Prenatal secondhand smoke exposure is associated with atopic dermatitis in school-aged children: COCOA study

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November 15, 2022

Abstract

Background: The effect of prenatal secondhand smoke (SHS) exposure on childhood atopic dermatitis (AD) remains controversial. We aimed to investigate the association between prenatal SHS and childhood AD in a general population-based birth cohort. Methods: Patients included 2,360 mother–child pairs from the Cohort for Childhood Origin of Asthma and Allergic diseases (COCOA), stratified into 0–3, 4–6, and 7–9 years age groups. Prenatal SHS exposure was assessed using questionnaires. AD diagnosis and symptom assessments were conducted through annual visits by pediatric allergists. Skin prick tests for 18 allergens were conducted. Serum total IgE and eosinophil levels were measured at birth and ages 3 and 7 years. Maternal urine cotinine concentrations were measured at week 36 of gestation. Multivariate logistic regression was performed. Results: Children aged 7–9 years exposed to prenatal SHS were significantly more likely to have an AD diagnosis (aOR 1.670, 95% CI: 0.995–2.804) and current AD (aOR 1.823, 95% CI: 1.051–3.161). This association in AD diagnosis was stronger in children with sensitization (aOR 2.205, 95% CI: 1.048–4.642). Higher maternal urine cotinine levels increased the risk of current AD at ages 4–6 (aOR 2.816, 95% CI: 1.053–7.529). Children exposed to prenatal SHS were more likely to have a late-onset phenotype of AD (aOR 1.663, 95% CI: 1.038–2.664). Conclusion: SHS exposure during pregnancy was associated with late childhood AD. Prevention of prenatal SHS exposure is necessary to reduce the risk of AD in schoolchildren.

Introduction

Changes in the prevalence of atopic dermatitis (AD) according to age in Korean children have been observed. Although the development of AD during infancy is decreasing, prevalence in late childhood is increasing, indicating rising rates of late-onset or early-onset phenotypes (1). AD is a persistent disease with a high burden on children and their families. In combination with genetic factors, environmental influence is an important contributor to the pathogenesis of AD, indicating the importance of early interventions to improve or prevent AD outcomes(2).

Smoking during pregnancy is a well-established risk factor for adverse outcomes such as low birth weight (3), spontaneous abortion(4), and preterm birth (5) and wheezing (6). However, the proportion of active

smokers among pregnant women in Korea is comparatively lower than that in western countries. A study conducted in South Korea reported that 0.55% of mothers admitted to actively smoking, while 3.03% were considered to be actively smoking based on their urine cotinine levels (7).

Approximately 35%, 33%, and 40% of nonsmoking females, males, and children are exposed to SHS daily (8). In Korea, 60.4% of pregnant non-smokers reported exposure to SHS during pregnancy(9). The Cohort for Childhood Origin of Asthma and Allergic diseases (COCOA) study revealed that prenatal SHS exposure increases susceptibility to lower respiratory tract infections in infancy(10). Given that AD is a disease affected by environmental factors, the possibility for SHS to be a potential contributor could not be ruled out.

Therefore, we investigated the relationship between prenatal SHS exposure and AD across various groups and the effects of sensitization in a prospective birth cohort study. Additionally, we aimed to validate our findings by determining the relationship between urine cotinine levels during pregnancy and AD.

Materials and methods

Study design

The COCOA is a general population-based prospective birth cohort study designed to assess the impact of environmental exposure on allergic diseases(11). Regularly scheduled follow-ups to the physician's office and self-reported questionnaires, laboratory examinations, and physical examinations were conducted at 36 weeks of gestation, birth, 6 months, 1 year, and then annually thereafter. Mothers completed a modified version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire(12). Of the 3,004 pairs, 2,360 (78.56%) were analyzed (Fig.1) in this study. A total of 1,405 and 659 children were followed up until the ages of 3 and 7, respectively.

Definition of AD and AD phenotype

Children were stratified into early childhood (0–3 years), preschool (4–6 years), and school-age (7–9 years) groups. AD was considered present if a diagnosis was constituted at any period within the specified age group. Current AD was determined as a diagnosis within the age group and the presence of AD symptoms confirmed by a physician within the previous 12 months. Our study targeted various phenotypes, including the: early-transient, defined as AD onset within 2 years of age and no further symptoms; early-persistent, defined as AD onset within 2 years of age and symptoms not improving within 2 years; and late-onset, defined as AD onset after 2 years of age(13).

Assessment of prenatal SHS exposure

SHS exposure after birth was assessed using self-reported questionnaires. Mothers were asked to complete the questionnaire at week 36 of pregnancy. They were considered to have been exposed to SHS if they answered "Yes" to the question: "Were you exposed to secondhand smoke during the period of this pregnancy?". For quantitative assessment, urine cotinine levels of the pregnant mothers were measured at gestational week 36.

Urine cotinine level

The median urine cotinine level was 0.55 ng/mL, and mothers were designated to the higher or lower half according to their urine cotinine levels. According to a previous report, the urinary samples were frozen at -70°C until analysis, and cotinine concentrations were measured by lipid chromatography-tandem mass spectrometry using electrospray ionization (14).

Skin prick tests

Skin prick tests (SPTs) were conducted at ages 3 and 7 years for 18 allergens using normal saline and histamine as a negative and positive control, respectively (15). The 18 allergens are specified in the online supplement. A positive SPT was defined by a mean wheal diameter of [?] 3 mm and at least as large as that of the positive control. ImmunoCAP tests were conducted at ages 1, 3, and 7 years. The target allergen for the ImmunoCAP test according to the child's age was: specific IgE to egg whites and milk for age 1; egg

white, milk, and *Der f* for age 3; and egg white, milk, *Der f* , birch, and *Alternaria* for age 7. Children were considered sensitized if they exhibited positive results for SPT or if any specific IgE levels were [?] 0.35kUA/L.

Statistical analysis

The chi-squared and Fisher's exact tests were performed to compare categorical variables of smoke exposure and cotinine levels between children with and without AD. Multivariate logistic regression analysis was used after adjusting for potential confounding factors to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CIs). The confounding factors were maternal education level, sex of the children, the type of milk given during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic diseases, the presence of a pet during the first year of life, daycare attendance during the first year of life, and mode of delivery. To analyze the effect of smoke exposure after birth, children in the school age (7–9 years) group were further adjusted for SHS exposure during their 4th to 6th year of age. The paramed command in STATA version 16.1 (STATA Corp. College Station Texas, USA) was used to perform causal mediation analysis using parametric regression models with SHS as exposure, AD as the outcome, and IgE level as mediators. All statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA), and a p-value of less than 0.05 was considered statistically significant.

Ethics Statement

This study was approved by the institutional review board of Asan Medical Center (IRB No. 2008-0616), Samsung Medical Center (IRB No. 2009-02-021), Severance Hospital (IRB No. 4-2008-0588), and CHA Medical Center (IRB No. 2010- 010). Written informed consent was confirmed by each IRB and obtained from the parents of each infant.

Results

Demographics

Statistically significant differences were observed in gestational age at birth, maternal age at birth, and SHS exposure during the first year of life between the prenatally exposed and the non-exposed groups (Table 1). No other differences were found, including in parental history of allergic diseases. Except for maternal age at birth, mode of delivery, and daycare attendance during the first year of life, no significant differences regarding general characteristics were observed between the participants whose data were and were not included in the analysis (Supplementary Table 1).

The association between prenatal SHS exposure and AD

Although no statistically significant differences were noted in AD diagnosis, symptoms, and current AD between the SHS-exposed and non-exposed groups until 6 years of age, children with prenatal SHS exposure had a significantly higher risk of AD diagnosis (aOR 1.670, 95% CI: 0.995–2.804), symptoms (aOR=1.483, 95%CI: 1.021–2.155), and current AD (aOR=1.823, 95% CI: 1.051–3.161) at school age (7–9 years) (Table 2).

The association between prenatal SHS exposure and AD according to sensitization

Prenatal SHS exposure increased the risk of AD in sensitized children at school age (7–9 years) (aOR=1.048, 95%CI: 1.048–4.642), while no statistically significant association was observed in non-sensitized children (aOR=1.126, 95% CI: 0.465–2.730). This discriminatory incremental effect was also true for current AD, for which increased risk was observed in sensitized children (aOR=2.557, 95% CI: 1.114–5.869) but not in non-sensitized children (aOR=1.059, 95% CI: 0.424–2.640) (Table 3). No significant association between prenatal SHS exposure and AD was observed until age 6, regardless of sensitization.

The association between maternal urine cotinine level and AD

Children born from mothers with higher urine cotinine levels had higher risks of AD symptoms (aOR=2.764, 95% CI: 1.486–5.140) and current AD (aOR=2.816, 95% CI: 1.053–7.529) at preschool age (age 4–6) compared

with those born from mothers with lower urine cotinine levels (Table 4). No significant association between urine cotinine level and AD was observed in early childhood (age 0–3), and the number of participants with necessary data was insufficient to be analyzed at school age (age 7–9).

The association between prenatal SHS exposure, maternal urine cotinine level, and the phenotype of AD

Children exposed to prenatal SHS had a higher risk of the late-onset AD phenotype (aOR 1.687, 95% CI: 1.028–2.770) than those who were not (Table 5). Children with a higher maternal urine cotinine level tended to have late-onset AD (aOR 2.884, 95% CI: 0.834–9.975).

Discussion

Our findings indicate that prenatal SHS exposure increases the risk of late-onset AD, especially in sensitized school-age children. While the relationship between maternal urine cotinine levels and AD in school-age children could not be explored, we noted a relationship between higher maternal urine cotinine levels and the risk of AD symptoms in preschool children (ages 4–6). These results provide strong scientific support for our observations. Our analyses of the AD phenotypes have reported the effects of prenatal SHS exposure on late-onset AD. The present study implies that children exposed to prenatal SHS are at a higher risk of developing AD with its onset after age 2, and that screening for these high-risk groups may help prevent childhood AD earlier. Further studies are warranted to understand the underlying mechanisms.

A study in Japan reported no relationship between prenatal smoke exposure and the risk of AD in early childhood up to 3 years, which is consistent with our observations despite the study's short follow-up period (16). Another prospective cohort study reported an association between prenatal smoke exposure and increased wheezing but decreased atopic eczema until age 3 (17). Our study investigated data from a longer follow-up period, allowing recognition of the late-onset manifestation of AD.

We found that the cumulative effect of SHS on AD was not apparent in early infancy and was only notable after reaching childhood. Additional analysis of AD phenotypes revealed that this effect is likely due to an increase in the late-onset AD phenotype, which develops after 2 years. While not statistically significant, an incremental relationship ($p<0.1$) was observed between higher cotinine levels and the late-onset AD phenotype. The prevalence of AD in Korea peaks during infancy and then decreases throughout early childhood (18), suggesting that AD aggravated by prenatal SHS may occur as the late-onset phenotype through a different mechanism from conventional AD.

Tobacco smoke induces the formation of hydrogen peroxide and activates the cellular NOX (nicotinamide adenine dinucleotide phosphatase oxidase), leading to the translocation and subsequent loss of SR-B1 or the HDL receptor. This may affect the stratum corneum, composed of 25% cholesterol (19). Tobacco smoke also exhibits oxidative effects in human skin fibroblasts (20). DNA methylation is reportedly induced by maternal smoking in pregnancy, which may mediate the effect of maternal smoking on AD (21). The methylation status of the TSLP 5'-CpG was significantly higher in the high-exposure group based on cord blood cotinine, and the degree of methylation was associated with decreased TSLP protein expression and increased AD (22). Hence, prenatal tobacco exposure may affect DNA methylation, leading to delayed AD occurrence. Only 0.23% of the mothers in the COCOA study reported smoking during pregnancy (data not shown). Therefore, a study focusing on the effect of SHS on AD will have high clinical significance in the Korean population.

The relationship between urine cotinine and AD was analyzed to determine the quantitative effect of prenatal SHS exposure on AD. The relationship between urine cotinine and smoking status (23) has been demonstrated, and a significant relationship between "smoking currently permitted in the whole house" and positive urine cotinine has been reported (9), indicating that maternal urine cotinine levels are a significant surrogate marker for SHS exposure. However, no significant relationship was observed between AD in early childhood (ages 0–3) and cotinine levels. The definition of AD in the earlier phase of childhood tends to vary, and a significant portion of patients undergo remission with various contributors. From this study, school-age (ages 7–9) data were insufficient for urine cotinine analysis, but a significant relationship was observed between

AD in preschool children (ages 4–6) and urine cotinine levels during pregnancy, indicating an association between higher doses of cotinine and AD in childhood.

We applied the mediation model with SHS as exposure, offspring AD as the outcome, and IgE level as mediator (Supplementary Fig. 1). Total effect of SHS on atopic AD at school age (ages 7–9) was significant (OR = 2.033, p = 0.029). IgE level at age 3 significantly mediated the relationship (indirect effect OR = 1.110, p = 0.010, the proportion mediated = 14.8%), but the level at the other ages (age 1 or 7) had no indirect effect. These results showed that the IgE level at 3 years of age is a mediating factor in the relationship between SHS exposure and AD in sensitized school children. However, further study is warranted given that this association was not mediated by IgE level at other ages, and the indirect effect of IgE level was weaker than expected (Supplementary Fig.1.). Discussion regarding mechanisms related to IgE are in the online supplement.

There are a few limitations to this study. First, data on SHS exposure were investigated using questionnaires, and the intensiveness of the exposure was not considered. While it is typical to measure cotinine in the second or third trimester to assess the level of smoke exposure during pregnancy (24, 25), we measured urine cotinine at week 36 per the COCOA protocol. Nevertheless, exposure status to prenatal SHS is expected to be consistent through pregnancy since most exposure is expected to have occurred at home or work.

The main strength of our study is its prospective design. Data on the SHS exposure of pregnant mothers, their urine cotinine levels, and other potential confounders were investigated before birth, reducing biases that may corrupt data. An additional strength is that the assessment of AD was examined by pediatric allergists using a standardized research data form, and that phenotypes of AD were assessed. Furthermore, all children were adjusted for SHS exposure during their first year of life to distinguish the effects of prenatal and postnatal SHS exposure since the latter is also a major risk factor for AD(26). Children in the school age (7–9 years) group were adjusted for SHS exposure from ages 4 to 6. The COCOA cohort is a general population cohort, allowing generalization of the results of this study, especially in Asian countries with a low rate of maternal smoking during pregnancy.

Conclusion

Prenatal SHS exposure during pregnancy increases the risk of AD in school-aged children. The late-onset AD phenotype was most strongly affected by SHS exposure. Our study highlights the importance of public health strategies to reduce SHS exposure in the prenatal period and for earlier AD diagnoses. Further studies that analyze the underlying mechanisms behind the delayed manifestation of the effect of SHS exposure on AD are warranted.

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Table 1. Characteristics of the study participants

Sex (boys)	
Gestational age at birth (weeks)	
Maternal education level++	
Highschool graduation	
College/University	
Graduate school	
Maternal age at birth (years)	
Mode of delivery	
Vaginal	
Cesarean	
Feeding type until 6 months	
Breastmilk only	
Formula and Breastmilk	
Parental history of allergic disease (yes)	
SHS exposure during the first year of life (yes)	
Daycare attendance during the first year of life (yes)	
Pet exposure during the first year of life (yes)	

Values are frequency (%). SHS, Secondhand smoke exposure. * n, the number of children with each characteristic; N, the to

Table 2. Association between prenatal maternal SHS exposure and offspring AD diagnosis, symptoms, and AD in childhood

Age	Prenatal SHS exposure	AD Diagnosis	AD Diagnosis	AD Symptoms	AD Symptoms	Current AD	Current AD	
		n/N* (%)	aOR [95% CI]	n/N* (%)	aOR [95% CI]	n/N* (%)	aOR [95% CI]	aOR [95 CI]
Early childhood (0 – 3 years)	No	269 / 886 (30.4)	1.00 (ref)	380 / 886 (42.9)	1.00 (ref)	254/745 (34.1)	1.00 (ref)	
	Yes	360 / 1216 (29.6)	1.108 [0.818–1.268]	527 / 1216 (43.4)	1.155 [0.941–1.416]	342/1013 (33.8)	1.083 [0.858–1.367]	
Preschool (4 – 6 years)	No	77 / 521 (14.8)	1.00 (ref)	168 / 521 (32.2)	1.00 (ref)	70 / 416 (16.8)	1.00 (ref)	
	Yes	141 / 803 (17.6)	1.301 [0.935–1.809]	276 / 803 (34.4)	1.103 [0.854–1.424]	134 / 654 (20.5)	1.322 [0.933–1.872]	
School-aged (7 – 9 years)**	No	26 / 940 (2.8)	1.00 (ref)	69 / 220 (31.4)	1.00 (ref)	24 / 173 (13.9)	1.00 (ref)	
	Yes	71 / 410 (17.3)	1.670 [0.995–2.804]	159 / 410 (38.8)	**1.483 [1.021–2.155]**	68 / 316 (21.5)	**1.823 [1.051–3.161]**	

Age	Prenatal SHS exposure	AD Diagnosis	AD Diagnosis	AD Symptoms	AD Symptoms	Current AD	Current AD
Current AD: doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare

Age	Prenatal SHS exposure	AD Diagnosis	AD Diagnosis	AD Symptoms	AD Symptoms	Current AD	Current AD

Table 3. The association between prenatal maternal SHS exposure and offspring AD according to sensitization

Age	Allergic Sensitization	Prenatal SHS exposure	AD Diagnosis	AD Diagnosis	AD Symptoms	AD Symptoms	Current AD	Current AD
			n/N* (%)	aOR [95%CI]	n/N* (%)	aOR [95%CI]	n/N* (%)	aOR [95%CI
Early childhood (0 – 3 years)	No	No	99/382 (25.9)	1.00 (ref)	156/382 (40.8)	1.00 (ref)	93/313 (29.7)	1.00 (re
Preschool (4 – 6 years)								
		Yes	151/536 (28.2)	1.138 [0.813– 1.592]	226/536 (42.2)	1.116 [0.822– 1.514]	143/445 (32.1)	1.132 [0.794– 1.614]
	Yes	No	98/261 (37.5)	1.00 (ref)	136/261 (52.1)	1.00 (ref)	95/217 (43.8)	1.00 (ref)
		Yes	109/345 (31.6)	0.798 [0.537– 1.186]	151/345 (43.8)	0.864 [0.589– 1.268]	104/293 (35.5)	0.801 [0.520– 1.233]
School-aged (7 – 9 years)**	No	No	34/249 (13.7)	1.00 (ref)	86/249 (34.5)	1.00 (ref)	31/191 (16.2)	1.00 (re
Early childhood (0 – 3 years)								
		Yes	55/369 (14.9)	1.129 [0.686– 1.859]	126/369 (34.1)	0.908 [0.625– 1.321]	55/298 (18.5)	1.169 [0.694– 1.969]
	Yes	No	40/227 (17.6)	1.00 (ref)	74/227 (32.6)	1.00 (ref)	37/187 (19.8)	1.00 (ref)
		Yes	77/356 (21.6)	1.334 [0.837– 2.124]	126/356 (35.4)	1.082 [0.734- 1.596]	70/293 (23.9)	1.239 [0.757– 2.028]
Preschool (4 – 6 years)	No	No	10/90 (11.1)	1.00 (ref)	28/90 (31.1)	1.00 (ref)	10/72 (13.9)	1.00 (ref)
		Yes	22/175 (12.6)	1.126 [0.465– 2.730]	63/175 (36.0)	1.269 [0.707– 2.277]	21/132 (15.9)	1.059 [0.424– 2.640]
	Yes	No	13/92 (14.1)	1.00 (ref)	31/92 (33.7)	1.00 (ref)	11/70 (15.7)	1.00 (ref)

Age	Allergic Sensitization	Prenatal SHS exposure	AD Diagnosis	AD Diagnosis	AD Symptoms	AD Symptoms	Current AD	Current AD
		Yes	43/173 (24.9)	**2.205 [1.048-4.642]**	76/173 (43.9)	1.623 [0.912–2.888]	41/136 (30.1)	**2.557 [1.114–5.869]**

Age	Allergic Sensitization	Prenatal SHS exposure	AD Diagnosis	AD Diagnosis	AD Symptoms	AD Symptoms	Current AD	Current AD
Current AD: doctor confirmed the coexistence of AD diagnosis and AD symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and AD symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and AD symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and AD symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and AD symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and AD symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and AD symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and AD symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare	Current AD: doctor confirmed the coexistence of AD diagnosis and AD symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare

Age	Allergic Sensitization	Prenatal SHS exposure	AD Diagnosis	AD Diagnosis	AD Symptoms	AD Symptoms	Current AD	Current AD

Table 4. Association between maternal urine cotinine level at week 36 of gestation and offspring AD

Age	Cotinine level	AD Diagnosis	AD Diagnosis	AD Symptoms	AD Symptoms	Current AD	Current AD
		n/N** (%)	aOR [95% CI]	n/N** (%)	aOR [95% CI]	n/N** (%)	aOR [95% CI]
Early childhood (0 – 3 years)	Low	111/437 (25.4)	1.00 (ref)	183/437 (41.9)	1.00 (ref)	110/363 (30.3)	1.00 (ref)
	High	132/451 (29.3)	1.146 [0.804–1.632]	217/451 (28.2)	1.334++ [0.972–1.831]	129/360 (35.8)	1.241 [0.856–1.799]
Preschool (4 – 6 years)	Low	7/86 (8.1)	1.00 (ref)	19/86 (22.1)	1.00 (ref)	6/72 (8.3)	1.00 (ref)
	High	30/220 (13.6)	1.885 [0.765–4.644]	91/220 (41.4)	**2.764 [1.486–5.140]**	29/157 (18.5)	**2.816 [1.053–7.529]**
School-aged (7 – 9 years)**	Low	Not available	Not available	Not available	Not available	Not available	Not available
	High						

Age	Cotinine level	AD Diagnosis	AD Diagnosis	AD Symptoms	AD Symptoms	Current AD	Current AD
Current AD: A doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare during the first year of life, and mode of delivery. * n, the number of children with each characteristic; N, the total number of children	Current AD: A doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare during the first year of life, and mode of delivery. * n, the number of children with each characteristic; N, the total number of children	Current AD: A doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare during the first year of life, and mode of delivery. * n, the number of children with each characteristic; N, the total number of children	Current AD: A doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare during the first year of life, and mode of delivery. * n, the number of children with each characteristic; N, the total number of children	Current AD: A doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare during the first year of life, and mode of delivery. * n, the number of children with each characteristic; N, the total number of children	Current AD: A doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare during the first year of life, and mode of delivery. * n, the number of children with each characteristic; N, the total number of children	Current AD: A doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare during the first year of life, and mode of delivery. * n, the number of children with each characteristic; N, the total number of children	Current AD: A doctor confirmed the coexistence of AD diagnosis and symptoms within the last 12 months. Logistic regression, adjusted for maternal education level, sex of the children, the type of milk during the first 6 months of life, SHS exposure during the first year of life, parental history of allergic disease, the presence of a pet during the first year of life, attendance of daycare during the first year of life, and mode of delivery. * n, the number of children with each characteristic; N, the total number of children

Age	Cotinine level	AD Diagnosis	AD Diagnosis	AD Symptoms	AD Symptoms	Current AD	Current AD

Table 5. The association between prenatal maternal SHS exposure, maternal urine cotinine level at week 36, and AD phenotypes

Prenatal SHS exposure
No
Yes
Cotinine level
Low
High

Early transient phenotype, AD onset within 2 years of age, and no further symptoms. Early persistent phenotype, AD onse

Fig. 1. Flow chart of the study patients