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Sex moderated interactions between IL4/IL13 pathway genes and prenatal environment on cord blood IgE levels

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Short running title: Sex moderated interactions on cord blood IgE levels

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ABSTRACT

Background: Elevated cord blood IgE (cIgE), a predictor of atopic diseases, is influenced by genetic and environmental factors. However, gene-environment interactions on cIgE elevation and their difference by sex remain largely unexplored.

Objective: This study aimed to determine if there are sex-moderated interactions between genetic variants in the IL4/IL13 pathway and prenatal environments on cIgE elevation.

Methods: Comprehensive information on environmental tobacco smoke (ETS), home dampness (indexed by combining mildewy odor, visible mold, and water stamp on the wall), and other household environments was obtained using a structured questionnaire during the third trimester of pregnancy in 1107 full-term newborns. The cord blood was collected for measuring cIgE levels, with elevation defined as ≥ 0.5 IU/mL, and for genotyping of five single nucleotide polymorphisms of three candidate genes (*IL-13* rs1800925, rs20541, rs848, *IL-4* rs2243250, and *STAT6* rs324011).

Results: Gene-environment interactions on cIgE elevation were observed in male but not female newborns, including those between ETS and *IL13* rs20541, between home dampness and *STAT6* rs324011, and between composite environmental exposure (combined ETS and the three home dampness indices) and *STAT6* rs324011 (*P* for interaction = .03, .006, and .001, respectively). Male newborns carrying *STAT6* rs324011 CT or TT genotype manifested with a significant dose-response association of the composite environmental exposure with cIgE elevation.

Conclusion and Clinical Relevance: Sex moderates the gene-environment interactions involving *IL4/IL13* pathway genes and prenatal household environments on cIgE elevation. Absence of prenatal exposure to ETS and home dampness in male neonates carrying the *STAT6* rs324011 CT or TT genotype is least likely associated with cIgE elevation.

Key words

cord blood, IgE, prenatal environment, single nucleotide polymorphism, sex

Abbreviations used

SNP: single nucleotide polymorphism

cIgE: cord blood IgE

ETS: environmental tobacco smoke

1 INTRODUCTION

Elevated IgE levels in the cord blood (cIgE) have been associated with higher risk of childhood allergic diseases¹ that are increasingly prevalent and have led to significant physical, social, and economic burden.^{2,3} For example, elevated cIgE has been a useful measure of subsequent risk of food allergy/urticaria at 12 months⁴ and allergic sensitization at varying ages during childhood.⁵⁻⁸ Both genetic influences and intrauterine exposure to allergens or triggers have been implicated in cIgE elevation.^{9,10} Among genetic influences, single nucleotide polymorphisms (SNPs) in the genes involved in the IL-4/IL-13 pathway, including the *IL-13*, *IL-4*, and *STAT6* genes, have been associated with increased IgE levels in children or adults.¹¹⁻¹⁴ Studies in newborns further demonstrated the associations of *IL4* and *IL-13* SNPs with elevated cIgE.¹⁵⁻¹⁷

In terms of prenatal environment, environmental tobacco smoke (ETS) is one of the most consistently reported risk factors for cIgE elevation.⁹ ETS can induce the production of IL4 and IL13,¹⁸ which then facilitates the switching of immunoglobulin class from M to E.¹⁹ Another potentially important household environment is home dampness, which has been shown to increase the risk for total IgE elevation,^{20,21} asthma,^{22,23} and rhinitis²² in children and adolescents. However, the influence of prenatal dampness on cIgE levels remains unclear.

Moreover, individual genetic or environmental factors cannot adequately explain cIgE elevation. Another influential factor may be gene-environment interaction, as demonstrated in our previous study wherein prenatal exposure to ETS synergistically enhanced the influence of *IL-13* SNPs on cIgE elevation.²⁴ To date, there has been no systematic examination on possible interactions between the genetic variants in the IL-4/IL-13 pathway and prenatal household environments on cIgE elevation.

One complicating factor on the relationship between cIgE elevation and its risk factors is the influence of male sex. Fetal immuno-regulatory function is different between male and female newborns.²⁵ Both cIgE elevation^{4,26,27} and allergic diseases²⁸ are more common in boys than in girls, and males are more susceptible to the effect of smoking on IgE production than females.^{29,30} Our previous study further showed that only male newborns exhibited a synergistic association of ETS and *IL-13* SNPs with cIgE levels.²⁴ Such findings emphasize the need to evaluate the possible moderation of male sex on the interactions between *IL4/IL13* pathway genes and prenatal environment on cIgE elevation.

To address this gap in the literature, we turned to a large birth cohort of 1107 Han Chinese term newborns in Taiwan, with information on prenatal household environmental exposures during the third trimester of pregnancy and cord blood collected. The aims of this study were to determine if there were interactions between genetic variants in the *IL4/IL13* pathway and prenatal household environments on cIgE elevation, and if such interactions were moderated by sex.

2 METHODS

2.1 Participants

Participants of this study were recruited in the Gene and Environment Interaction Birth Cohort Study (GEIBCS) from March 2008 to April 2011, with a total of 1178 newborns collected. The procedure of recruitment was described in detail in a previous study using the participants recruited in the early phase (till February 2010) of the project.²⁴ Briefly, the structured questionnaires were completed during the third trimester of pregnancy by pregnant women who underwent prenatal care in Min-Sheng General Hospital. The fetuses were prospectively followed. After each baby was born, the umbilical cord was ligated before placental delivery. Cord blood was then collected into sterile EDTA-coated tubes and kept at 4°C and specimens were processed within 20 h of collection.

Serum cIgE levels were measured using a CAP FEIA system (Pharmacia, Uppsala, Sweden) and a level ≥ 0.5 IU/mL was defined as elevated.^{16,31} Written informed consent was obtained from all participants. This study was approved by the Institutional Review Board of Min-Sheng General Hospital (approval number: MSEIRB0980109).

Among 1178 newborns recruited, 71 (6%) were excluded due to prematurity (n = 30), respiratory distress (n = 31), Apgar score < 7 at 5 min after birth (n = 7), Tetralogy of Fallot (n = 1), severe subgaleal hemorrhage (n = 1), and severe abdominal distention (n = 1). A total of 1107 term newborns (94%) of Han Chinese ethnicity were included for this study.

2.2 Questionnaire and medical records

The questionnaire included questions on maternal age, parental race/ethnicity, parental education (the highest education among parents), household income per year, sibling number, family history of atopic diseases in the parents or siblings, and prenatal environmental exposures. The latter covered the presence of carpet, cockroach, furred pet, number of smokers at home, home dampness indices (including mildewy odor, visible mold, and water stamp on the wall), incense burning, and perception of outdoor air pollution. ETS was defined as having any smoker at home. Mildewy odor, visible mold, and water stamp on the wall were defined as the perception of any moldy odor, visible mold on the walls, and wet stamps due to moisture in the ceilings, floors, or walls of the house, respectively. Home dampness was indexed by combining mildewy odor, visible mold, and water stamp on the wall. Composite environmental exposure was indexed by combining ETS and the three home dampness indices.²⁴

Information on birth history (i.e., mode of delivery, Apgar score, sex, gestational age, birth weight, birth height, and birth head circumference of the newborn, and prior obstetric history of the mother) was obtained from medical records.

2.3 Validation of self-reported ETS

To verify if ETS reported by the pregnant women was appropriate to represent the real passive smoking status, we conducted a subsample analysis. Because all of the cord blood samples have been stored for at least 8 years, the decay of cotinine by time was evaluated first. We selected 39 newborns from our study population to see if the cotinine levels were still measurable or not. Their serum cotinine levels were measured using a human cotinine ELISA kit (Boster Biological Technology, 3942 B Valley Ave, Pleasanton, CA) and a level $\geq 1\text{ng/ml}$ was defined as elevated.³² Among the 39 newborns, 33 was exposed to at least one smokers at home (Table S1). However, only 7 (21%) of the 33 exposed newborns had elevated cotinine levels ($>1\text{ ng/ml}$). Both babies not exposed to any smoker and babies exposed to only one smoker at home prenatally had no elevated cotinine levels. The seven newborns with elevated cotinine levels were all exposed to at least 2 smokers at home prenatally. Although the results reflected the exposure dose, their values indicated substantial decay of cotinine in the stored cord bloods by time. Hence we did not perform cotinine analysis further in the remaining samples.

2.4 Measuring mould-specific IgE

A previous study on the impact of age on IgE production found that the levels of mould-specific IgE were undetectable until 2 years of age, although the levels of some other allergen-specific IgE were detectable at 0 and 1 year of age.³³ As a preliminary exploration, we measured mould-specific IgE from cord bloods in 46 selected newborns. Among them, 22 (48%) were prenatally exposed to at least one index of home dampness (including mildewy odor, visible mold, and water stamp on the wall), and 24 (52%) were not exposed to any one of home dampness indexes prenatally. Serum levels of mould-specific IgE were measured using an automated microfluidic-based immunoassay system (BioICTM Allergen-specific IgE Detection Kit; Agnitio Science and Technology, Hsinchu,

Taiwan). The following 8 allergens in the mould category were included: *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Candida albicans*, *Alternaria alternate*, *Cladosporium cladosporioides*, *Cladosporium oxysporum*, and *Penicillium brevicompactum*. The results revealed that all of the 46 newborns, irrespective of the presence or absence of prenatal home dampness exposure, did not have any detectable cIgE to the 8 allergens in the mould category.

2.5 DNA collection and genotyping

DNA was extracted from blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). The rationale for choosing three *IL-13* SNPs (rs1800925, rs20541, and rs848) was described in our previous study.²⁴ *IL-4* rs2243250 and *STAT6* rs324011 were chosen due to their well-studied associations with serum IgE levels and atopic diseases.^{12,15,34,35}

Genotyping of the five SNPs was done using TaqMan probe assays (TaqMan® SNP Genotyping Assays, Applied Biosystems, Foster City, CA, USA) on a 7900 Fast Real-Time PCR System (Applied Biosystems). Genotyping of each sample was automatically attributed using the SDS 1.3 software for allelic discrimination.

2.6 Independent sample for replication

To replicate the findings from GEIBCS, similar analyses were applied to an independent sample from Taiwan Birth Panel Study (TBPS), which aims to investigate the development of child disease in relation to in-utero environmental exposures.³⁶ Among a total of 486 newborns enrolled from one medical hospital in Taipei city (Taipei), one area hospital (Sansia), and two clinics in Taipei County (Sinjhuang, Lujhou) during the period from April 2004 to January 2005, cord blood IgE levels were measured in 236 newborns, in which 17 (7%) were excluded from this study due to prematurity. Due

to inadequate amount of stored cord blood DNA, we did not conduct the genotyping of the 5 SNPs in the IL4/IL13 pathway.

We were able to obtain information on neonatal sex, gestational age, birth body weight, maternal age, parental education, ETS, and family history of atopic diseases from TBPS, but not on mildewy odor, visible mold, and water stamp on the wall, because the related questions were not included in their questionnaires.

2.7 Statistical analysis

Deviations of genotypes from the Hardy-Weinberg equilibrium were assessed using the χ^2 test. Based on the findings of our previous study,²⁴ the dominant genetic models were implemented in logistic regression analyses to evaluate the relationship of each SNP to elevated cIgE.

To further investigate if neonatal sex and genetic polymorphisms modified the influences of prenatal household environmental exposures on cIgE elevation, we stratified the association analyses by neonatal sex and genotypes of each genetic polymorphism simultaneously, with adjustments for known predictors of atopy (i.e., gestational age, birth body weight, maternal age, and family history of atopic diseases) and correlates of cIgE elevation in this study (i.e., parental education, environmental tobacco smoke, and mildewy odor) in multivariable logistic regression analysis. Significance levels of the associations in each stratification strategy were corrected for multiple testing by means of false discovery rate (FDR). A two-tailed *P* value < .05 after FDR correction was considered significant. The significance level of statistical interaction was determined using the likelihood ratio tests that compared the model with an interaction term versus the model without it.

All statistical analyses were performed using the SAS software version 9.1 and the SAS/Genetics module (SAS Institute Inc., Cary, NC, USA). A two-tailed *P* < .05 was considered significant.

3 RESULTS

3.1 Characteristics of newborns

The study sample of 1107 full-term newborns had a mean gestational age of 38.6 ± 1.1 weeks and a female-to-male ratio of around 1:1.03. Among them, 226 (20.4%) had elevated cIgE. As shown in Table 1, male babies had larger mean birth body weight and greater prevalence of cIgE elevation than female babies ($P \leq .0001$ and $.002$, respectively). Other demographic variables were not different between male and female babies.

Table 2 displays the distribution of family and birth characteristics of babies with normal cIgE versus that of babies with elevated cIgE. Newborns with elevated cIgE had a smaller proportion of parental education of college level or higher, a larger proportion of prenatally perceived mildewy odor, and a larger proportion of males than newborns with normal cIgE. The other covariates were not different between the two groups.

3.2 Environmental risk factors for cIgE elevation

Among the several exposures examined in the study, only mildewy odor exhibited a significant association with cIgE elevation in the univariate logistic regression analysis (Table 3). After adjusting for covariates, including neonatal sex, gestational age, birth body weight, maternal age, parental education, ETS, and family history of atopic diseases, mildewy odor was still the only environmental factor associated with cIgE elevation (adjusted OR = 1.89, 95% CI:1.01- 3.53; $P = .047$).

3.3 Distribution of genotypes and their associations with clgE elevation

Genotypes of *IL13* rs1800925, rs20541, rs848, *IL4* rs2243250, and *STAT6* rs324011 were in Hardy-Weinberg equilibrium, and their distributions are listed in Table S2. There were no significant differences in genotype distributions between male and female neonates. When we evaluated the relations of these SNPs to clgE elevation using univariate logistic regression analysis, only *IL13* rs848 ($P = .03$) showed a significant association (Table 4).

3.4 Replication of the association of sex with clgE

To replicate the finding about the association of male sex with clgE elevation, we analyzed the subsample from TBPS that had information of clgE. Demographic characteristics (Table S3) and distribution of covariates of the full-term newborns (Table S4) from TBPS were shown in this article's online supporting information. Among the 219 full-term newborns of TBPS, 58 (26.5%) had elevated clgE. Male sex was the only factor significantly associated with clgE elevation (OR [95% CI] = 2.16 [1.15-4.06], $P = .02$).

3.5 Sex-moderated associations of prenatal environment with clgE elevation

Because male sex augmented the production of clgE, we examined whether neonatal sex moderated the relationship between prenatal environmental exposures and clgE elevation. After stratification by neonatal sex (Table 3), the associations of both ETS and visible mold with clgE elevation were synergistically increased by male sex (P for interaction = .03 and .03, respectively). In the replication sample of full-term newborns of TBPS, however, sex-environment interactions on clgE elevation did not reach significance.

3.6 Sex-moderated gene-environment interactions

Because the associations of prenatal environmental risk factors with cIgE elevation were moderated by neonatal sex, the prenatal gene-environment interactions for cIgE elevation and the role of sex were further explored. In the whole sample, the interactions between genetic variants in the IL4/IL13 pathway and ETS/home dampness did not reach significance (data not shown).

Gene-environment interactions were then examined for male and female neonates separately. For males (Table 5), there were significant dose-response associations of ETS with cIgE elevation for those carrying the *IL13* rs1800925 CT or TT, *IL13* rs20541 GA or AA, *IL13* rs848 CA or AA, *IL4* rs2243250 TC or CC, and *STAT6* rs324011 CT or TT genotypes (*P* for trend = .03, .004, .02, .03, and .01, respectively), with significant interaction between ETS and *IL13* rs20541 (*P* for interaction = .03).

When home dampness was indexed by combining mildewy odor, visible mold, and water stamp on the wall, there were dose-response associations of home dampness with cIgE elevation in those carrying the *IL13* rs1800925 CC, *IL13* rs20541 GG, *IL13* rs848 CC, *IL4* rs2243250 TT, and *STAT6* rs324011 CT or TT genotypes (*P* for trend = .003, .002, .001, .005, and .0002, respectively), with significant interaction between home dampness and *STAT6* rs324011 (*P* for interaction = .006).

In contrast, female newborns did not show any dose-response association of ETS or home dampness with cIgE elevation regardless of genotypes (Table S5).

Lastly, ETS and the three home dampness indices were compiled into a composite index, and the participants were divided into four subgroups (i.e., none, one, two, or $\geq$ three environmental exposures) to evaluate the relationship between environmental “load” and cIgE elevation. Among male neonates, the corresponding proportion in each exposure loading category was 212 (37.8%) for none, 247 (44.0%) for one, 76 (13.5%) for two, and 26 (4.6%) for three or more.

When the total sample was subjected to multivariable logistic regression analysis of cIgE elevation with a model containing the three-way interaction among the composite environmental exposure, *STAT6* rs324011 genotype, and neonatal sex, the three-way interaction term reached statistical significance (P for interaction = .001). Hence, subgroup analyses stratified by sex were conducted. For male newborns, as shown in Figure 1, there was a significant dose-response association of the composite environmental exposure with cIgE elevation in newborns carrying the *STAT6* rs324011 CT or TT genotype (P for trend < .0001) but not in newborns carrying the *STAT6* rs324011 CC genotype (P for trend = .95), with a significant interaction between the composite environmental exposure and *STAT6* rs324011 (P for interaction = .001). Whereas for female newborns, there was no such interaction between the composite environmental exposure and *STAT6* rs324011 genotype (data not shown).

4 DISCUSSION

4.1 Main findings

To date, this is the first study to document that only male newborns have several gene-environment interactions involving IL4/IL13 pathway genes and prenatal household environmental exposures on cIgE elevation. In particular, a composite household environmental exposure that includes ETS and home dampness exhibits a dose-response association with cIgE elevation for male newborns carrying the *STAT6* rs324011 allele T. These findings help shed new light on the underlying mechanism of cIgE elevation and have implications in the early detection and prevention of atopy or allergic diseases.

4.2 Comparison with other studies

Although the study participants of GEIBCS were enrolled only from one hospital, most of the cIgE-related characteristics are generally comparable to those in the literature. For example, the percentage of cIgE elevation in the present study (20.4%) is close to that in the replication sample (26.5%) and those reported in other studies in Taiwan (22%)¹⁶ and in Canada (18%).⁴ Other results consistent with previous studies include the positive associations of male sex²⁶ and *IL13* rs848^{24,37} with cIgE elevation and the inverse association of high parental education^{26,38} with cIgE elevation.

The household environment examined in this study included two types of common prenatal exposures. Although pregnant women infrequently smoke (2%), 46% of them have been exposed to ETS, with more than 10% of their homes having two or more smokers. The association of ETS with cIgE elevation appears only in male newborns, which is the same as that observed in our earlier study of smaller sample size²⁴ and is compatible with previous findings in adult smokers.^{29,30}

Nevertheless, the interaction between sex and ETS was not significant in the replication sample of TBPS, probably resulting from its relatively small sample size (n=219) and low prevalence of ETS (26%). The prevalence of ETS in our study sample was 46%. The differential ETS prevalences might be attributed to time and spatial differences. The babies of our study sample were born in Taoyuan during the period between 2008 and 2011, whereas the babies of TBPS were born in Taipei during the period between 2004 and 2005.

The second type of prenatal exposure, home dampness, is also common in a subtropical country like Taiwan, which has a mean monthly humidity ranging from 68% to 80%. Among the three dampness indices in the present study, visible mold is the most frequent one, followed by water stamp and then mildewy odor. Their relations to cIgE elevation vary, with mildewy odor having the strongest association, whereas the associations for visible mold and water stamp tend to be observed only in male newborns. Intra-nasal and intra-tracheal administration of mold extracts to mice has been demonstrated to induce a robust serum IgE response.^{39,40} Homes with mildewy

odor^{41,42} and homes with reported molds/dampness⁴² have higher concentrations of molds/fungus in the dust and air. Both mildewy odor²¹⁻²³ and visible mold^{20,22,43,44} have been associated with higher total serum IgE levels and increased risks of childhood asthma and rhinitis. Taken together, these dampness indices likely reflect a partially overlapping indoor environment that is associated with atopy or atopic diseases. However, none of these previous studies investigated whether the effects of home dampness were moderated by sex.

4.3 Differences between sexes and possible mechanisms

The findings that the associations of ETS and visible mold with cIgE elevation are more prominent in male than in female neonates in the current study probably reflect early differences in prenatal immune responses to environmental stimuli between sexes, which hence contribute to the known predominance of atopic diseases among boys.²⁸ The results here further show that in male newborns, the associations of ETS and home dampness with cIgE elevation depended on the particular genotypes of *IL4* and *IL13* SNPs, although the associated genotypes were different for ETS and home dampness, respectively. For male newborns carrying the minor allele of either *IL4* or any one of the three *IL13* SNPs, there were significant dose-response associations of ETS with cIgE elevation, with a significant interaction identified between *IL13* rs20541 and ETS. Whereas the dose-response associations of home dampness were noted only in male newborns not carrying the minor allele of the *IL4* or *IL13* SNPs.

One possibility is that these *IL4* or *IL13* SNPs are merely in linkage disequilibrium with some unknown culprit genetic change, and hence, the associated genotypes are not directly related to the underpinning functions. Nevertheless, there is some evidence indicating that these SNPs do lead to different levels of gene expression. For example, the genetic variants of *IL13* rs20541, *IL13* rs1800925, and *IL4* rs2243250 have been associated with increased *IL13* levels,⁴⁵ enhanced *IL13* promoter activities,⁴⁶ and augmented *IL4* promoter activities,⁴⁷ respectively.

Thus, it is worthwhile to postulate some hypotheses regarding the differential associations of the genotypes of *IL4* and *IL13* SNPs. One explanation is that ETS and home dampness probably exert their associations with cIgE production via different pathways. ETS is a complex mixture of chemicals, whereas home dampness is a biological environment containing microbes like molds. ETS can modulate immune responses,¹⁸ resulting in substantially higher production of Th2 cytokines, including IL4 and IL-13.^{18,48} ETS can also synergistically enhance the influences of the minor alleles of *IL-13* SNPs on IgE production in children.⁴⁹ Regarding home dampness, such environment has been associated with higher amounts of (1→3)-β-D-glucan,⁵⁰ which is a complex carbohydrate found in the cell wall of mold. If the Th2 response is diminished in male newborns not carrying the minor allele of *IL4* and *IL13* SNPs, this may drive a Th1 protective effect against molds⁵¹ and therefore decrease the functional dosage of (1→3)-β-D-glucan affecting individuals at home with dampness. Instead of the high functional dosage-mediated Th1 immune response, the low functional dosage of (1→3)-β-D-glucan leads to enhance Th2 immune response,⁵² which thus promotes IgE production.

Intriguingly, for *STAT6*, the dose-response effects of ETS and home dampness on cIgE elevation in male newborns depended on the presence of T allele, i.e., genotype CT or TT, implying that the different pathways of Th2 cytokine production driven by ETS^{18,48} and home dampness⁵⁰⁻⁵² may ultimately merge and link intracellularly to the essential signaling element, STAT6. Activated STAT6 can dimerize and translocate to the nucleus, where it activates the transcription of a number of target genes, and in turn induce IgE production.⁵³ Studies have shown that the T allele of *STAT6* rs324011 increases STAT6 promoter activity *in vitro*,⁵⁴ enhances the gene expression of STAT6 splice variants *ex vivo*,⁵⁴ augments IgE production in children,^{34,54,55} and leads to atopic disease.³⁵ However, no human study has reported a gene-environment interaction involving *STAT6* and prenatal household environmental exposures.

After combining ETS and the three home dampness indices into a composite variable, we further demonstrate that, in male newborns carrying *STAT6* rs324011 CT or TT genotype, prenatal exposure to ≥ 3 environmental risk factors greatly increases the proportion of cIgE elevation to 58%, whereas the absence of any prenatal exposure substantially reduces the prevalence of cIgE elevation to 8%. These novel findings imply that *STAT6* is probably a plasticity gene that leads to differential susceptibility of individuals to environmental influences.⁵⁶ In other words, individuals with genetic susceptibility may respond to negative prenatal environment (more ETS and dampness) with IgE elevation in a greater magnitude than others, but respond to positive environment (neither ETS nor home dampness) with IgE depreciation also in a greater magnitude than others. Hence, eliminating ETS and home dampness may provide the utmost benefit for male neonates with the *STAT6* rs324011 CT or TT genotype.

All of the gene-environment interactions identified in this study occur only in male neonates, providing a new insight to explain the phenomenon of increased cIgE production in male babies.^{4,26,27} The associations of the environments with IgE production has been more prominent in males than in females.^{29,30} Furthermore, sex may modify the gene-maternal atopy interaction in newborns of Chinese ethnicity, with male newborns more unfavorably affected.²⁷ One possible mechanism may be related to the lower cord blood regulatory T cell numbers in male than in female newborns.²⁵ Detailed biological mechanisms of these sex-related modifications warrant further investigations.

4.4 Strengths and limitations

The present study, with a larger sample size, showed a significant three-way interaction among the composite environmental exposure, genotype, and neonatal sex and then adopted a different stratification strategy from that of our previous study.²⁴ Nevertheless, the interaction between ETS and *IL13* rs20541 in male but not female babies were similarly found in both studies. Our previous

study stratified the babies by neonatal sex and prenatal ETS simultaneously to investigate the associations of SNPs with cIgE elevation in different strata. Meanwhile, the present study examined the associations of prenatal household environments with cIgE elevation in babies stratified by neonatal sex and different SNPs, emphasizing the importance of finding the preventable risk factors. In addition to ETS, the current study for the first time discovers home dampness as a significant factor to enhance cIgE levels in male babies with certain SNPs.

This study has some limitations. First, not all SNPs of all candidate genes in the IL4/IL13 pathway were examined. Instead, information on prenatal environment was comprehensively collected, and the moderation of their associations with cIgE production by five SNPs was investigated. Second, the participants' environmental exposures were evaluated using a self-reported questionnaire, but not by direct measurement. Thus, associations of certain environmental exposures might have been underestimated. Third, cord blood IgA levels were not measured to exclude newborns whose circulation was contaminated by maternal blood. However, studies that concurrently measured cord blood IgA and IgE concentrations reported a low rate of maternal contamination.⁹ Such contamination at most causes a non-differential misclassification which leads to underestimation, but not overestimation, of associations. Fourth, the sex-environment interactions on cIgE elevation and sex-moderation on the interactions involving genotypes were not replicated either due to small sample size or lack of genotyping in our replication sample. Further investigation is warranted.

4.5 Conclusions

In conclusion, this study for the first time demonstrates that all of the gene-environment interactions involving the IL4/IL13 pathway genes and prenatal household environments on cIgE production occur only in male neonates. There is a significant dose-response association of the composite environmental exposure with cIgE elevation in male newborns carrying the *STAT6*

rs324011 CT or TT genotype. Prenatal avoidance of ETS and home dampness in genetically susceptible male babies may help in the early prevention of allergy.

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CONFLICTS OF INTEREST

The authors do not have any conflict of interest on this paper.

AUTHOR CONTRIBUTIONS

All authors planned the study. CHC, MHW, PJC, and TSW collected and processed the specimens. CIT and MHW did the measurement of the specimens. PCC provided the data from TBPS. CHC conducted the statistical analysis, interpreted the data, and drafted the manuscript under supervision of YLL and WJC. All authors read the different versions of the manuscript, participated in the critical revision of the manuscript, and approved the final version.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed in the current study are not publicly available due to ethical concerns but are available from the corresponding author on reasonable request.

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TABLE 1 Demographic characteristics of the study sample of full-term newborns in the Gene and Environment Interaction Birth Cohort Study in northern Taiwan

Variable	Total (N = 1107)	Female (n = 546)	Male (n = 561)	<i>P</i> [†]
Maternal age (years), mean (SD)	31.2 (4.2)	31.0 (4.2)	31.4 (4.3)	.15
Parental education, n (%)				.76
Not higher than senior high school	348 (31.4)	174 (31.9)	174 (31.0)	
College or higher	759 (68.6)	372 (68.1)	387 (69.0)	
Household income per year, n (%)				.98
0~4 hundred thousand NTD	59 (5.4)	29 (5.4)	30 (5.4)	
4~6 hundred thousand NTD	120 (10.9)	61 (11.3)	59 (10.5)	
6~10 hundred thousand NTD	727 (66.1)	356 (65.9)	371 (66.3)	
≥ 10 hundred thousand NTD	194 (17.6)	94 (17.4)	100 (17.9)	
Sibling numbers, mean (SD)	0.5 (0.7)	0.5 (0.7)	0.5 (0.7)	.97
Family history of atopic disease, n (%)	680 (61.4)	340 (62.3)	340 (60.6)	.57
Gestational age (weeks), mean (SD)	38.6 (1.1)	38.7 (1.0)	38.6 (1.1)	.34
Cesarean section, n (%)	305 (27.6)	143 (26.2)	162 (28.9)	.32
Birth body weight (kg), mean (SD)	3.1 (0.4)	3.1 (0.4)	3.2 (0.4)	<.0001
Apgar score in 1 min, mean (SD)	8.1 (0.4)	8.0 (0.4)	8.1 (0.4)	.16
Apgar score in 5 min, mean (SD)	9.0 (0.2)	9.0 (0.2)	9.1 (0.3)	.06
clgE level (IU/ml), mean (SD)	0.5 (1.7)	0.4 (0.9)	0.6 (2.3)	.04
clgE level ≥ 0.5 IU/ml, n (%)	226 (20.4)	91 (16.7)	135 (24.1)	.002

clgE, cord blood immunoglobulin E; SD, standard deviation; NTD, new Taiwan dollar.

[†]t-test for continuous variables and chi-square test for categorical variables.

TABLE 2 Distribution of covariates in the study sample by the status of cord blood IgE (cIgE) elevation

Variable	cIgE levels		<i>P</i> [†]
	normal (<0.5 IU/mL) (n = 881)	elevated (≥0.5 IU/mL) (n = 226)	
Maternal age (years), mean (SD)	31.3 (4.1)	30.8 (4.6)	.13
Parental education, n (%)			
College or higher	620 (70.4)	139 (61.5)	.01
Household income per year, n (%)			.72
4~6 hundred thousand NTD	95 (10.8)	25 (11.2)	
6~10 hundred thousand NTD	579 (66.1)	148 (66.1)	
≥10 hundred thousand NTD	158 (18.0)	36 (16.1)	
Family history of atopic disease, n (%)	541 (61.4)	139 (61.5)	.98
Having any sibling, n (%)	390 (44.3)	88 (38.9)	.15
Prenatal environmental exposures			
Maternal smoking during pregnancy, n (%)	14 (1.6)	7 (3.1)	.14
Environmental tobacco smoke ^a , n (%)	398 (45.2)	112 (49.6)	.24
Number of smokers at home, n (%)			.10
1	315 (35.8)	80 (35.4)	
≥2	83 (9.4)	32 (14.2)	
Incense, n (%)	248 (28.2)	71 (31.4)	.33
Furred pet, n (%)	179 (20.3)	51 (22.6)	.46
Cockroach, n (%)	387 (43.9)	96 (42.5)	.70
Carpet, n (%)	80 (9.1)	14 (6.2)	.17
Mildewy odor, n (%)	33 (3.8)	16 (7.1)	.03
Visible mold, n (%)	200 (22.7)	57 (25.2)	.42
Water stamp on the walls, n (%)	75 (8.5)	24 (10.6)	.32
Air pollution, n (%)	147 (16.7)	39 (17.3)	.84

Birth history

Cesarean section, n (%)	248 (28.2)	57 (25.2)	.38
Male sex, n (%)	426 (48.4)	135 (59.7)	.002
Gestational age (weeks), mean (SD)	38.6 (1.1)	38.7 (1.0)	.52
Birth body weight (kg), mean (SD)	3.1 (0.4)	3.1 (0.4)	.76

IgE, immunoglobulin E; SD, standard deviation; NTD, new Taiwan dollars.

^aEnvironmental tobacco smoke is defined as having any smoker at home.

[†]t-test for continuous variables and chi-square test for categorical variables.

TABLE 3 Univariate logistic regression analysis of cord blood IgE elevation on prenatal environmental exposures and their interactions with sex in the study sample

Exposure	Total (N = 1107)	Female (n = 546)	Male (n = 561)	<i>P</i> for interaction
	OR (95% CI)	OR (95% CI)	OR (95% CI)	
Environmental tobacco smoke	1.19 (0.89-1.60)	0.82 (0.52-1.30)	1.57 (1.06-2.31)*	.03
Mildewy odor	1.96 (1.06-3.63)*	1.86 (0.58-5.96)	1.79 (0.86-3.72)	.96
Visible mold	1.15 (0.82-1.61)	0.68 (0.37-1.22)	1.53 (0.998-2.35)	.03
Water stamp on the walls	1.28 (0.79-2.07)	0.71 (0.29-1.73)	1.77 (0.97-3.26)	.09

**P* < .05

TABLE 4 Univariate logistic regression of *IL13*, *IL4*, and *STAT6* genetic polymorphisms on cord blood IgE (cIgE) elevation in the study sample of 1107 full term newborns in northern Taiwan

Polymorphisms	N	cIgE elevation	
		n (%)	OR (95% CI)
<i>IL13</i> rs1800925			
CC	797	156 (19.6)	1
CT or TT	310	70 (22.6)	1.20 (0.87-1.65)
<i>IL13</i> rs20541			
GG	496	90 (18.2)	1
GA or AA	611	136 (22.3)	1.29 (0.96-1.74)
<i>IL13</i> rs848			
CC	501	88 (17.6)	1
CA or AA	605	138 (22.8)	1.39 (1.03-1.87)*
<i>IL4</i> rs2243250			
TT	724	152 (21.0)	1
TC or CC	381	72 (18.9)	0.88 (0.64-1.20)
<i>STAT6</i> rs324011			
CC	628	139 (22.1)	1
CT or TT	479	87 (18.2)	0.78 (0.58-1.05)

* $P < .05$

TABLE 5 Relationship of cumulative exposure to environmental tobacco smoke and home dampness, respectively, to cord blood IgE elevation in male newborns, stratified by genotypes of *IL13*, *IL4*, and *STAT6* genetic polymorphisms

	<i>IL13</i> rs1800925		<i>IL13</i> rs20541		<i>IL13</i> rs848	
	CC (n = 405) aOR (95% CI)	CT or TT (n = 156) aOR (95% CI)	GG (n = 250) aOR (95% CI)	GA or AA (n = 311) aOR (95% CI)	CC (n = 255) aOR (95% CI)	CA or AA (n = 306) aOR (95% CI)
Exposure						
Smokers at home ^a						
None	1	1	1	1	1	1
1	0.93 (0.55-1.60)	2.15 (0.86-5.40)	0.47 (0.22-1.01)	2.22 (1.21-4.07)*†	0.54 (0.25-1.15)	1.98 (1.09-3.61)*
≥2	1.71 (0.79-3.68)	3.17 (1.06-9.49)*	1.34 (0.48-3.70)	2.80 (1.24-6.37)*†	1.73 (0.65-4.57)	2.34 (1.01-5.39)*
<i>P</i> for trend	.34	.03	.73	.004	.78	.02
<i>P</i> for interaction		.07		.03		.12
Dampness index ^{#b}						
None	1	1	1	1	1	1
1	1.61 (0.92-2.79)	1.16 (0.48-2.80)	1.49 (0.70-3.16)	1.62 (0.88-3.00)	1.57 (0.74-3.32)	1.50 (0.81-2.75)
≥2	2.99 (1.36-6.56)**†	0.90 (0.16-4.95)	5.63 (2.04-15.53)**‡	1.22 (0.41-3.64)	5.72 (2.09-15.62)**‡	1.15 (0.39-3.40)
<i>P</i> for trend	.003	.91	.002	.24	.001	.35

P for interaction

.12

.19

.10

[#]dampness indexes include mildewy odor, visible mold, and water stamp on the wall.

^aAdjusted for gestational age, birth body weight, maternal age, parental education, family history of atopic diseases, and perception of mildewy odor.

^bAdjusted for gestational age, birth body weight, maternal age, parental education, family history of atopic diseases, and environmental tobacco smoke.

P* < .05, *P* < .01, †*P* < .05 after FDR correction, ‡*P* < .01 after FDR correction

(To be continued)

TABLE 5 (continued) Relationship of cumulative exposure to environmental tobacco smoke and home dampness, respectively, to cord blood IgE elevation in male newborns, stratified by genotypes of *IL13*, *IL4*, and *STAT6* genetic polymorphisms

Exposure	<i>IL4</i> rs2243250		<i>STAT6</i> rs324011	
	TT (n = 363) aOR (95% CI)	TC or CC (n = 197) aOR (95% CI)	CC (n = 325) aOR (95% CI)	CT or TT (n = 236) aOR (95% CI)
Smokers at home ^a				
None	1	1	1	1
1	1.11 (0.63-1.98)	1.13 (0.52-2.47)	0.90 (0.50-1.61)	1.76 (0.83-3.74)
≥2	1.38 (0.62-3.09)	3.73 (1.34-10.43)*†	1.42 (0.63-3.24)	3.35 (1.25-8.97)*
<i>P</i> for trend	.44	.03	.60	.01
<i>P</i> for interaction		.24		.19
Dampness index ^{#b}				
None	1	1	1	1
1	1.55 (0.87-2.75)	1.36 (0.62-2.99)	0.99 (0.54-1.80)	3.03 (1.40-6.57)**†
≥2	3.38 (1.40-8.16)**†	1.26 (0.37-4.25)	1.11 (0.40-3.10)	5.35 (1.92-14.93)**‡
<i>P</i> for trend	.005	.50	.90	.0002
<i>P</i> for interaction		.23		.006

[#]dampness indexes include mildewy odor, visible mold, and water stamp on the wall.

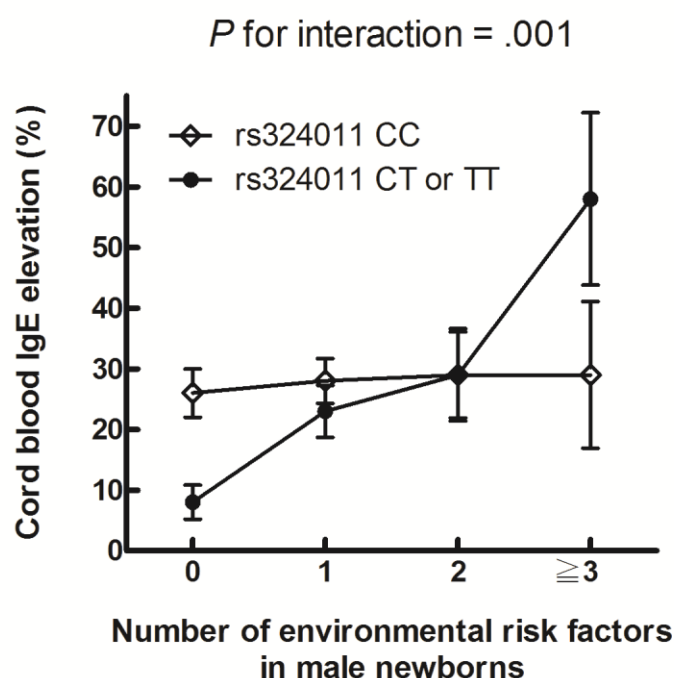
^aAdjusted for gestational age, birth body weight, maternal age, parental education, family history of atopic diseases, and perception of mildewy odor.

^bAdjusted for gestational age, birth body weight, maternal age, parental education, family history of atopic diseases, and environmental tobacco smoke.

P* < .05, *P* < .01, †*P* < .05 after FDR correction, ‡*P* < .01 after FDR correction

FIGURE LEGEND

Figure 1 Gene-environment interaction between *STAT6* rs324011 genotypes and the composite environmental exposure, which was indexed by the number of environmental risk factors (i.e., environmental tobacco smoke, mildewy odor, visible mold, and water stamp on the wall), on the status of cord blood IgE elevation in male newborns. Data were proportions $\pm$ standard error of the proportion.



Online Supporting Information

Sex moderated interactions between IL4/IL13 pathway genes and prenatal environment on cord blood IgE levels

C. H. Chen, Y. L. Lee, M. H. Wu, P. J. Chen, T. S. Wei, P. C. Chen, C. I. Tseng, W. J. Chen

TABLE S1 The relationship between environmental tobacco smoke and cotinine levels

TABLE S2 Distributions of *IL13*, *IL4*, and *STAT6* genotypes in the study sample by sex

TABLE S3 Demographic characteristics of the full-term newborns in the Taiwan Birth Panel Study

TABLE S4 Distribution of covariates in the full-term newborns from Taiwan Birth Panel Study by the status of cord blood IgE (cIgE) elevation

TABLE S5 The relations of cumulative exposure of environmental tobacco smoke and home dampness, respectively, on cord blood IgE elevation in female newborns, separately for different genotypes of *IL13*, *IL4*, and *STAT6* genetic polymorphisms

TABLE S1 The relationship between environmental tobacco smoke and cotinine levels

	Number of smokers at home			<i>P</i> [†]
	None (n =6)	1 (n =8)	≥2 (n =25)	
Elevated cotinine levels (≥ 1 ng/mL) , n (%)	0 (0)	0 (0)	7 (28)	0.1
Cotinine levels (ng/ml), mean (SE)	0.23 (8.8)	0.11 (7.6)	11.6 (4.3)	0.3

[†]chi-square test for categorical variables and ANOVA for continuous variables.

TABLE S2 Distributions of *IL13*, *IL4*, and *STAT6* genotypes in the study sample by sex

	Total (N = 1107)	Female (n = 546)	Male (n = 561)	<i>P</i> ^a
	n (%)	n (%)	n (%)	
<i>IL13</i> rs1800925				.99
CC	797 (72.0)	392 (71.8)	405 (72.2)	
CT	282 (25.5)	140 (25.6)	142 (25.3)	
TT	28 (2.5)	14 (2.6)	14 (2.5)	
<i>IL13</i> rs20541				.58
GG	496 (44.8)	246 (45.1)	250 (44.6)	
GA	488 (44.0)	234 (42.9)	253 (45.1)	
AA	124 (11.2)	66 (12.1)	58 (10.3)	
<i>IL13</i> rs848				.69
CC	501 (45.3)	246 (45.1)	255 (45.5)	
CA	486 (43.9)	236 (43.3)	250 (44.6)	
AA	119 (10.8)	63 (11.6)	56 (10.0)	
<i>IL4</i> rs2243250				.84
TT	724 (65.5)	361 (66.2)	363 (64.8)	
TC	327 (29.6)	159 (29.2)	168 (30.0)	
CC	54 (4.9)	25 (4.6)	29 (5.2)	
<i>STAT6</i> rs324011				.54
CC	628 (56.7)	303 (55.5)	325 (57.9)	
CT	404 (36.5)	202 (37.0)	202 (36.0)	
TT	75 (6.8)	41 (7.5)	34 (6.1)	

^achi-square test to compare the distributions of genotypes between females and males

TABLE S3 Demographic characteristics of the full-term newborns in the Taiwan Birth Panel Study

Variable	Total (N = 219)	Female (n = 101)	Male (n = 118)	<i>P</i> [†]
Maternal age (years), mean (SD)	31.9 (4.4)	32.2 (4.7)	31.6 (4.2)	0.30
Parental education, n (%)				0.61
Not higher than senior high school	94 (43.1)	45 (45.0)	49 (41.5)	
College or higher	124 (56.9)	55 (55.0)	69 (58.5)	
Family history of atopic disease, n (%)	82 (37.4)	36 (35.6)	46 (39.0)	0.61
Gestational age (week), mean (SD)	38.8 (1.1)	38.8 (1.1)	38.8 (1.1)	0.93
Birth body weight (kg), mean (SD)	3.2 (0.4)	3.1 (0.4)	3.3 (0.4)	0.0002
cIgE level (IU/ml), mean (SD)	0.7 (1.7)	0.6 (1.6)	0.8 (1.8)	0.55
cIgE level $\geq$ 0.5 IU/ml, n (%)	58 (26.5)	19 (18.8)	39 (33.1)	0.02

cIgE, cord blood immunoglobulin E; SD, standard deviation.

[†]t-test for continuous variables and chi-square test for categorical variables.

TABLE S4 Distribution of covariates in the full-term newborns from Taiwan Birth Panel Study by the status of cord blood IgE (cIgE) elevation

Variable	cIgE levels		<i>P</i> [†]
	normal (<0.5 IU/mL) (n = 161)	elevated (≥0.5 IU/mL) (n = 58)	
Maternal age (years), mean (SD)	32.0 (4.3)	31.7 (4.9)	0.63
Parental education, n (%)			
College or higher	89 (55.6)	35 (60.3)	0.53
Family history of atopic disease, n (%)	55 (34.2)	27 (46.6)	0.09
Prenatal environmental exposures			
Maternal smoking during pregnancy, n (%)	2 (1.25)	0 (0)	0.4
Environmental tobacco smoke ^a , n (%)	43 (26.7)	14 (24.1)	0.7
Birth history			
Male sex, n (%)	79 (49.1)	39 (67.2)	0.02
Gestational age (weeks), mean (SD)	38.8 (1.1)	38.9 (1.2)	0.71
Birth body weight (kg), mean (SD)	3.2 (0.4)	3.3 (0.3)	0.13

IgE, immunoglobulin E; SD, standard deviation.

^aEnvironmental tobacco smoke is defined as having any smoker at home.

[†]t-test for continuous variables and chi-square test for categorical variables.

TABLE S5 The relations of cumulative exposure of environmental tobacco smoke and home dampness, respectively, on cord blood IgE elevation in female newborns, separately for different genotypes of *IL13*, *IL4*, and *STAT6* genetic polymorphisms

Environmental risk factors	<i>IL13</i> rs1800925		<i>IL13</i> rs20541		<i>IL13</i> rs848	
	CC	CT or TT	GG	GA or AA	CC	CA or AA
	(n = 392)	(n = 154)	(n = 246)	(n = 300)	(n = 246)	(n = 299)
	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Smokers at home ^a						
None	1	1	1	1	1	1
1	0.81 (0.43-1.54)	0.73 (0.28-1.87)	1.23 (0.57-2.65)	0.57 (0.28-1.19)	1.27 (0.58-2.78)	0.57 (0.27-1.18)
≥2	0.54 (0.18-1.68)	0.85 (0.15-4.90)	0.37 (0.05-2.97)	0.47 (0.15-1.46)	0.75 (0.15-3.65)	0.37 (0.11-1.25)
<i>P</i> for trend	.26	.62	.75	.10	.91	.06
Dampness index ^{#b}						
None	1	1	1	1	1	1
1	0.35 (0.14-0.87)*	1.36 (0.43-4.30)	0.18 (0.04-0.78)*	1.14 (0.51-2.59)	0.29 (0.08-1.01)	0.95 (0.41-2.22)
≥2	0.79 (0.25-2.48)	1.59 (0.37-6.83)	0.55 (0.12-2.58)	1.76 (0.58-5.32)	0.60 (0.13-2.85)	1.62 (0.54-4.87)
<i>P</i> for trend	.10	.46	.05	.34	.10	.53

[#]dampness indexes include mildewy odor, visible mould, and water stamp on the wall.

^aAdjusted for gestational age, birth body weight, maternal age, parental education, family history of atopic diseases, and perception of mildewy odor.

^bAdjusted for gestational age, birth body weight, maternal age, parental education, family history of atopic diseases, and environmental tobacco smoke.

**P* < .05

(To be continued)

TABLE S5 (Continued) The relations of cumulative exposure of environmental tobacco smoke and home dampness, respectively, on cord blood IgE elevation in female newborns, separately for different genotypes of *IL13*, *IL4*, and *STAT6* genetic polymorphisms

Environmental risk factors	<i>IL4</i> rs2243250		<i>STAT6</i> rs324011	
	TT (n = 361)	TC or CC (n = 184)	CC (n = 303)	CT or TT (n = 243)
	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
Smokers at home ^a				
None	1	1	1	1
1	0.66 (0.34-1.29)	0.99 (0.41-2.39)	0.87 (0.42-1.80)	0.67 (0.31-1.45)
≥2	0.54 (0.17-1.71)	0.63 (0.12-3.28)	0.96 (0.33-2.78)	0.00 (0.00-999)
<i>P</i> for trend	.17	.68	.83	0.06
Dampness index ^{#b}				
None	1	1	1	1
1	0.39 (0.16-0.96)*	1.03 (0.34-3.07)	1.04 (0.44-2.43)	0.29 (0.09-1.01)
≥2	1.86 (0.71-4.85)	0.00 (0.00-999)	1.50 (0.45-4.97)	0.79 (0.21-2.98)
<i>P</i> for trend	.87	.29	.58	.18

[#]dampness indexes include mildewy odor, visible mould, and water stamp on the wall.

^aAdjusted for gestational age, birth body weight, maternal age, parental education, family history of atopic diseases, and perception of mildewy odor.

^bAdjusted for gestational age, birth body weight, maternal age, parental education, family history of atopic diseases, and environmental tobacco smoke.

**P* < .05