, and *K4022X*) in Japanese populations (Nemoto-Hasebe et al., 2009a; b; Nomura et al., 2008; Nomura et al., 2009; Nomura et al., 2007). This allowed us to perform comprehensive analysis of *FLG* mutations and investigate whether children with *FLG* mutations are a more vulnerable to the exposure to indoor environmental chemicals/factors such as phthalates, PFRs, and mite allergens, resulting in higher risk of eczema and wheeze than those without mutations.

The limitations of this study also need to be considered. First, because of the cross-sectional design, we were unable to discern any causal relationships between indoor environmental chemicals/mite allergens and eczema/wheeze among children. This study design did not allow us to consider covariates such as history of eczema, medical treatments, or use of moisturizer, which could be related to eczema outcome. In addition, although parental allergies seem to affect exposure levels, these were not directly correlated. On the other hand, children with allergies were associated with parental attitude/lifestyle related with phthalates/PFRs, such as reducing use of floor WAX and compressed wooden flooring. Therefore, underestimation of the associations between exposure levels and the risk of eczema may have occurred due to these parental attitudes and lifestyle. However, this is a cross-sectional study, we could not control for the effects of parental attitude/lifestyle on children's

allergies, to address this, further studies should focus on these points, and prospective study designs should be implemented. Moreover, collection of house dust for measurement of indoor environmental chemicals occurred only once, when the children were 7 years old. Eczema and wheeze were assessed using an ISAAC questionnaire, which represents past 12 months allergic symptoms. The timing of dust sampling may not fully cover outcome assessments (past 12 months): 10 children lived in their current house for less than 12 months. However, the sensitivity analysis, of which excluded the children who lived in their current house for less than 12 months, did not change the results. Therefore, we believe this difference in period of dust sampling and outcome assessments did not affect our findings. Second, although ISAAC questionnaire is of international standards and has been validated for defining allergic illness, it cannot assess the severity of eczema and wheeze. Misclassification of outcomes may have occurred because of parental-report-assessments. Third, the parents in this study may have relatively higher interest in their health and home environments. Compared to the original cohort, the participants in this study are have a lower prevalence of maternal smoking, a higher household income, and living in a newer house (Supplemental Table 1). Fourth, false positives or false negatives might have occurred due to the large number of statistical analyses performed. Fifth, the sample size of this study was relatively small, with only 27 children having one or more *FLG* mutations carriers. It is possible that there is inadequate variability in the group with *FLG* mutations to detect an association between exposure and outcome. In addition to this, we were also unable to consider mutation severity or the effect of combined loss-of-function mutations since we defined *FLG* mutation as the presence of at least one mutation. This small sample size also did not allow us to thoroughly perform stratified analyses among children with *FLG* mutations using binominal or quartile of exposure levels in logistic regression models. Moreover, the presented results of the odds ratio may have overestimated the risk because the prevalence rates of both eczema and wheeze in this study are more than 10%. We estimated risk ratios for the associations between phthalates/PFRs and allergies. Due to non-convergence of some analyses and use of mostly stratified analyses, due to limited sample size and low detection frequency of compounds (BBzP, TPP, TEHP, TDCiPP, and

TPhP), we presented the results as odds ratio. However, the differences of the estimates between odds ratio and risk ratio were minute. Thus, the results need to be interpreted with care, and further studies should be performed in larger populations to address these limitations. Lastly, in this study, we only demonstrated associations of house dust levels as exposure assessments. House dust levels represent indoor exposure levels, whereas urinary metabolites of phthalates and PFRs represent personal exposure levels. It is reported that *FLG* mutations may have higher internal exposure to phthalates due to increased transdermal absorption (Joensen et al., 2014; Joensen et al., 2017). Thus, we should investigate the associations of *FLG* mutations with urinary phthalates/PFRs metabolites and wheeze and eczema as our further studies.

5. Conclusions

The present study demonstrated that children without loss-of-function gene mutations for the epidermal barrier protein filaggrin showed clearer effects of indoor environmental factors on childhood eczema and wheeze. Among children without any *FLG* mutations, TBOEP decreased the OR of eczema; DiNP and TDCiPP increased the ORs of eczema and wheeze, respectively. Children with one or more *FLG* mutations might already be receiving different care in terms of medication, skin protection, and/or parental lifestyle, and this might affect how indoor environmental factors relate to their allergy symptoms. Our limited sample size produced several borderline associations and prevented us from considering *FLG* mutations in greater depth. Further studies with larger sample sizes and with urinary phthalates/PFRs metabolites are required to elucidate these points.

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Competing interests

The authors have no competing interests to declare.

References

Abb, M.; Heinrich, T.; Sorkau, E.; Lorenz, W. Phthalates in house dust. Environ Int 2009;35:965-970

Ait Bamai, Y.; Araki, A.; Kawai, T.; Tsuboi, T.; Saito, I.; Yoshioka, E.; Kanazawa, A.; Tajima, S.; Shi, C.; Tamakoshi, A.; Kishi, R. Associations of phthalate concentrations in floor dust and multi-surface dust with the interior materials in Japanese dwellings. Sci Total Environ 2013;468-469C:147-157

Ait Bamai, Y.; Araki, A.; Kawai, T.; Tsuboi, T.; Yoshioka, E.; Kanazawa, A.; Cong, S.; Kishi, R. Comparisons of urinary phthalate metabolites and daily phthalate intakes among Japanese families. Int J Hyg Environ Health 2015;218:461-470

Ait Bamai, Y.; Shibata, E.; Saito, I.; Araki, A.; Kanazawa, A.; Morimoto, K.; Nakayama, K.; Tanaka, M.; Takigawa, T.; Yoshimura, T.; Chikara, H.; Saijo, Y.; Kishi, R. Exposure to house dust phthalates in relation to asthma and allergies in both children and adults. Sci Total Environ 2014;485-486C:153-163

Ali, N.; Dirtu, A.C.; Van den Eede, N.; Goosey, E.; Harrad, S.; Neels, H.; 't Mannetje, A.; Coakley, J.; Douwes, J.; Covaci, A. Occurrence of alternative flame retardants in indoor dust from New Zealand: indoor sources and human exposure assessment. Chemosphere 2012a;88:1276-1282

Ali, N.; Van den Eede, N.; Dirtu, A.C.; Neels, H.; Covaci, A. Assessment of human exposure to indoor organic contaminants via dust ingestion in Pakistan. Indoor Air 2012b;22:200-211

Araki, A.; Bamai, Y.A.; Kishi, R. [Epidemiological studies on the environmental pollutants and allergies: focused on indoor air quality]. Arerugi 2014a;63:1075-1083; quiz 1084

Araki, A.; Saito, I.; Kanazawa, A.; Morimoto, K.; Nakayama, K.; Shibata, E.; Tanaka, M.; Takigawa, T.; Yoshimura, T.; Chikara, H.; Saijo, Y.; Kishi, R. Phosphorus flame retardants in indoor dust and their relation to asthma and allergies of inhabitants. Indoor Air 2014b;24:3-15

Beko, G.; Weschler, C.J.; Langer, S.; Callesen, M.; Toftum, J.; Clausen, G. Children's Phthalate Intakes and Resultant Cumulative Exposures Estimated from Urine Compared with Estimates from Dust Ingestion, Inhalation and Dermal Absorption in Their Homes and Daycare Centers. PLoS One 2013;8:e62442

Bertelsen, R.J.; Carlsen, K.C.; Calafat, A.M.; Hoppin, J.A.; Håland, G.; Mowinckel, P.; Carlsen, K.H.; Lovik, M. Urinary biomarkers for phthalates associated with asthma in Norwegian children. Environ Health Perspect 2013;121:251-256

Bisgaard, H.; Simpson, A.; Palmer, C.N.; Bønnelykke, K.; McLean, I.; Mukhopadhyay, S.; Pipper, C.B.; Halkjaer, L.B.; Lipworth, B.; Hankinson, J.; Woodcock, A.; Custovic, A. Gene-environment interaction in the onset of eczema in infancy: filaggrin loss-of-function mutations enhanced by neonatal cat exposure. PLoS Med 2008;5:e131

Bornehag, C.G.; Sundell, J.; Weschler, C.J.; Sigsgaard, T.; Lundgren, B.; Hasselgren, M.; Hägerhed-Engman, L. The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case-control study. Environ Health Perspect 2004;112:1393-1397

Brommer, S.; Harrad, S.; Van den Eede, N.; Covaci, A. Concentrations of organophosphate esters and brominated flame retardants in German indoor dust samples. J Environ Monit 2012;14:2482-2487

Callesen, M.; Beko, G.; Weschler, C.J.; Sigsgaard, T.; Jensen, T.K.; Clausen, G.; Toftum, J.; Norberg, L.A.; Host, A. Associations between selected allergens, phthalates, nicotine, polycyclic aromatic

hydrocarbons, and bedroom ventilation and clinically confirmed asthma, rhinoconjunctivitis, and atopic dermatitis in preschool children. Indoor Air 2014;24:136-147

Canbaz, D.; Logiantara, A.; van Ree, R.; van Rijt, L.S. Immunotoxicity of organophosphate flame retardants TPHP and TDCIPP on murine dendritic cells in vitro. Chemosphere 2017;177:56-64

Candi, E.; Schmidt, R.; Melino, G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 2005;6:328-340

Cao, J.; Weschler, C.J.; Luo, J.; Zhang, Y. C(m)-history method, a novel approach to simultaneously measure source and sink parameters important for estimating indoor exposures to phthalates. Environ Sci Technol 2016;50:825-834

Cao, J.; Zhang, X.; Zhang, Y. Predicting dermal exposure to gas-phase semi-volatile organic compounds (SVOCs): a further study of SVOC mass transfer between clothing and skin surface lipids. Environ Sci Technol 2018;52:4676-4683

Chan, A.; Terry, W.; Zhang, H.; Karmaus, W.; Ewart, S.; Holloway, J.W.; Roberts, G.; Kurukulaaratchy, R.; Arshad, S.H. Filaggrin mutations increase allergic airway disease in childhood and adolescence through interactions with eczema and aeroallergen sensitization. Clin Exp Allergy 2018;48:147-155

International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 1998;351:1225-1232

Debinska, A.; Danielewicz, H.; Drabik-Chamerska, A.; Kalita, D.; Boznanski, A. Filaggrin loss-of-function mutations as a predictor for atopic eczema, allergic sensitization and eczema-associated asthma in Polish children population. Adv Clin Exp Med 2017;26:991-998

Dirtu, A.C.; Ali, N.; Van den Eede, N.; Neels, H.; Covaci, A. Country specific comparison for profile of chlorinated, brominated and phosphate organic contaminants in indoor dust. Case study for Eastern Romania, 2010. Environ Int 2012;49:1-8

Fromme, H.; Lahrz, T.; Piloty, M.; Gebhart, H.; Oddoy, A.; Rüden, H. Occurrence of phthalates and musk fragrances in indoor air and dust from apartments and kindergartens in Berlin (Germany). Indoor Air 2004;14:188-195

Guo, Y.; Kannan, K. Comparative assessment of human exposure to phthalate esters from house dust in China and the United States. Environ Sci Technol 2011;45:3788-3794

Joensen, U.N.; Jorgensen, N.; Meldgaard, M.; Frederiksen, H.; Andersson, A.M.; Menne, T.; Johansen, J.D.; Carlsen, B.C.; Stender, S.; Szecsi, P.B.; Skakkebaek, N.E.; Rajpert-De Meyts, E.; Thyssen, J.P. Associations of filaggrin gene loss-of-function variants with urinary phthalate metabolites and testicular function in young Danish Men. Environ Health Perspect 2014;122:345-350

Joensen, U.N.; Jorgensen, N.; Thyssen, J.P.; Petersen, J.H.; Szecsi, P.B.; Stender, S.; Andersson, A.M.; Skakkebaek, N.E.; Frederiksen, H. Exposure to phenols, parabens and UV filters: Associations with loss-of-function mutations in the filaggrin gene in men from the general population. Environ Int 2017;105:105-111

Kanazawa, A.; Saito, I.; Araki, A.; Takeda, M.; Ma, M.; Saijo, Y.; Kishi, R. Association between indoor

exposure to semi-volatile organic compounds and building-related symptoms among the occupants of residential dwellings. Indoor Air 2010;20:72-84

Kang, J.; Song, J.; Shen, S.; Li, B.; Yang, X.; Chen, M. Diisononyl phthalate aggravates allergic dermatitis by activation of NF-kB. Oncotarget 2016;7:85472-85482

Kishi, R.; Araki, A.; Minatoya, M.; Hanaoka, T.; Miyashita, C.; Itoh, S.; Kobayashi, S.; Ait Bamai, Y.; Yamazaki, K.; Miura, R.; Tamura, N.; Ito, K.; Goudarzi, H. The Hokkaido Birth Cohort Study on Environment and Children's Health: cohort profile—updated 2017. Environmental health and preventive medicine 2017;22:46

Koike, E.; Yanagisawa, R.; Sadakane, K.; Inoue, K.; Ichinose, T.; Takano, H. Effects of diisononyl phthalate on atopic dermatitis in vivo and immunologic responses in vitro. Environ Health Perspect 2010;118:472-478

Kolarik, B.; Naydenov, K.; Larsson, M.; Bornehag, C.G.; Sundell, J. The association between phthalates in dust and allergic diseases among Bulgarian children. Environ Health Perspect 2008;116:98-103

Kono, M.; Nomura, T.; Ohguchi, Y.; Mizuno, O.; Suzuki, S.; Tsujiuchi, H.; Hamajima, N.; McLean, W.H.; Shimizu, H.; Akiyama, M. Comprehensive screening for a complete set of Japanese-population-specific filaggrin gene mutations. Allergy 2014;69:537-540

Nagorka, R.; Scheller, C.; Ullrich, D. Plasticizer in house dust. Gefahrst Reinhalt Luft 2005;65:99-105

Nemoto-Hasebe, I.; Akiyama, M.; Nomura, T.; Sandilands, A.; McLean, W.H.; Shimizu, H. Clinical severity correlates with impaired barrier in filaggrin-related eczema. J Invest Dermatol 2009a;129:682-689

Nemoto-Hasebe, I.; Akiyama, M.; Nomura, T.; Sandilands, A.; McLean, W.H.; Shimizu, H. FLG mutation p.Lys4021X in the C-terminal imperfect filaggrin repeat in Japanese patients with atopic eczema. Br J Dermatol 2009b;161:1387-1390

Li, M.C.; Chen, C.H.; Guo, Y.L. Phthalate esters and childhood asthma: A systematic review and congener-specific meta-analysis. Environ Pollut 2017;229:655-660

Nomura, T.; Akiyama, M.; Sandilands, A.; Nemoto-Hasebe, I.; Sakai, K.; Nagasaki, A.; Ota, M.; Hata, H.; Evans, A.T.; Palmer, C.N.; Shimizu, H.; McLean, W.H. Specific filaggrin mutations cause ichthyosis vulgaris and are significantly associated with atopic dermatitis in Japan. J Invest Dermatol 2008;128:1436-1441

Nomura, T.; Akiyama, M.; Sandilands, A.; Nemoto-Hasebe, I.; Sakai, K.; Nagasaki, A.; Palmer, C.N.; Smith, F.J.; McLean, W.H.; Shimizu, H. Prevalent and rare mutations in the gene encoding filaggrin in Japanese patients with ichthyosis vulgaris and atopic dermatitis. J Invest Dermatol 2009;129:1302-1305

Nomura, T.; Sandilands, A.; Akiyama, M.; Liao, H.; Evans, A.T.; Sakai, K.; Ota, M.; Sugiura, H.; Yamamoto, K.; Sato, H.; Palmer, C.N.; Smith, F.J.; McLean, W.H.; Shimizu, H. Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. J Allergy Clin Immunol 2007;119:434-440

Oddy, W.H.; Holt, P.G.; Sly, P.D.; Read, A.W.; Landau, L.I.; Stanley, F.J.; Kendall, G.E.; Burton, P.R. Association between breast feeding and asthma in 6 year old children: findings of a prospective birth cohort study. BMJ 1999;319:815-819

Overgaard, L.E.K.; Main, K.M.; Frederiksen, H.; Stender, S.; Szecsi, P.B.; Williams, H.C.; Thyssen, J.P. Children with atopic dermatitis and frequent emollient use have increased urinary levels of low-molecular-weight phthalate metabolites and parabens. Allergy 2017;72:1768-1777

Palmer, C.N.; Irvine, A.D.; Terron-Kwiatkowski, A.; Zhao, Y.; Liao, H.; Lee, S.P.; Goudie, D.R.; Sandilands, A.; Campbell, L.E.; Smith, F.J.; O'Regan, G.M.; Watson, R.M.; Cecil, J.E.; Bale, S.J.; Compton, J.G.; DiGiovanna, J.J.; Fleckman, P.; Lewis-Jones, S.; Arseculeratne, G.; Sergeant, A.; Munro, C.S.; El Houate, B.; McElreavey, K.; Halkjaer, L.B.; Bisgaard, H.; Mukhopadhyay, S.; McLean, W.H. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 2006;38:441-446

Pelletier, M.; Bonvallot, N.; Ramalho, O.; Blanchard, O.; Mercier, F.; Mandin, C.; Le Bot, B.; Glorennec, P. Dermal absorption of semivolatile organic compounds from the gas phase: Sensitivity of exposure assessment by steady state modeling to key parameters. Environ Int 2017;102:106-113

Rodriguez, E.; Baurecht, H.; Herberich, E.; Wagenpfeil, S.; Brown, S.J.; Cordell, H.J.; Irvine, A.D.; Weidinger, S. Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. J Allergy Clin Immunol 2009;123:1361-1370.e7

Saijo, Y.; Kanazawa, A.; Araki, A.; Morimoto, K.; Nakayama, K.; Takigawa, T.; Tanaka, M.; Shibata, E.; Yoshimura, T.; Chikara, H.; Kishi, R. Relationships between mite allergen levels, mold concentrations, and sick building syndrome symptoms in newly built dwellings in Japan. Indoor Air 2011;21:253-263

Sandilands, A.; Terron-Kwiatkowski, A.; Hull, P.R.; O'Regan, G.M.; Clayton, T.H.; Watson, R.M.; Carrick, T.; Evans, A.T.; Liao, H.; Zhao, Y.; Campbell, L.E.; Schmuth, M.; Gruber, R.; Janecke, A.R.; Elias, P.M.; van Steensel, M.A.; Nagtzaam, I.; van Geel, M.; Steijlen, P.M.; Munro, C.S.; Bradley, D.G.; Palmer, C.N.; Smith, F.J.; McLean, W.H.; Irvine, A.D. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. Nat Genet 2007;39:650-654

Schuttelaar, M.L.; Kerkhof, M.; Jonkman, M.F.; Koppelman, G.H.; Brunekreef, B.; de Jongste, J.C.; Wijga, A.; McLean, W.H.; Postma, D.S. Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction with cat exposure. Allergy 2009;64:1758-1765

Sears, M.R.; Greene, J.M.; Willan, A.R.; Taylor, D.R.; Flannery, E.M.; Cowan, J.O.; Herbison, G.P.; Poulton, R. Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. Lancet 2002;360:901-907

Smit, L.A.; Lenters, V.; Høyer, B.B.; Lindh, C.H.; Pedersen, H.S.; Liermontova, I.; Jönsson, B.A.; Piersma, A.H.; Bonde, J.P.; Toft, G.; Vermeulen, R.; Heederik, D. Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. Allergy 2015;70:653-660

Tajima, S.; Araki, A.; Kawai, T.; Tsuboi, T.; Ait Bamai, Y.; Yoshioka, E.; Kanazawa, A.; Cong, S.; Kishi, R. Detection and intake assessment of organophosphate flame retardants in house dust in Japanese dwellings. Sci Total Environ 2014;478:190-199

Tanaka, M.; Inoue, K.; Momoi, T.; Takano, H. In vivo immunoamplifying effects of di-(2-ethylhexyl) phthalate on cytokine response. Immunopharmacol Immunotoxicol 2013;35:147-150

U.S.EPA. Child-Specific Exposure Factors Handbook. in: Development O.o.R.a., ed; 2002

Van den Eede, N.; Dirtu, A.C.; Neels, H.; Covaci, A. Analytical developments and preliminary assessment of human exposure to organophosphate flame retardants from indoor dust. Environ Int 2011;37:454-461

van den Oord, R.A.; Sheikh, A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. BMJ 2009;339:b2433

Whyatt, R.M.; Perzanowski, M.S.; Just, A.C.; Rundle, A.G.; Donohue, K.M.; Calafat, A.M.; Hoepner, L.A.; Perera, F.P.; Miller, R.L. Asthma in inner-city children at 5-11 years of age and prenatal exposure to phthalates: the Columbia Center for Children's Environmental Health Cohort. Environ Health Perspect 2014;122:1141-1146

Table 1

Characteristics of children

		Total		With eczema		Without eczema			With wheeze		Without wheeze		
		N	%	n	%	n	%		n	%	n	%	
Sex	Boy	148	50.0	37	25.0	111	75.0	†	27	18.2	121	81.8	*
	Girl	148	50.0	24	16.2	124	83.8		14	9.5	134	90.5	
FLG mutation	Yes	27	9.1	5	18.5	22	81.5		3	7.3	24	9.4	
	No	269	90.9	56	20.8	213	79.2		38	92.7	231	90.6	
Duration of breast feeding	Mean, SD	15.1,	7.6	14.0,	1.1	15.4,	0.5		14.8,	6.2	15.2,	7.8	
Maternal tobacco smoking	Yes	28	9.5	9	32.1	19	67.9		5	12.2	23	9.0	
	No	268	90.5	52	19.4	216	80.6		36	87.8	232	91.0	
Paternal tobacco smoking	Yes	44	14.9	8	18.2	36	81.8		5	12.2	39	15.3	
	No	245	82.8	52	21.2	193	78.8		35	85.4	210	82.4	
	Missing	7	2.4	1	14.3	6	85.7		1	2.4	6	2.4	
ETS at home	Yes	104	35.1	23	22.1	81	77.9		17	41.5	87	34.1	
	No	192	64.9	38	19.8	154	80.2		24	58.5	168	65.9	
Maternal history of allergies	Yes	173	58.4	38	22.0	135	78.0		30	73.2	143	56.1	*
	No	123	41.6	23	18.7	100	81.3		11	26.8	112	43.9	
Paternal history of allergies	Yes	140	47.3	35	25.0	105	75.0	†	22	53.7	118	46.3	
	No	156	52.7	26	16.7	130	83.3		19	46.3	137	53.7	
Maternal history of eczema	Yes	44	14.9	19	43.2	25	56.8	***	8	19.5	36	14.1	
	No	252	85.1	42	16.7	210	83.3		33	80.5	219	85.9	
Paternal history of eczema	Yes	33	11.5	14	42.4	19	57.6	**	7	17.1	26	10.2	
	No	263	88.5	47	17.9	216	82.1		34	82.9	229	89.8	
Household income	< 3 million yen	32	10.8	8	25.0	24	75.0		7	17.1	25	9.8	
	>= 3million yen	250	84.5	52	20.8	198	79.2		33	80.5	217	85.1	
	Missing	14	4.7	1	7.1	13	92.9		1	2.4	13	5.1	
Pet keeping	Yes	65	22	10	15.4	55	84.6		10	24.4	55	21.6	
	No	229	77.4	50	21.8	179	78.2		31	75.6	198	77.7	
	Missing	2	0.7	1	50.0	1	50.0		0	0.0	2	0.8	
Wall-to-wall carpet	Yes	164	55.4	34	20.7	130	79.3		23	56.1	141	55.3	
	No	131	44.3	27	20.6	104	79.4		18	43.9	113	44.3	
	Missing	1	0.3	0	0.0	1	100.0		0	0.0	1	0.4	
Visible mold	Yes	165	55.7	36	21.8	129	78.2		21	51.2	144	56.5	
	No	131	44.3	25	19.1	106	80.9		20	48.8	111	43.5	
Moldy odor	Yes	15	5.1	5	33.3	10	66.7		2	4.9	13	5.1	
	No	281	94.9	56	19.9	225	80.1		39	95.1	242	94.9	
Water leakage	Yes	45	15.2	10	22.2	35	77.8		5	12.2	40	15.7	
	No	251	84.8	51	20.3	200	79.7		36	87.8	215	84.3	
Condensation	Yes	172	58.1	33	19.2	139	80.8		20	48.8	152	59.6	
	No	124	41.9	28	22.6	96	77.4		21	51.2	103	40.4	

X2-test; †: p< 0.1; *: p< 0.05; **: p< 0.01; ***: p< 0.001

ETS: environmental tobacco smoke

Table 2

Distribution of Phthalates, PFRs, and mite allergen in house dust (ug/g dust)

	LOQ	>LOQ%	Min	25%	Median	75%	Max
Phthalates							
DMP	0.28	24.4	<LOQ	<LOQ	<LOQ	0.42	11.77
DEP	0.68	23.5	<LOQ	<LOQ	<LOQ	0.71	15.86
DiBP	0.31	79.0	0.67	2.08	4.50	8.30	158.80
DnBP	0.46	99.5	2.22	26.66	47.45	89.35	1,084.23
BBzP	0.15	95.2	<LOQ	0.38	1.31	3.73	134.69
DEHP	0.57	99.8	<LOQ	940.94	1,350.26	2,254.32	21,849.03
DiNP	0.66	100.0	2.03	30.72	63.91	152.50	3,264.94
DEHA	0.74	44.3	<LOQ	4.41	9.75	20.04	1,670.19
PFRs							
TMP	0.4	8.1	<LOQ	<LOQ	<LOQ	<LOQ	13.37
TEP	0.13	43.9	<LOQ	<LOQ	<LOQ	0.30	11.94
TPP	0.18	89.5	<LOQ	0.31	0.47	0.80	11.19
TBP	0.26	41.6	<LOQ	<LOQ	<LOQ	0.75	36.21
TCIP	0.20	92.9	<LOQ	0.42	0.85	2.05	68.60
TCEP	0.25	85.8	<LOQ	0.36	0.79	1.78	756.08
TEHP	0.18	89.9	<LOQ	0.30	0.45	0.80	15.93
TBEP	0.15	87.8	<LOQ	11.71	43.18	125.13	1,507.90
TDCPP	0.28	50.5	<LOQ	<LOQ	<LOQ	0.82	504.23
TPhP	0.52	52.6	<LOQ	<LOQ	<LOQ	0.90	13.27
TCP	0.23	60.8	<LOQ	<LOQ	0.35	0.86	28.05
Mite allergen							
Der1	0.1	98.2	<LOQ	0.86	2.63	7.14	90.1

LOQ: limit of qualification

Table 3

Associations between eczema and phthalates, PFRs, and mite allergens in house dust

	Total			*p for gene-environment interaction*	With *FLG* mutation			Without *FLG* mutation		
	OR[#]	95% CI			OR[$]	95% CI		OR[$]	95% CI	
DiBP	0.98	0.72	1.35	*n.s.*	0.56	0.13	2.46	1.06	0.76	1.49
DnBP	1.04	0.77	1.41	*n.s*	0.63	0.13	3.09	1.08	0.78	1.48
BBzP	1.05	0.88	1.25	*n.s*	2.19	0.93	5.16†	1.00	0.82	1.21
DEHP	0.92	0.67	1.27	*n.s*	0.32	0.05	2.10	1.03	0.74	1.41
DiNP	1.17	0.91	1.52	*	0.27	0.06	1.28†	1.29	0.98	1.69†
TPP	1.04	0.76	1.41	*n.s*	0.86	0.37	1.97	1.01	0.72	1.42
TCIP	0.88	0.69	1.11	*n.s*	1.77	0.56	5.61	0.84	0.65	1.09
TCEP	0.92	0.74	1.14	*n.s*	1.21	0.50	2.96	0.93	0.74	1.16
TEHP	0.85	0.62	1.17	*n.s*	0.73	0.25	2.09	0.85	0.61	1.18
TBEP	0.91	0.81	1.02†	*n.s*	0.90	0.63	1.29	0.92	0.81	1.04
TDCPP	1.10	0.92	1.32	*n.s*	1.51	0.68	3.32	1.10	0.91	1.33
TPhP	0.93	0.64	1.34	*n.s*	1.14	0.41	3.13	0.89	0.59	1.35
Der1	1.04	0.85	1.27	†	0.60	0.23	1.55	1.10	0.89	1.36

The odds ratios were calculated using loge-transformed variables.

†: p< 0.1; *: p< 0.05; *n.s.*: not significant.

#: Adjusted for sex, household income, maternal smoking, parental history of atopy, and FLG mutation.

$: Adjusted for sex, household income, maternal smoking, and parental history of atopy.

Table 4

Associations between wheeze and phthalates, PFRs, and mite allergens in house dust

	Total			*p for gene-environment interaction*	**With *FLG* mutation**			**Without *FLG* mutation**		
	OR[#]	95% CI			OR[$]	95% CI		OR[$]	95% CI	
DiBP	1.15	0.81	1.63	*n.s.*	3.19	0.49	20.64	1.09	0.75	1.58
DnBP	1.00	0.70	1.44	*n.s.*	1.29	0.14	11.58	1.00	0.70	1.44
BBzP	1.06	0.86	1.31	*n.s.*	0.57	0.20	1.61	1.08	0.87	1.34
DEHP	1.18	0.79	1.76	*n.s.*	-	-	-	1.11	0.75	1.63
DiNP	0.93	0.69	1.27	*n.s.*	1.17	0.19	7.00	0.91	0.66	1.24
TPP	1.06	0.75	1.49	*n.s.*	0.76	0.19	2.98	1.11	0.77	1.59
TCIP	0.97	0.73	1.27	*n.s.*	-	-	-	0.93	0.70	1.23
TCEP	0.94	0.73	1.20	*n.s.*	1.57	0.35	7.03	0.92	0.71	1.18
TEHP	0.99	0.69	1.41	*n.s.*	1.61	0.41	6.39	0.94	0.65	1.37
TBEP	1.10	0.94	1.27	*n.s.*	1.09	0.64	1.85	1.11	0.95	1.31
TDCPP	1.26	1.04	1.52*	*n.s.*	1.57	0.55	4.55	1.22	1.00	1.48*
TPhP	1.26	0.83	1.90	*n.s.*	1.56	0.42	5.76	1.20	0.77	1.89
Der1	1.00	0.80	1.26	*n.s.*	0.71	0.18	2.85	1.05	0.83	1.33

The odds ratios were calculated using loge-transformed variables.

*: p< 0.05; -: statistical analyses did not converge.; n.s.: not significant.

#: Adjusted for sex, household income, maternal smoking, parental history of allergies, and *FLG* mutation.

$: Adjusted for sex, household income, maternal smoking, and parental history of allergies.

Supplemental Table 1.

	Children in original cohort (n=2697)		Children included in this study (n=296)	
	n	%	n	%
Wheeze	357	13.2	41	13.8
Eczema	522	19.4	61	20.5
Rhino-conjunctivitis	333	12.3	35	11.8
Maternal smoking	397	14.7	28	9.4
Paternal smoking	1139	42.2	124	42.9
Environmental tobacco smoke	1034	38.4	104	35.0
Household income (< 3 million yen/year)	333	13.3	32	11.3
Maternal history of allergies	1514	56.1	173	58.4
Paternal history of allergies	1174	43.5	140	47.3
Maternal history of eczema	408	15.1	44	14.9
Paternal history of eczema	265	9.8	33	11.1
Wall-to-wall carpet	1497	55.7	164	55.2
Pet keeping	596	22.2	65	21.9
*Building characteristics**				
Detached house	727	72.8	215	72.6
Wooden house	755	75.7	222	75.0
Building age (year; mean,SD)	15.0	12.0	7.6	5.8
Renovation	40	4.0	7	2.3
Visible mold	505	50.6	165	55.7
Moldy odor	66	6.6	15	5.1
Water leakage	156	15.7	45	15.2
Condensation	594	59.8	172	58.1

Supplementary Table 2

Associations between phthalates, PFRS, and mite allergen and eczema, wheeze, and *FLG* mutations

	With eczema		Without eczema		With wheeze		Without wheeze		With *FLG* mutations		Without *FLG* mutations	
	Median	(IQR)	Median	(IQR)	Median	(IQR)	Median	(IQR)	Median	(IQR)	Median	(IQR)
Phthalates												
DiBP	5.17	(2.09, 9.57)	4.39	(2.08, 8.06)	5.76	(2.94, 9.25)	4.26	(2.02, 8.06)	5.06	(1.98, 7.33)	4.45	(2.1, 8.48)
DnBP	41.32	(24.33, 102.92)	48.07	(27.41, 88.31)	54.04	(26.98, 102.3)	46.48	(26.39, 89.35)	33.59	(19.12, 65.8)	48.83	(27.18, 92.11)*
BBzP	1.16	(0.43, 5.34)	1.34	(0.38, 3.73)	1.88	(0.68, 4.17)	1.25	(0.36, 3.71)	1.04	(0.31, 5.71)	1.36	(0.4, 3.72)

DEHP	1319	(882, 2016)	1365	(941.7, 2312)	1524	(942.9, 2231)	1336	(935.9, 2292)	1123	(913.1, 1777)	1374	(942.2, 2309)
DINP	82.77	(32.81, 205.08)	61.61	(30.38, 128.76)	73.45	(26.98, 134.9)	63.8	(31.85, 154.0)	76.01	(26.85, 165)	63.8	(31.36, 151.7)
PFRs												
TPP	0.48	(0.35, 0.85)	0.47	(0.3, 0.8)	0.53	(0.34, 1.01)	0.46	(0.3, 0.75)	0.5	(0.09, 0.64)	0.47	(0.33, 0.82)
TCiPP	0.74	(0.38, 2.77)	0.9	(0.43, 1.89)	1.04	(0.37, 1.82)	0.82	(0.42, 2.12)	0.74	(0.32, 1.52)	0.86	(0.42, 2.2)
TCEP	0.67	(0.33, 2.06)	0.81	(0.37, 1.74)	0.62	(0.24, 1.63)	0.79	(0.38, 1.84)	0.68	(0.32, 2.33)	0.79	(0.37, 1.73)
TEHP	0.44	(0.31, 0.72)	0.46	(0.3, 0.81)	0.5	(0.39, 0.67)	0.45	(0.3, 0.81)	0.32	(0.22, 0.56)	0.47	(0.32, 0.81)†
TBOEP	23.13	(7.5, 69.88)	49.09	(13.13, 144.2)*	46.83	(13.11, 204.8)	42.77	(10.07, 117.8)	24.57	(0.08, 90.59)	46.61	(12.48, 137.2)†
TDCiPP	<LOQ	(<LOQ, 1.09)	<LOQ	(<LOQ, 0.77)	<LOQ	(0.32, 1.1)	<LOQ	(<LOQ, 0.75)†	0.14	(0.14, 0.89)	0.14	(0.14, 0.82)
TPhP	<LOQ	(<LOQ, 0.75)	<LOQ	(<LOQ, 0.92)	<LOQ	(<LOQ, 0.93)	<LOQ	(<LOQ, 0.89)	0.26	(0.26, 0.8)	0.26	(0.26, 0.91)
Mite allergen												
Der1	11.615	(0.77, 3.03)	6.8	(0.88, 2.59)	2.025	(0.99, 8.34)	2.725	(0.85, 7.08)	0.455	(1.44, 3.99)	0.905	(2.73, 7.69)

Mann-Whitney test

†: p< 0.1; *: p< 0.05

Figure legends

Figure 1. DiNP in house dust and eczema

The ORs and 95% CIs of the prevalence of eczema are shown as black squares and whiskers, respectively for (a) DiNP and eczema among total children, (b) DiNP and eczema among children with and without any of FLG gene mutations.

The levels of DiNP in house dust were categorized as in quartiles as quartile (Q) 1 ≤ 30.67, 30.67 < Q2 ≤ 63.80, 63.80 < Q3≤ 150.9, Q4 > 150.9 (μg/g dust). The OR was calculated for 1 st quartile as a reference, and adjusted for sex, household income, maternal smoking, parental history of atopy, and FLG gene mutations. The stratified model was adjusted for sex, household income, maternal smoking, and parental history of atopy.

DiNP, di-iso-nonyl phthalate; OR, odds ratio; CI, confidence interval

Figure 2. TBOEP in house dust and eczema

The ORs and 95% confidence intervals of the prevalence of eczema are shown as black squares and whiskers, respectively for (a) TBOEP and eczema among total children, (b) TBOEP and eczema among children without any of FLG gene mutations.

The levels of TBOEP in house dust were categorized in quartiles as Q 1 ≤ LOQ, 0.075 < Q2 ≤ 24.42, 24.42 < Q3 ≤ 100.93, Q4 > 100.93 (μg/g dust). LOQ for TBOEP was 0.15 μg/g dust.

The ORs were calculated for the 1 st quartile as reference, and adjusted for sex, household income, maternal smoking, parental history of atopy, and *FLG* gene mutations. The stratified model was adjusted for sex, household income, maternal smoking, and parental history of atopy.

TBOEP, tris(2-butoxyethyl) phosphate; LOQ, limit of quantification.

Figure 3. TDCiPP in house dust and wheeze

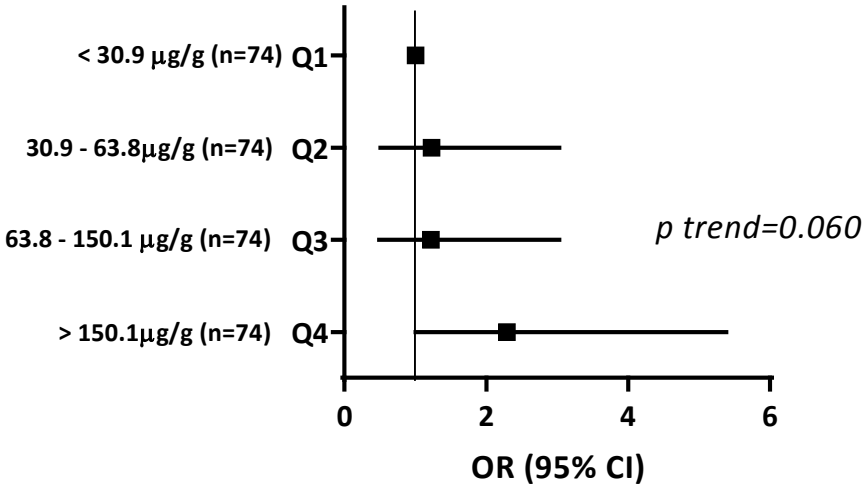
The ORs and 95% confidence intervals of the prevalence of eczema are shown as black squares and whiskers, respectively for (a) TDCIPP and wheeze among total children, (b) TDCIPP and wheeze among children without any of FLG gene mutations.

The levels of TDCIPP in house dust were categorized as either undetected (< LOQ) or detected (>= LOQ). LOQ for TDCIPP was 0.28 μg/g dust.

The ORs were calculated for the undetected levels as reference, and adjusted for sex, household income, maternal smoking, parental history of allergies, and *FLG* gene mutations. The stratified model was adjusted for sex, household income, maternal smoking, and parental history of allergies.

TDCIPP, tris(1,3-dichloro-2-propyl) phosphate; LOQ, limit of quantification.

(a) DiNP and eczema among total children



(b) DiNP and eczema among children with and w/o *FLG* mutation

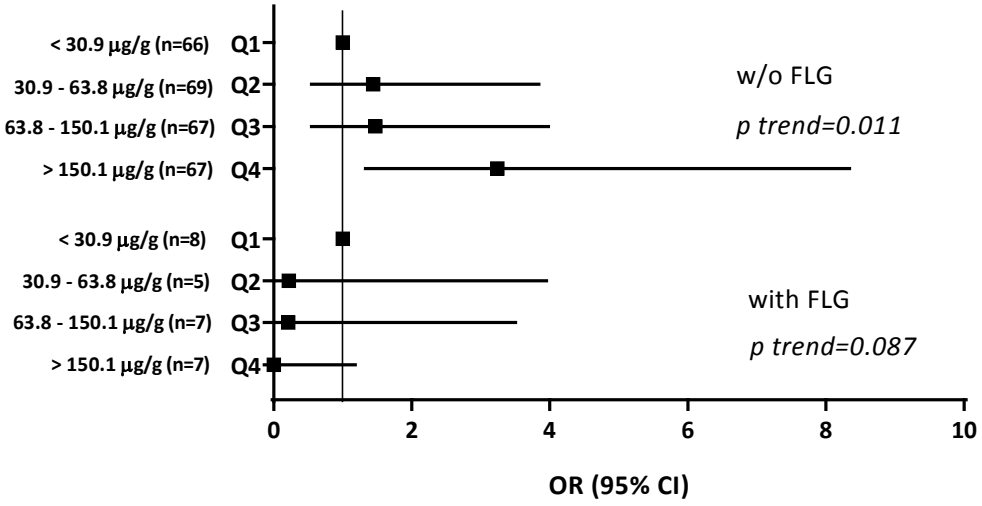
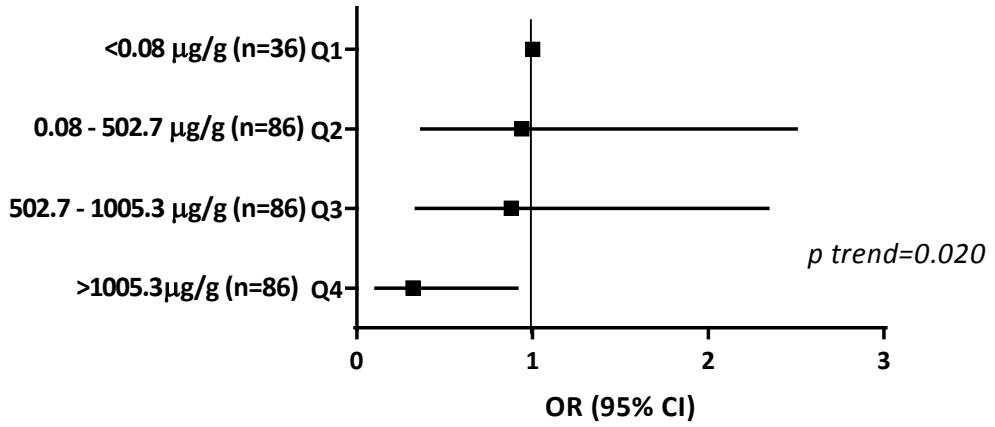


Figure 1. DiNP in house dust and eczema

(a) TBOEP and eczema among total children



(b) TBOEP and eczema among children w/o FLG

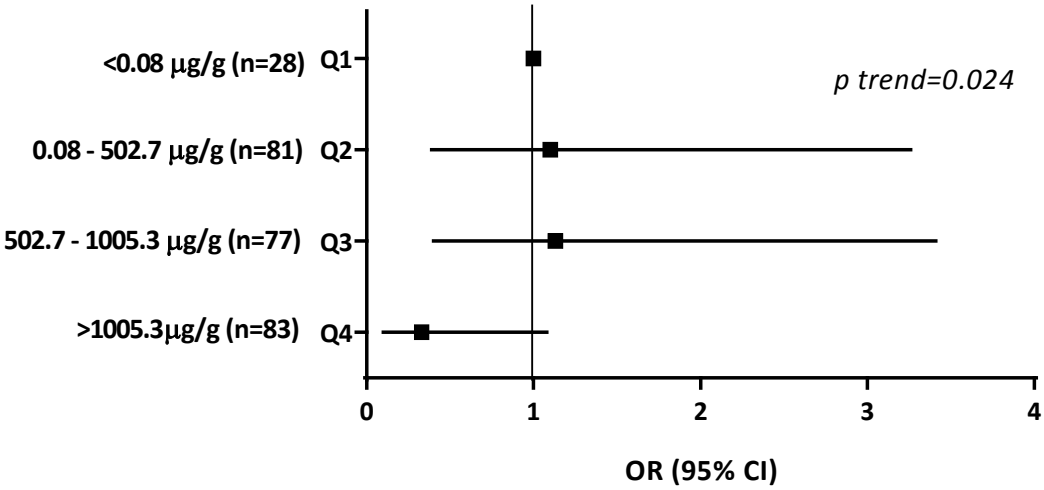
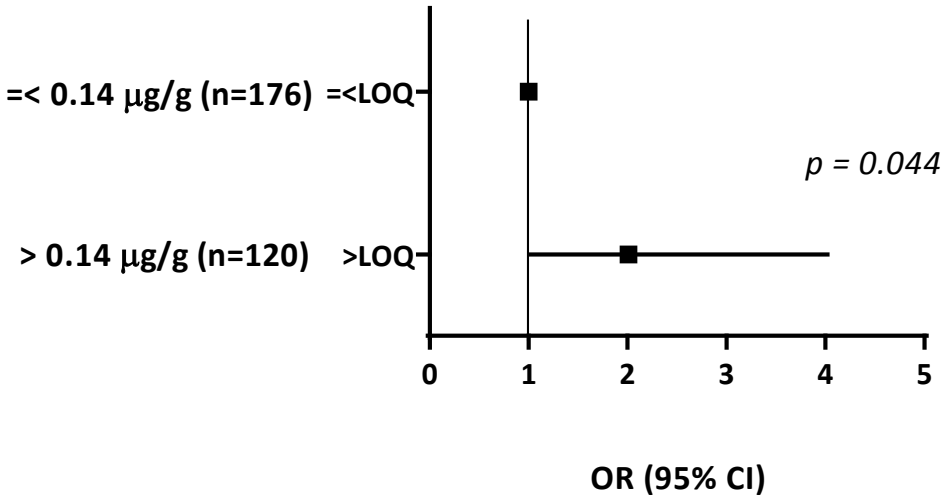


Figure 2. TBOEP in house dust and eczema

(a) TDiCPP and wheeze among total children



(b) TDiCPP and wheeze among children w/o *FLG* mutation

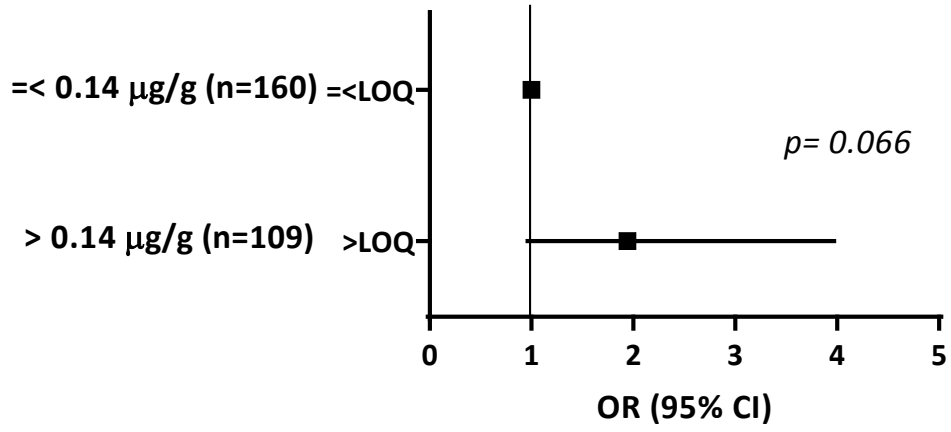


Figure 3. TDCiPP in house dust and wheeze