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ORIGINAL ARTICLE

Atopic Dermatitis, Urticaria and Skin Disease

Prenatal paraben exposure and atopic dermatitis-related outcomes among children

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Abstract

Background: Parabens, widely used as preservatives in cosmetics, foods, and other consumer products, are suspected of contributing to allergy susceptibility. The detection of parabens in the placenta or amniotic fluid raised concerns about potential health consequences for the child. Recently, an increased asthma risk following prenatal exposure has been reported. Here, we investigated whether prenatal paraben exposure can influence the risk for atopic dermatitis (AD).

Methods: 261 mother-child pairs of the German mother-child study LINA were included in this analysis. Eight paraben species were quantified in maternal urine obtained at gestational week 34. According to the parental report of physician-diagnosed AD from age 1 to 8 years, disease onset, and persistence, childhood AD was classified into four different phenotypes.

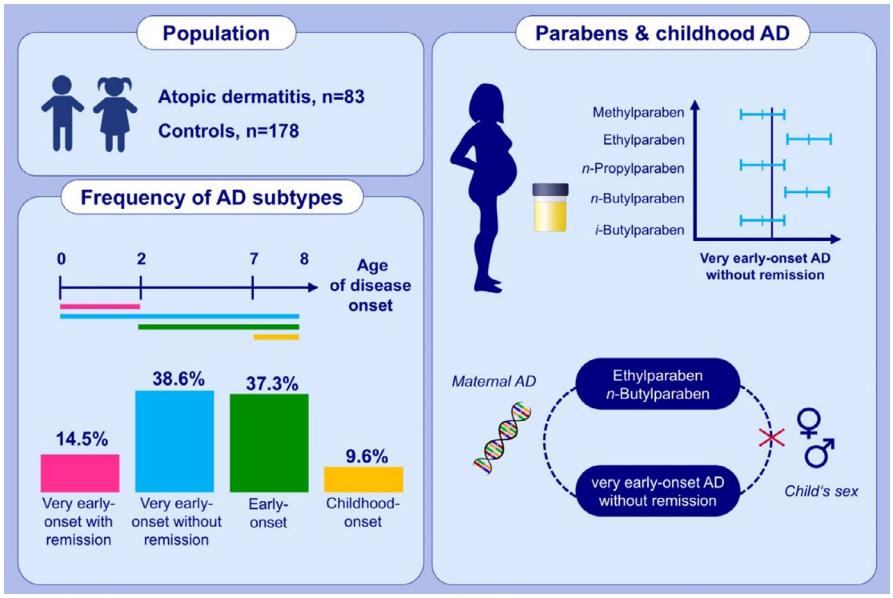
Results: 4.6% (n = 12) and 12.3% (n = 32) of the children were classified as having very early-onset AD (until age two) either with or without remission, 11.9% (n = 31) as early-onset (after age two), and 3.1% (n = 8) as childhood-onset AD (after age six). Exposure to ethylparaben and n-butylparaben was associated with an increased risk to develop very early-onset AD without remission (EtP: adj.OR/95% CI:1.44/1.04–2.00,nBuP:adj.OR/95% CI:1.95/1.22–3.12). The effects of both parabens were predominant in children without a history of maternal AD and independent of children's sex.

Conclusion: Prenatal EtP or nBuP exposure may increase children's susceptibility for persistent AD with disease onset at very early age. This association was particularly pronounced in children without a history of maternal AD, indicating that children without a genetic predisposition are more susceptible to paraben exposure.

Abbreviations: AD, atopic dermatitis; CHAMACOS, The Center for the Health Assessment of Mothers and Children of Salinas; CI, confidence interval; CoRAP, Community rolling action plan; ECHA, European Chemicals Agency; EDC, endocrine-disrupting chemicals; EDEN, Etude des Determinants pre et post natals du development et de la sante de l'Enfant; IQR, interquartile range; LINA, Lifestyle and Environmental Factors and their Influence on Newborns Allergy risk; LOQ, limit of quantification; Me-/Et-/iPr-/nPr-/nBu-/iBu-/sBu/BzP, methyl-, ethyl-,isopropyl-,n-propyl-, isobutyl-, n-butyl-, sec-butyl-, benzylparaben; NHANES, US National Health and Nutrition Examination Surveys; OR, odds ratio; UPLC, Ultra-Performance Liquid Chromatography.

Loreen Thürmann, Gunda Herberth, Bettina Seiwert, Saskia Trump, Thorsten Reemtsma, and Irina Lehmann equal contribution.

KEYWORDS
allergy, atopic dermatitis, paraben, prenatal, sex



GRAPHICAL ABSTRACT

Different paraben species, widely used as preservatives in consumer products, were detected in the urine of pregnant women. Prenatal ethylparaben and *n*-butylparaben exposure are associated with an increased risk for very early-onset atopic dermatitis without remission independent of the child's sex. This risk increase predominantly affects children without a history of maternal atopic dermatitis.
Abbreviation: AD, atopic dermatitis

1 | INTRODUCTION

Parabens are preservatives widely used in cosmetics, pharmaceuticals, and food products due to their antimicrobial properties.[1,2] As a result of the extensive usage, parabens are now omnipresent in the environment[3,4] and numerous studies confirmed a ubiquitous presence of parabens among various body fluids and tissues.[5-8] Parabens are classified as endocrine-disrupting chemicals (EDCs) shown to bind to the estrogen receptor.[9] In line, exposure to parabens has been linked to reproductive malfunctions,[10] while also obesogenic effects have been described.[11,12] Due to the immunomodulatory capacity[13] and the antimicrobial activity of parabens[14] probably affecting the microbiome,[15] it has been speculated that the extensive usage of paraben-containing consumer products could have contributed to the increased prevalence of allergic diseases observed in the last decades.[16]

The first pieces of evidence that parabens might increase the risk for pediatric allergic diseases came from cross-sectional studies. In NHANES (US National Health and Nutrition Examination Surveys), a positive association of urinary parabens to aeroallergen sensitization[17,18] and asthma-related emergency department visits[19] has been identified in children aged between six and 19 years with a more pronounced effect among boys. In the same survey, no association was found between urinary paraben concentration and atopic dermatitis (AD) frequency or asthma diagnosis in 6–18 -year-old children.[18] On the contrary, increased urinary paraben concentrations were observed in Japanese adolescents with current AD and in trend also with wheezing symptoms.[20]

Since there are even substantial paraben concentrations measurable in the placenta, cord blood, or amniotic fluid,[21] concerns about potential adverse health consequences following a paraben exposure during early development arose. To date, the contribution of a prenatal paraben exposure on allergic diseases in particular to atopic dermatitis is largely unexplored, and results of the limited number of studies are inconsistent. The three previous studies mainly focused on asthma/wheeze and IgE sensitization as an allergic outcome.[22-24] In the two independent US studies, no positive association was observed between a prenatal MeP, PrP, or BuP exposure and allergic sensitization or asthma/wheezing among 3-[22] or 7-year-old children.[24] In the French EDEN (Etude des Determinants pre et post natals du development et de la sante de l'Enfant) mother-child study, a weak association of prenatal EtP exposure to asthma was observed in 5-year-old boys, while no association for BuP or PrP was identified with wheezing, bronchitis, or asthma until the age of 5.[23] Only the US CHAMACOS (The Center for the Health Assessment of Mothers and Children of Salinas) study included AD present at the age of 7 years (current AD) in the examination of effects related to prenatal paraben exposure and allergic diseases and identified no association.[24] However, one recent study showed that the presence of BuP in breast milk is associated with AD at the age of 1 year.[25] As such, more research is needed to explore the prenatal effects of parabens on AD at earlier ages.

AD is regarded as the first manifestation of allergies, and a disease onset at a very early age is a major risk factor for the progression in the atopic march.[26] This highly heterogeneous, chronic inflammatory skin disease has a multifactorial etiology and a peak age of disease onset before 2 years of life. Therefore, for a valid evaluation of a potential contribution of prenatal paraben exposure on AD risk in childhood, considering AD subtypes based on the disease onset is warranted.

To address this knowledge gap, the current study aimed to examine whether prenatal paraben exposure was associated with an increased risk for children to develop AD up to the age of 8 years with a particular focus on specific AD subtypes. Using samples and data from the longitudinal prospective mother-child cohort LINA (Lifestyle and Environmental Factors and their Influence on Newborns Allergy risk), the potential association between maternal urine paraben concentrations and children's AD risk was studied. Measurements of eight parabens were available. We focused on those five parabens, namely methylparaben (MeP), ethylparaben(EtP), propylparaben (PrP), isobutylparaben (iBuP), and *n*-butylparaben (nBuP), which showed concentrations above the lowest limit of quantification in over 70% of the maternal urine samples.

2 | METHODS

2.1 | Study design

This study is based on a subcohort of 261 children of the prospective mother-child cohort LINA for whom longitudinal AD information and relevant covariate data until the age of 8 years as well as maternal paraben urine measurements at the 34th week of pregnancy were available (Figure 1).

In LINA, 629 mother-child pairs were recruited from March 2006 until December 2008 in Leipzig, Germany. Standardized questionnaires (self-administered by the parents) on family history of atopy, housing/environmental conditions, and atopic outcomes of the children were recorded during pregnancy and annually thereafter. Participation in the study was voluntary, and written informed consent was given by the parents. The LINA study was approved by the Institutional Review Board of the University of Leipzig and the Saxonian Board of Physicians (046-2006, 160-2008, 160b/2008, 144-10-31052010, 113-11-18042011, 206-12-02072012, 169/13-ff, 150/14-ff, EK-allg-28/14-1).

2.2 | Paraben measurement

Urinary concentrations of eight paraben species namely methylparaben (MeP), ethylparaben (EtP), isopropylparaben (iPrP), *n*-propylparaben (nPrP), *sec*-butylparaben (sBuP), isobutylparaben (iBuP), *n*-butylparaben (nBuP), and benzylparaben(BzP) were determined in maternal urine samples of the 34th week of gestation as previously described using a UPLC™ system (ACQUITY I-Class, Waters Cooperation Milford, MA, USA) coupled to a triple quadrupole mass spectrometer (Xevo TQ-S, Waters Cooperation, Manchester, UK) and equipped with an electrospray ionization (ESI) source.[27] For MeP, the limit of quantification (LOQ) was 0.5 µg/L and 0.1 µg/L for the other parabens (EtP, iPrP, nPrP, sBuP, iBuP, nBuP, and BzP). For iPrP, sBuP, and BzP, more than 80% of samples had concentrations

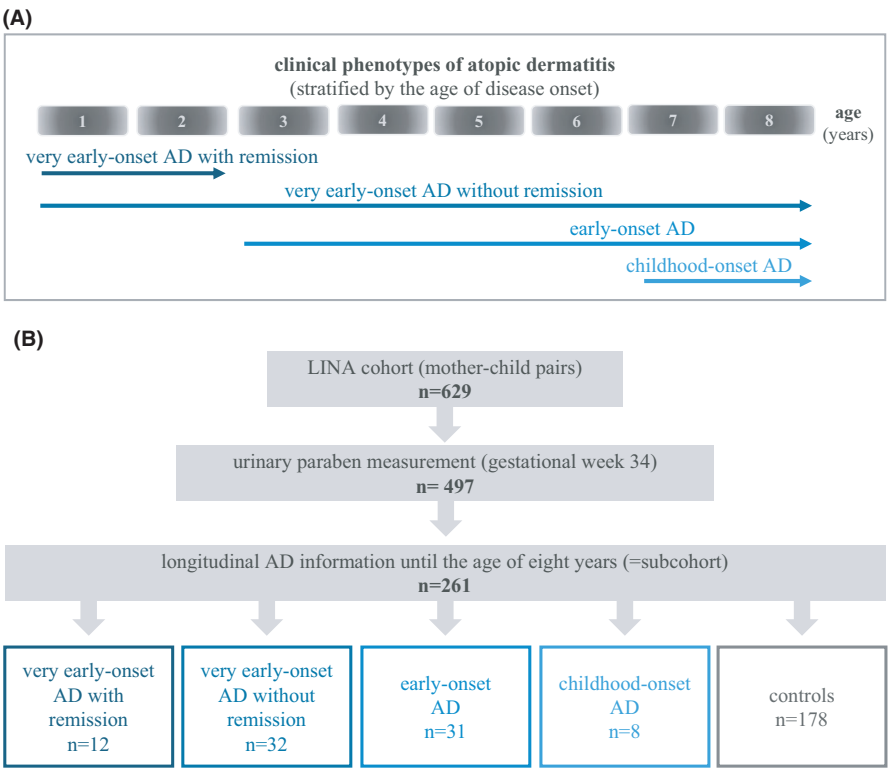


FIGURE 1 Overview of the analyzed AD subtypes and the LINA subcohort. A, Classification of AD phenotypes according to age at disease onset and disease persistence based on Bieber et al. 2017. B, Flow chart illustrating the selection of participants enrolled in the LINA study, which were included in this study. Only children with a valid maternal paraben measurement and longitudinal information on AD outcome and covariates were considered for this analysis

below the LOQ and were therefore excluded from further analyses (Table S1). For the remaining parabens, concentrations below LOQ were assigned a value that was half of the LOQ.

2.3 | IgE measurement

Concentrations of total serum IgE were determined by the Pharmacia CAP System (Pharmacia Diagnostics, Freiburg, Germany).

2.4 | Dependent variable: Atopic dermatitis classification

Information on physician-diagnosed AD manifestation until the age of 8 years was obtained annually from questionnaires: "Has a physician-diagnosed your child with allergic or atopic dermatitis/eczema or neurodermatitis in the past year?". The classification of AD subtypes was based on the age of disease onset as proposed by Bieber et al.[28] To this end, the children were classified in either (1) "very early-onset AD without remission" (AD in the first 2 years and onset at least once after the age of two), (2) "very early-onset AD with remission" (AD diagnosis exclusively in the first 2 years and no AD manifestation after the age of two), (3) "early-onset AD" (first AD diagnosis after the age of two and until the age of six), or (4) "childhood-onset AD" (first AD diagnosis after the age of six). Controls were never diagnosed with AD until the age of 8 years (Figure 1).

Maternal AD was defined according to the question "Did you ever suffer from atopic dermatitis/eczema?" included in the pregnancy questionnaire. Accordingly, the maternal control group for AD did not report AD.

2.5 | Independent variables: Covariates

To control for potential confounding factors, we *a priori* tested for associations between all AD subtypes and environmental and genetic factors, which have previously been associated with childhood AD, by applying crude binary logistic regressions. These factors included prenatal exposure and life style factors such as parental education level, renovation activities during pregnancy,[29] prenatal tobacco smoke exposure,[30] cat ownership,[31] as well as genetic influences like the child's sex, and the maternal history of AD.[32] Maternal AD, which was shown to be the strongest risk factor for early AD in the LINA study,[33] was used to account for a genetic predisposition. Whenever one factor was significantly associated with any AD subtype, it was considered as a covariate.

Following this approach, we retained prenatal tobacco smoke exposure, renovation activities during pregnancy, the child's sex, and the maternal history of AD as influencing factors for AD. As illustrated by the directed acyclic graph (Figure 2), the parental education level was not directly associated with AD and therefore not included in downstream analyses. However, smoking and renovation activities during pregnancy were associated with the parental education level.

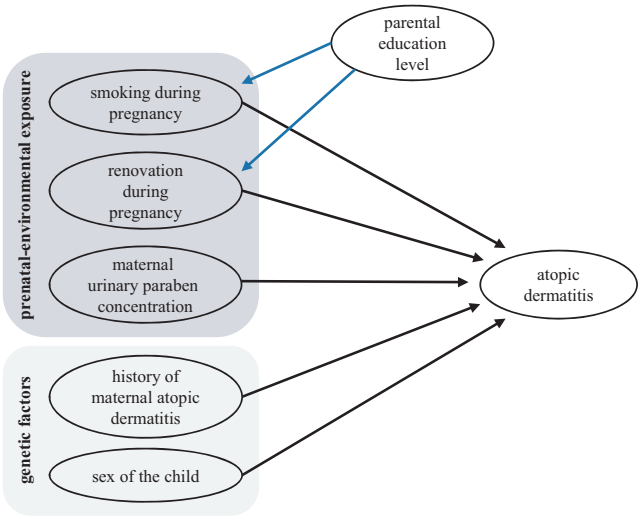


FIGURE 2 Directed acyclic graph depicts the hypothesized relationship between prenatal paraben exposure and atopic dermatitis and shows the potential confounders classified in genetic factors and lifestyle factors during pregnancy (black arrows). As the parental education level was significantly associated with smoking and renovation during pregnancy, it was omitted as a covariate (blue arrows)

Information on covariates was derived from questionnaires, except for prenatal tobacco smoke exposure, where the ln-transformed maternal urinary cotinine concentration at the 34th gestational week was used.[34]

To adjust urinary paraben concentrations for creatinine, we included ln-transformed creatinine concentrations measured at the 34th week of pregnancy as an independent variable in any statistical model.[35]

2.6 | Statistics

Chi-squared and Mann-Whitney *U* tests were used to test for equal parameter distribution and paraben concentrations between the analyzed subcohort and the entire LINA cohort.

To elucidate the potential contribution of a maternal history of AD on the relationship between prenatal EtP or nBuP exposure and the development of childhood AD, a moderation model was conducted using Andrew Hayes' process macro(process macro v3.4 for SPSS,[36] www.processmacro.org) with a bootstrap approach of 5000 samples to estimate the statistical significance of the moderating effect as bias-corrected 95% CIs. In this model, EtP and nBuP exposure were dichotomized, where high was defined as an exposure above and low exposure as below the geometric mean. The moderation analyses were adjusted using the covariates described above.

To test for a potential association of prenatal paraben exposure and the different AD subtypes, multinomial logistic regression models were applied. Since paraben concentrations are highly correlated (Table S2), models were conducted for each paraben separately to exclude multicollinearity. Models were adjusted for covariates selected as described above, and paraben concentrations were z-score-transformed to allow for better comparability of the effect size between the different parabens. The association of EtP and nBuP on very early-onset AD in different subgroups of the study cohort was assessed by adjusted binary logistic regression. Linearity in our logistic regression models was tested by the Box-Tidwell procedure.

Continuous variables were either z-score- or ln-transformed before analyses. As we investigated *a priori* hypotheses, no multiple test correction was performed.[37] *P*-values ≤ 0.05 were considered significant. Analyses were performed in either STATISTICA 13.3 for Windows (Dell Inc., USA), GraphPad Prism version 7.00 for Windows (GraphPad Software, USA), or IBM SPSS Statistics version 25 (IBM Corps., USA).

3 | RESULTS

3.1 | Study characteristics

For this explorative study, longitudinal data on the onset of AD were used to classify the different AD phenotypes according to Bieber et al.[28] (Figure 1A). Only children with valid maternal paraben measurements at the 34th week of pregnancy and with information on longitudinal AD manifestation and confounding factors up to the age of 8 were included. To this end, 261 children were considered in subsequent analyses (Figure 1B). There was no general selection bias in the analyzed subcohort compared to the whole LINA cohort, except a minor difference in parental education level with a tendency that higher educated parents continued to participate in the 8-year follow-up study of LINA (Table 1).

The maternal urinary paraben concentrations differed among the analyzed species. MeP (median (IQR) = 38.2 µg/L (10.8–147.0), nPrP = 4.6 µg/L (1.0–21.5)) was most abundant, followed by EtP (median (IQR) = 2.5 µg/L (0.8–11.5)) and the two BuP derivatives (median (IQR):iBuP = 0.2 µg/L (0.1–0.75), nBuP = 0.7 µg/L (0.2–2.7), Table 1, Table S1).

Until the age of 8 years, 68.2% (*n* = 178) of the children were never diagnosed with AD. The children, who ever developed AD, were stratified based on their age of disease onset as proposed by Bieber et al.[28] The majority of the children with AD developed first symptoms within the first 2 years of life and were classified as very early-onset AD, either with (*n* = 12, 4.6%), or without remission (*n* = 32, 12.3%). 11.9% of the children (*n* = 31) developed AD after the age of two, but before the age of seven (early-onset AD), whereas only a minor part was diagnosed after the age of six and was subsequently classified as childhood-onset AD (*n* = 8, 3.1%, Figure 1B).

3.2 | Prenatal paraben exposure and AD manifestation during childhood

To elucidate the contribution of a prenatal paraben exposure on the children's risk to develop different types of AD, multinomial logistic regression models adjusted for AD covariates were applied (Figure 2, Table 2, Table S3). Z-scores were used in this regression analysis, so odds ratios correspond to a one unit change in the SD of the paraben concentration. Increased urinary gestational EtP and nBuP concentrations were associated with an increased risk for the children to develop very early-onset AD without remission (EtP: adj. OR/95% CI: 1.44/1.04–2.00), *P*-value = 0.029; nBuP: adj.OR/95% CI: 1.95 (1.22–3.12), *P*-value = 0.005, *n* = 32 vs. 178, Table 2, Figure 3), but not with the other AD subtypes. Only children with this very early and persistent AD subtype showed elevated longitudinal serum IgE concentrations from age 1 onwards compared to controls (Figure S1).

3.3 | Influence of a maternal history of AD and child's sex

Besides prenatal EtP and nBuP exposure, a significant association to very early-onset AD without remission was also observed for renovation activities during pregnancy, prenatal tobacco smoke exposure, and maternal AD, with the latter being the major risk factor with the highest OR (OR/95% CI: 3.08/1.35–7.02, *P*-value = 0.007, *n* = 32 vs. 178, Table 2B). Note that the paternal history of AD was not associated with very early-onset AD without remission (OR/95% CI: 1.78/0.54–5.90, *P*-value = 0.344).

Separating the children in those with and those without a history of maternal AD, the influence of prenatal EtP or nBuP was apparent in children without a history of maternal AD (EtP: adj. OR/95% CI = 1.43/1.09–1.89, nBuP: adj.OR/95% CI = 1.37/1.06–1.78), while in children with a maternal predisposition, the effect of EtP or nBuP was less pronounced (EtP: adj.OR/95% CI = 1.05/0.70–1.57, nBuP: adj.OR/95% CI = 1.07/0.69–1.64; Figure S2, Table S4).

Subsequently, to elucidate whether a maternal history of AD indeed influences the effect of a prenatal EtP or nBuP exposure and the risk for very early-onset AD without remission, we implemented a moderation model considering both parabens as a binary variable. In contrast to mediators directly associated with the independent variable (paraben exposure), a moderator modifies the strength or direction of the relationship between the independent and dependent (childhood AD) variable without being causally affected by the independent variable.

In the moderation model, a significant interaction term was found for maternal history of AD and EtP or nBuP (EtP: β(95% CI) = −2.10(−3.91/−0.29), *P*-value = 0.023; nBuP: β(95% CI) = −1.68 (−3.47/−0.10), *P*-value = 0.065, Table 3A-B, Figure 4), suggesting that the effect of a prenatal EtP or nBuP exposure on very early-onset AD without remission is different in children without a maternal

TABLE 1 Characteristics of the entire LINA cohort and the investigated subcohort

	Entire cohort *n* = 629 (%)[a]	Subcohort *n* = 261 (%)	*P*-value[b]
Sex of the child			
Male	330 (52.5)	130 (49.8)	0.517
Female	299 (47.5)	131 (50.2)	
Parental education level[c]			
Low	16 (2.5)	3 (1.1)	0.034
Intermediate	144 (22.9)	43 (16.5)	
High	469 (74.6)	215 (82.4)	
Living and household conditions during pregnancy			
Renovation activities			
Yes	328 (52.1)	138 (52.9)	0.791
No	278 (44.2)	123 (47.1)	
Tobacco smoke exposure[d]			
(almost) daily	48 (7.6)	10 (3.8)	0.101
Occasionally	47 (7.5)	18 (6.9)	
Never	534 (84.9)	233 (89.3)	
Cat ownership			
Yes	95 (81.2)	33 (12.6)	0.294
No	511 (15.1)	228 (87.4)	
Prevalence of atopic dermatitis	**n/N (%)**	**n/N (%)**	
Maternal history of atopic dermatitis	112/623 (18.0)	50/261 (19.2)	0.750
Children's atopic dermatitis phenotypes			
Very early-onset AD with remission	14/351 (4.0)	12/261 (4.6)	0.899
Very early-onset AD without remission	35/351 (10.0)	32/261 (12.3)	
Early-onset AD	42/351 (12.0)	31/261 (11.9)	
Childhood-onset AD	10/351 (2.8)	8/261 (3.1)	
Maternal chemical exposure (urine concentration at gestational week 34)	**Median (IQR)**	**Median (IQR)**	***P*-value[e]**
	n = 613		
Cotinine [µg/g creatinine]	2.05 (0.78–5.62)	1.92 (0.89–5.04)	0.474
	n = 497		
Creatinine [g/L]	0.71 (0.51–1.05)	0.72 (0.53–1.01)	0.750
MeP [µg/L]	39.2 (11.05–156.70)	38.2 (10.80–147.00)	0.822
EtP [µg/L]	2.32 (0.62–13.00)	2.50 (0.75–11.52)	0.865
nPrP [µg/L]	5.00 (1.00–21.50)	4.60 (1.00–21.50)	0.873
iBuP [µg/L]	0.20 (0.05–0.80)	0.17 (0.05–0.75)	0.976
nBuP [µg/L]	0.70 (0.15–3.30)	0.70 (0.16–2.70)	0.803

[a]Numbers may be different from the total sum due to missing data.

[b]*P*-value from chi-squared test for cross-relationship.

[c]Low: 9 years of schooling or less ("Hauptschulabschluss"), intermediate: 10 years of schooling ("MittlereReife"), high: 12 years of schooling or more ("(Fach-)hochschulreife").

[d]Answers based on the question "Did you or anybody else smoke inside your home during the last 12 months?"

[e]*P*-value from Mann-Whitney *U* test.

The bold values are the number of samples with available measurement of cotinine (n = 613) or creatinine+parabens (n = 497).

predisposition and those who had a mother suffering from AD. Since EtP or nBuP exposure was positively related to very early-onset AD without remission solely in children without a maternal history of AD (EtP: β(95% CI) = 1.03(0.02/2.05), *P*-value = 0.045; nBuP: β(95% CI) = 1.19 (0.14/2.24), *P*-value = 0.027), but not in children with a maternal history of AD (EtP: β(95% CI) = −1.07(−2.56/0.43),

TABLE 2 Association between prenatal paraben exposure and the risk for the development of different AD subtypes

(A)								
	very early-onset AD with remission (*n* = 178/12)		very early-onset AD without remission (*n* = 178/32)		early-onset AD (*n* = 178/31)		childhood-onset AD (*n* = 178/8)	
paraben	adj.OR[a] (95% CI)	*P*-value	adj.OR[a] (95% CI)	*P*-value	adj.OR[a] (95% CI)	*P*-value	adj.OR[a] (95% CI)	*P*-value
MeP	0.90 (0.34, 2.38)	0.839	1.28 (0.90, 1.83)	0.174	1.15 (0.83, 1.60)	0.407	0.64 (0.14, 2.98)	0.566
EtP	0.82 (0.34, 1.99)	0.655	1.44 (1.04, 2.00)	0.029	1.06 (0.72, 1.55)	0.784	0.31 (0.04, 2.50)	0.269
nPrP	1.11 (0.65, 1.89)	0.711	1.04 (0.69, 1.56)	0.856	1.17 (0.86, 1.58)	0.315	0.50 (0.05, 4.74)	0.548
iBuP	0.28 (0.01, 5.45)	0.401	1.39 (0.99, 1.93)	0.054	1.16 (0.83, 1.62)	0.380	0.90 (0.32, 2.55)	0.840
nBuP	0.23 (0.01, 4.63)	0.338	1.95 (1.22, 3.12)	0.005	1.55 (0.99, 2.43)	0.058	0.72 (0.14, 3.75)	0.692

(B) Full adjusted multinomial logistic regression model for very early-onset AD without remission as the dependent variable				
	EtP		nBuP	
independent variable	adj.OR[a] (95% CI)	*P*-value	adj.OR[a] (95% CI)	*P*-value
maternal history of AD	3.50 (1.46, 8.39)	0.005	4.10 (1.68, 10.0)	0.002
prenatal EtP exposure	1.44 (1.04, 2.00)	0.029	NA	NA
prenatal nBuP exposure	NA	NA	1.95 (1.22, 3.12)	0.005
renovation activities	2.64 (1.12, 6.24)	0.027	3.17 (1.29, 7.80)	0.012
prenatal tobacco smoke exposure	1.32 (1.00, 1.75)	0.052	1.36 (1.02, 1.80)	0.035
child's sex	0.89 (0.40, 1.99)	0.785	1.01 (0.45, 2.30)	0.976

Note: Multinomial logistic regression model. Z-scores of paraben concentrations were used for analysis.

[a]OR from multinomial regression adjusted for the child's sex, renovation activities during pregnancy, prenatal tobacco smoke exposure, maternal history of AD, and creatinine concentration at gestational week 34.

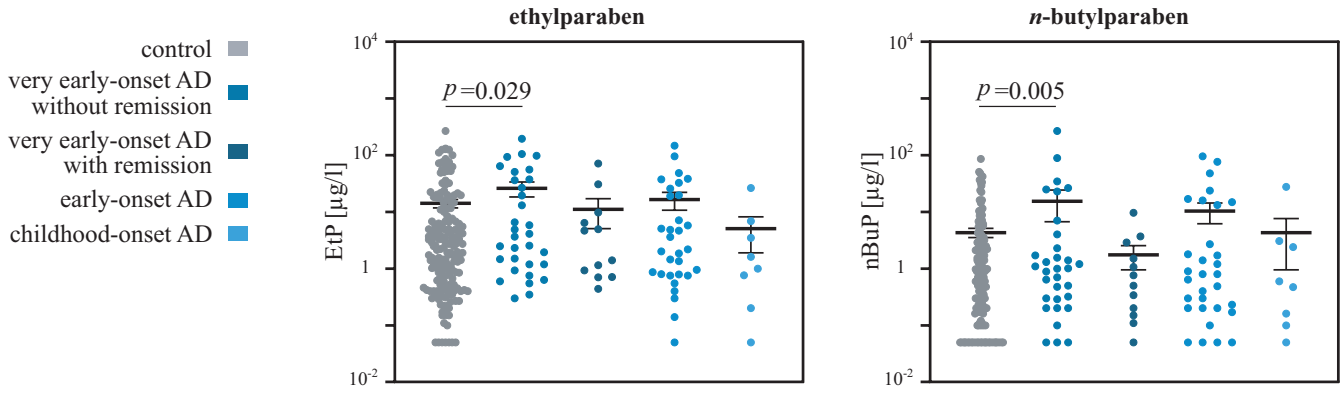


FIGURE 3 Maternal urinary EtP and nBuP concentration according to children's AD subtypes (mean ± SEM). *P*-value from multinomial logistic regression adjusted for the child's sex, maternal AD history, renovation activities during pregnancy, prenatal tobacco smoke exposure, and creatinine concentration at gestational week 34

P-value = 0.163; nBuP, β(95% CI) = −0.49(−1.98/0.99), *P*-value = 0.514, Table 3), a prenatal paraben exposure seems to be associated with AD dominantly in children not being genetically predisposed. Note that for none of the other parabens, a moderating effect by the maternal history of AD was observed (data not shown).

When we studied the influence of a prenatal paraben exposure on very early-onset AD without remission, we did not see a significant impact of the child's sex on this AD phenotype (Table 2B). In agreement, neither for EtP or nBuP (Table S5 and Table S6) nor for any other paraben species (data not shown) a moderating effect by child's sex was observed.

4 | DISCUSSION

The prevalence of childhood AD has steadily increased, and this observation is attributed to changing environmental influences.[38] The current study provides evidence for a contribution

TABLE 3 Moderation analysis evaluating the moderation effect of a maternal history of AD on the relationship between a prenatal EtP or nBuP exposure and very early-onset AD without remission (n = 178 vs. 32)

(A) Moderation analysis for EtP and very early-onset AD without remission				
	***b* coefficient (95% CI)**	**SE (*b*)**	**Z-value**	***P*-value**
Prenatal EtP exposure[a]	1.03 (0.02,2.05)	0.52	2.00	0.045
History of maternal AD	4.47 (1.62,7.32)	1.46	3.07	0.002
Maternal AD × EtP exposure (interaction)	−2.10 (−3.91, −0.29)	0.92	−2.28	0.023
Covariates				
Child's sex	−0.16 (−0.97, 0.65)	0.41	−0.39	0.693
Renovation activities	0.95 (0.08,1.82)	0.44	2.15	0.032
Prenatal tobacco smoke exposure	0.23 (−0.05, 0.52)	0.15	1.62	0.106
Conditional effect moderated by maternal AD				
No history of maternal AD	1.03 (0.02,2.05)	0.52	2.00	0.045
History of maternal AD	−1.07 (−2.56, 0.43)	0.76	−1.40	0.163
(B) Moderation analysis for nBuP and very early-onset AD without remission				
	***b* coefficient (95% CI)**	**SE (*b*)**	**Z-value**	***P*-value**
Prenatal nBuP exposure[b]	1.19 (0.14, 2.24)	0.54	2.21	0.027
History of maternal AD	3.89 (1.07, 6.72)	1.44	2.70	0.007
Maternal AD × nBuP exposure (interaction)	−1.68 (−3.47, 0.10)	0.91	−1.85	0.065
Covariates				
Child's sex	−0.09 (−0.90, 0.72)	0.41	−0.21	0.831
Renovation activities	0.85 (0.00, 1.71)	0.44	1.95	0.051
Prenatal tobacco smoke exposure	0.25 (−0.05, 0.54)	0.15	1.64	0.102
Conditional effect moderated by maternal AD				
No history of maternal AD	1.19 (0.14, 2.24)	0.54	2.21	0.027
History of maternal AD	−0.49 (−1.98, 0.99)	0.76	−0.65	0.514

[a]high/low EtP prenatal exposure (>/< geometric mean of urinary EtP concentration within the subcohort).

[b]high/low nBuP prenatal exposure (>/< geometric mean of urinary nBuP concentration within the subcohort).

of prenatal exposure to different parabens, namely EtP and nBuP, to the risk of developing very early-onset AD without remission, which is the most prevalent form of AD in the general population, and relevant for the atopic march.[28] This agrees with our observation that 39% of the AD children in our study suffered from this persistent AD subtype and that those children showed the highest total serum IgE concentrations longitudinally.

Among the five parabens investigated (MeP, EtP, nPrP, iBuP, nBuP), prenatal exposure to EtP and nBuP was associated with an increased risk to develop the persistent AD phenotype already manifested before the age of 2 years. This association was predominant in children without a history of maternal AD indicating that children who are not genetically predisposed might be more susceptible to paraben exposure. No association was observed to any other AD sub-phenotype developed later in life. These results agree with the US CHAMACOS study, in which urinary MeP, PrP, and BuP were analyzed, and which did not observe an association of a prenatal paraben exposure to AD at the age of 7 years.[24]

In the NHANES national study, which investigated the influence of different EDCs among 6–18 years old children on allergic outcomes, an association with aeroallergen sensitization was observed only for EDCs with antimicrobial activity, such as parabens, but not for EDCs without an antimicrobial capacity.[17] This observation might provide some evidence that in terms of allergies, the antimicrobial properties rather than the role as an EDC might be crucial for the association seen between paraben exposure and allergic outcomes.

During the prenatal period, the penetration of antimicrobial active compounds like parabens across the placenta and accumulation in breast milk might influence the composition of the maternal microbiome as well as the developing microbiome of the infant known to be crucial for atopic manifestations.[39] Transplacental passage of BuP,[40] accumulation of maternal EtP and BuP in amniotic fluid as previously seen in rats[41] as well as the presence of EtP and BuP in breast milk,[42-45] may increase the exposure burden of the fetus and prolong the exposure time to the perinatal phase.[46] In an explorative study, high concentrations of BuP in breast milk were associated with AD in 1-year-old children.[25] Although the study was unable to detect EtP in breast milk samples, it supports the general notion that parabens might be involved in the early pathophysiology of AD.

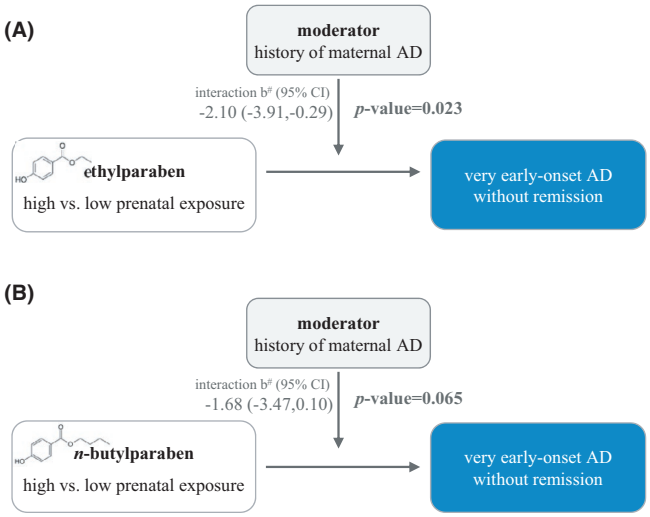


FIGURE 4 Impact of the maternal history of AD on the relationship between prenatal EtP or nBuP exposure and the risk for very early-onset AD without remission. The graphical illustration shows moderating effect of the child's history of maternal AD on the association between a prenatal (A) EtP or (B) nBuP exposure and the development of very early-onset AD without remission. # *b* interaction coefficient between EtP or nBuP and the history of maternal AD from moderation model adjusted for the child's sex, renovation activities during pregnancy, prenatal tobacco smoke exposure, and creatinine concentration at gestational week 34. For details, refer to Table 3

Besides a potential impact on the microbiome, parabens might play an additional role in AD pathophysiology. This idea is supported by an in vitro study demonstrating that MeP affects keratinocyte differentiation following long-term exposure.[47] Thus, parabens might also have the potential to directly perturb skin development. In addition, low amounts of IL-1β in breast milk have been associated with an increased risk of early AD.[48] Since EtP was shown to be associated with reduced IL-1β in pregnant women,[49] a perturbation in maternal immune signaling by EtP might contribute to the child's increased AD risk.

Although BuP is thought to have a stronger estrogenic activity than EtP,[50,51] for both parabens the association to very early-onset AD was not dependent on the child's sex. This contrasts with previous studies, which have implied sex-dimorphic effects following paraben exposure in relation to allergic outcomes.[17,19,22] Since uterotrophic effects were observed for EtP in a rat model together with the upregulation of estrogen-responsive genes[52] and BuP is considered an endocrine disrupter,[53] it cannot be excluded that also early EtP or nBuP exposure might induce sex-dimorphic effects in the context of other diseases than AD.

This study has to be seen in the light of some limitations. Paraben measurements have been performed at only one time-point during late gestation and might therefore not represent the overall exposure. However, we previously confirmed in our LINA study that the usage of paraben-containing leave-on cosmetics by the mother during the entire pregnancy period coincided with high paraben urine concentrations at this single time-point measurement.[11] This result suggests that a one-time spot-urine measurement could be a valid surrogate for an overall paraben exposure. The diagnosis of AD cases is based on parent-reported questionnaires, which might introduce misclassifications. However, the longitudinal study design including 8-year follow-up data increased the validity of the applied classification, which is supported by the observed prevalence of the AD subtypes being in agreement with the distribution in the general population according to Bieber et al.[28] In this study, only eight children (9.6% of the AD children) showed AD symptoms after the age of seven. As such, results regarding this subtype need to be interpreted with caution. Childhood-onset AD represents an AD subtype, which is not well investigated, probably because only ~10% of the AD children develop this rare AD form.[28] Therefore, large population-based studies including school-aged children are needed to evaluate the effect of a prenatal paraben exposure on childhood-onset AD. Due to the nature of explorative epidemiological studies, causality cannot be derived and future studies are needed to confirm the results of the current study, and to elucidate potential molecular mechanisms, for example, whether indeed a perturbed microbiome is involved in the observed effects of parabens.

The longitudinal design and the availability of maternal urine samples together with 8-year follow-up data on children's health outcomes are the major strength of this study.

Taken together, we here show for the first time an association between prenatal EtP and BuP exposure and the development of very early-onset AD without remission. This persistent type of AD manifestation is considered the most severe AD phenotype, and those children suffering from AD at this very early age are at high risk for the progression into the atopic march.[26,54] Lowering risk factors such as paraben exposure in the developmental period might therefore not only attenuate the risk for very early-onset AD but even reduce the likelihood of other atopic manifestations.

There is an ongoing debate about the safety of parabens used in consumer products in particular for cosmetics. To date, the EU already banned five parabens in cosmetic products and lowered the concentration limits for the use of the remaining parabens. EtP is considered safe in the maximum concentration of 0.4% and as a non-potent allergen.[55] Contradictory, EtP is currently on the European ECHA CoRAP (Community rolling action plan), which identifies EtP[56] as a high-priority chemical for evaluation due to concerns regarding potential environmental and health consequences. Denmark has banned BuP in cosmetic products for children younger than 3 years of age.[57] Although the 2013 SCCS report considered BuP as a safe preservative in cosmetic products for adults and children,[57] the ECHA added BuP to their list of substances of very high concern.[53] Based on the current stage of risk assessment, further epidemiological studies evaluating the effects of prenatal paraben exposure and the consequences for the immune system are highly warranted to provide valid data for safety recommendations. However, although more studies and subsequent risk assessments are necessary regarding the usage

of parabens, precautionary approaches are advisable when dealing with paraben-containing consumer products. As we and others have demonstrated that high urinary paraben concentration is associated with cosmetic usage,[11,42] one strategy might be the avoidance of paraben-containing leave-on products during critical developmental time windows.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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