Effect of early-life antibiotic exposure and IL-13 polymorphism on atopic dermatitis phenotype

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Abstract

Background: Although atopic dermatitis (AD) is associated with certain gene variants, the rapidly increasing incidence of AD suggests that environmental factors contribute to disease development. In this study, we investigated the association of AD incidence and phenotype with antibiotic exposure within 6 months of age, considering the dose administered and genetic risk. Methods: This study included 1,637 children from the COCOA birth cohort. Pediatric allergists assessed the presence of AD at each visit and obtained information about antibiotic exposure for more than 3 days. IL-13 (rs20541) polymorphism was genotyped by the TaqMan method. We stratified the AD phenotypes into 4 groups and used multinomial logistic regression models for analysis. Results: Antibiotic exposure within 6 months of age was found to increase the risk of AD within 3 years of life (aOR=1.40, 95%, CI 1.09–1.81) in dose-dependent manner. Antibiotic exposure more than twice increased the risk of the early-persistent AD phenotype (aOR=2.50, 95% CI 1.35–4.63). There was a weak interaction between genetic polymorphisms and environmental factors on the development of AD (p for interaction=0.06). Children with the IL-13 (rs20541) GA+ AA genotype have a higher risk of the early-persistent AD phenotype when exposed to antibiotics more than twice than those with the IL-13 (rs20541) GG genotype and without exposure to antibiotics (aOR=4.73, 2.01–11.14). Conclusion: Antibiotic exposure within 6 months was related to the incidence of early-persistent AD and a dose-dependent increase in the incidence of AD in childhood, whose effect was modified by the IL-13 (rs20541) genotype.

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Key message: Being wary of unwarranted prescription of antibiotics, especially in vulnerable children, might be helpful in preventing AD development in infants and modification of its clinical course.

Conflicts of interest

The authors declare no conflicts of interest in relation to this study.

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Ethical approval

The study was approved by the Institutional Review Boards of Asan Medical Center (IRB No. 2008-0616), the Samsung Medical Center (IRB No. 2009-02-021), the Severance Hospital (IRB No. 4-2008-0588), and the CHA Medical Center (IRB No. 2010-010). Written informed consent was obtained from each mother and her husband before the study-related interview was conducted. The obtainment of consent was confirmed by the IRB.

Abstract

Background: Although atopic dermatitis (AD) is associated with certain gene variants, the rapidly increasing incidence of AD suggests that environmental factors contribute to disease development. In this study, we investigated the association of AD incidence and phenotype with antibiotic exposure within 6 months of age, considering the dose administered and genetic risk.

Methods: This study included 1,637 children from the COCOA birth cohort. Pediatric allergists assessed the presence of AD at each visit and obtained information about antibiotic exposure for more than 3 days. *IL-13* (rs20541) polymorphism was genotyped by the TaqMan method. We stratified the AD phenotypes into 4 groups and used multinomial logistic regression models for analysis.

Results: Antibiotic exposure within 6 months of age was found to increase the risk of AD within 3 years of life (aOR=1.40, 95%, CI 1.09–1.81) in dose-dependent manner. Antibiotic exposure more than twice increased the risk of the early-persistent AD phenotype (aOR=2.50, 95% CI 1.35–4.63). There was a weak interaction between genetic polymorphisms and environmental factors on the development of AD (p for interaction=0.06). Children with the *IL-13 (rs20541)* GA+ AA genotype have a higher risk of the early-persistent AD phenotype when exposed to antibiotics more than twice than those with the *IL-13 (rs20541)* GG genotype and without exposure to antibiotics (aOR=4.73, 2.01–11.14).

Conclusion: Antibiotic exposure within 6 months was related to the incidence of early-persistent AD and a dose-dependent increase in the incidence of AD in childhood, whose effect was modified by the*IL-13* (rs20541) genotype.

Key words: dermatitis, atopic; phenotype; anti-bacterial agents; *IL-13* ; polymorphism

Introduction

Atopic dermatitis (AD) is a chronic, recurrent inflammatory skin disease characterized by itching eczematous lesions, and it is the leading cause of health burden due to non-fatal skin-related disease globally.(1) The prevalence rate of childhood AD ranges from 15% to 20%(2) and varies widely from one country to another globally. In Korea, primary surveys of children and adolescents demonstrated increasing trends in the prevalence of AD symptoms within the last 12 months.(3)

To identify the cause of AD and to effectively prevent and manage it, many epidemiological studies have been conducted on hygiene hypothesis. This hypothesis states that the Western lifestyle not only limits infection and microbial exposure but also alters the colonization of the gut microbiome, thereby disrupting the development of the immune system and leading to allergic disease. As part of this concept, there is growing evidence that the increased prevalence of allergic diseases can be attributed to increased exposure to antibiotics.(4) In particular, the antibiotic prescription rate for all pediatric patients increased from 34.8% in 2010 to 70.4% in 2014 in Korea.(5) Therefore, it is necessary to investigate the relationship between increasing exposure to antibiotics and the development of AD. Previous studies have demonstrated that the composition and function of the gut microbiome at 6 months of age could affect the course of AD in early childhood (6) and antibiotic administration aggravates clinical signs in a mouse model of AD (7). No other studies have analyzed the severity and natural course of AD according to the frequency of early-life antibiotic exposure. Thus, considering these factors, we hypothesized that antibiotic exposure within the first 6 months of life affects not only the severity of AD but also the natural course of AD through changes in the microbiome. In this prospective birth cohort study, we focused on the relationship between the development of AD and antibiotic exposure within 6 months, a critical period in the development of the microbiome and the immune system, and further examined whether the frequency of antibiotic exposure is differently associated with the severity and phenotypes of AD.

AD was considered a TH_2 disease characterized by interleukin (IL)-4 and 13 signaling. *IL-13* has a significant impact on the alteration of the skin microbiome, causing the deterioration in barrier function of the skin, and it may be a more important mediator for the TH_2 response in the skin than*IL-4*. (8) Previous studies have identified that polymorphisms in the *IL-13* promoters are associated with the development of AD,(9, 10) and among them, many studies have focused on rs20541, especially in terms of its relationship with AD, in the existing Asian population.(11-13) Following on from the above considerations, we hypothesized that antibiotic exposure influences the AD phenotype and this relationship can be modified by *IL-13* polymorphism. In this study, we explored the association of the occurrence and phenotype of AD with antibiotic exposure within 6 months of age, considering the dose administered and *IL-13* (rs20541) polymorphism.

Methods

Study population

The COCOA (COhort for Childhood Origin of Asthma and Allergic diseases) comprised the general Korean population and aimed to investigate the causal contribution of genetics, perinatal environment, maternal lifestyle, and psychosocial stress of the mother and child on pediatric susceptibility to allergic diseases.(14) (See "study population" in the Online Repository)

Of the 2,846 enrolled infants, 488 were lost to follow-up; children with incomplete questionnaire data about AD and antibiotic exposure until 5 years of age were excluded. The remaining 1,637 children were included in this study.

DNA Collection and SNP Genotyping

Genomic DNA was extracted from the cord blood mononuclear cells of each child and genotyped for *IL-13* (rs20541) polymorphisms using the TagMan assay (ABI, Foster City, CA, USA). The endpoint fluorescent readings were measured on an ABI 7900HT Sequence Detection System (ABI, Foster City, CA, USA). The details of the methods are described in a previous study.(15) Duplicate samples and negative controls were included to ensure genotyping accuracy.

Physician's assessment of allergic diseases and antibiotic exposure

The presence of AD was clinically diagnosed by pediatric allergists on the basis of the Hanifin and Rajka criteria.(16) Presence of AD was defined as physician-diagnosed AD in the preceding 12 months during each follow-up annually, and the incidence of AD was defined as the number of new cases of physician-diagnosed AD that developed since birth over a defined period. At consecutive visits, AD patients were assessed by pediatric allergists using the SCORing Atopic Dermatitis (SCORAD). Higher numbers indicate greater severity, and the scale ranges from 0 to 103.(17) AD severity was categorized as mild (<15) and moderate to severe ([?]15) according to the objective components of the index (clinical signs and disease extent).(18)

Early antibiotic treatment was defined as exposure to antibiotics for more than 3 days within the first 6 months of life, regardless of whether the infant was hospitalized or not, and pediatric allergists obtained the information about the antibiotics at each visit after birth.

Outcomes

Our primary outcome of interest was AD incidence at the age of 6 months and 1 to 3 years according to the frequency of antibiotic exposure within 6 months as assessed by pediatric allergists. Other primary outcomes were the influence of antibiotic exposure on AD phenotypes and investigator-reported clinical signs (SCORAD).

The secondary outcomes were AD incidence at the age of 6 months and 1 to 3 years according to IL-13 genetic variations. Other secondary outcomes were combined effects of IL-13 genetic variations and antibiotic exposure on the AD incidence and phenotypes. Two transition periods were considered, namely, age from 6 months to 2 years and from 2 to 5 years, to classify AD phenotypes. We defined four different phenotypes of AD: the early-transient phenotype, with AD onset within 2-years of age and no further symptoms later; the early-persistent phenotype, with onset within 2 years of age and symptoms do not improve within less than 2 years; the late phenotype, with onset after 2 years of age; and the non-AD.(19)

Statistical analysis

Data are presented as frequencies and proportions for categorical variables. Chi-square test and Fisher's exact test were performed to evaluate the associations between the incidence of AD at the age of 1 year and the variables. To assess effect modification according to*IL-13* polymorphisms and early-life antibiotic exposure, subjects were divided into 4 groups according to environmental factors (antibiotic use within 6 months for more than 3 days) and genetic background (genotypes) and multivariable logistic regression models were performed with the data to estimate adjusted odd ratios (aOR) and the corresponding 95% confidence intervals (95% CI) for a comparison of AD occurrence risk by relevant covariates after adjusting for potential confounders: sex, maternal education level, family history of allergic diseases, history of breastfeeding, and mode of delivery. Multinomial logistic regression analysis was also used to identify the effects of the use of antibiotics within 6 months of age and the combined effect of the use of antibiotics and IL-13 genetic variations on AD phenotypes. Finally, we tested for trends regarding the effect of the risk factors (early-life environmental factors and genetic polymorphisms) on the development of AD by two-factor analysis of variance (two-way ANOVA). All statistical tests were two-sided, and significance levels of p values were set at <0.05. Statistical analyses were performed using SPSS Statistics, version 23.0 (IBM SPSS Statistics, Inc., Chicago, IL).

Results

Study population

The demographic characteristics of the participants are shown in Table 1. Children diagnosed with AD at 1 year accounted for 25.3% of the study subjects, with a higher proportion of boys, when compared to non-AD children. No significant differences were observed in the prevalence of antibiotic exposure during pregnancy and history of acute bronchiolitis within 6 months (p=0.85 and 0.17, respectively).

Association between antibiotic exposure within 6 months of age and AD

In multivariable regression analyses, antibiotic exposure within 6 months of age increased the risk of AD at each visit after adjusting for sex, maternal education, family history of allergic disease, breastfeeding, and mode of delivery (Fig. 1; at the age of 6 months: aOR=1.30, 95% CI 0.99–1.71, p=0.06; at 1 year: aOR=1.28, 95% CI 1.00–1.65, p=0.05 at 2 years: aOR=1.38, 95% CI 1.08–1.77, p=0.01; at 3 years: aOR=1.40, 95% CI 1.09–1.81, p=0.01). Furthermore, the more the antibiotics were prescribed, the higher was the dose-dependent risk of AD at 6 months to 3 years (Fig. E1).

Association between antibiotic exposure within 6 months of age and AD phenotypes

We define 4 different phenotypes of AD(19): the early-transient phenotype (n = 111; 14.8%), the early-persistent phenotype (n = 199; 26.5%), the late phenotype (n = 70; 14.8%), and the never/infrequent phenotype (n = 370; 49.3%).

Antibiotic exposure within 6 months of life was associated with early-persistent AD compared with non-AD (Table 2; aOR=1.84, 95% CI 1.18–2.86), and the odds increased with a greater number of antibiotic treatment courses (once: aOR=1.51, 95% CI 0.90–2.55; [?]2 times: aOR=2.50, 95% CI 1.35–4.63; p=0.049). There was no relationship between antibiotic exposure and phenotypes other than early-persistent AD.

Association between antibiotic exposure within 6 months of age and severity of AD

According to the SCORAD index, when antibiotic exposure occurred within 6 months of age, 55 patients (66.3%) had mild AD and 28 patients (33.7%) had moderate to severe AD at the age of 1 year. In contrast, when antibiotic exposure did not occur, the severity of AD at 1 year was predominantly mild, accounting for 82.5% of the cases (Table 3, p<0.01). Mean SCORAD score was 9.42 (SD = 10.00) in 1 year children with AD who were exposed to antibiotics, which was significantly higher than that of AD patients who did not have exposure to antibiotics within the first 6 months of life (p=0.02).

Combined effects of *IL-13* gene polymorphism and antibiotic exposure within 6 months of age on AD development

We found some evidence for the interaction between genetic polymorphisms and environmental factors on the development of AD (p for interaction=0.06). Compared to infants who were not exposed to antibiotics within the first 6 months of age and had the *IL-13*(rs20541) GG genotype, those who were exposed to antibiotics and had the*IL-13* (rs20541) GA+AA genotype had a higher risk of AD at a young age (Table 4).

Regarding antibiotics, the dose–response relationship was particularly notable in children with *IL-13* (rs20541) GA+AA genotype (Fig. E2). The higher the number of prescriptions, the higher was the risk of AD at 2- and 3-year-old children (at 2 years, once: aOR=1.49, 95% CI 0.96–2.32; [?]2 times: aOR=2.00, 95% CI 1.18–3.42; at 3 years, once: aOR=1.57, 95% CI 1.00–2.46; [?]2 times: aOR=1.96, 95% CI 1.13–3.38)

Association between antibiotic exposure within 6 months of age and *IL-13* gene polymorphism on the phenotypes of AD

In the final model, we considered the combined effects of *IL-13*gene polymorphism and antibiotic exposure within the first 6 months of age on the phenotypes of AD. The*IL-13* (rs20541) GA+AA genotype was associated with early-persistent AD when the non-AD with GG genotype was considered as a reference (Table 5). Of children with the *IL-13* (rs20541) GA+AA genotype, children who were exposed with antibiotics more than 2 times was associated with a higher risk of early-persistent AD than those who were exposed with antibiotics only once, and this effect was even pronounced in infants who were never exposed with antibiotics (never: aOR=2.06, 95% CI 1.13-3.77; once: aOR=2.99, 95% CI 1.44–6.22; [?]2 times: aOR=4.73, 95% CI 2.01–11.14).

Discussion

This prospective birth cohort study revealed that most children who were at risk of developing AD, especially the early-persistent AD phenotype, had antibiotic exposure within 6 months of life, which occurred in a dose-dependent manner. Moreover, the relationship between antibiotic exposure and the development and

persistency of AD can be modified by*IL-13* genetic susceptibility. These findings suggest that early life antibiotic exposure to a certain extent contributes to the development of AD and phenotype, especially in susceptible infants, and this can be modified by efforts toward primary prevention.

Despite antibiotic exposure in early life being a plausible risk factor for the development of AD in children, existing epidemiological evidence is controversial. Our finding on the association of antibiotics with AD in dose-response manner was similar to those in previous reports. A systematic review reported a significant positive dose–response association and approximately 7% increase in the risk of AD due to antibiotic exposure during the first year of life.(20) Another prospective cohort study from Japan reported that an increase in the current AD risk in 5-year-old children is due to antibiotic exposure within the first 2 years of life.(21) However other studies concluded that there was no such relationship between antibiotic exposure in infancy and the development of AD.(22, 23) Differences in results could be due to differences in exposure to antibiotic dose, types of antibiotics, exposure time, definition and timing of outcome, and target sample size.

Although the basic etiology of AD is not fully known, it is thought to be attributable to complex, but interrelated biologic pathways, such as dysfunction of the skin barrier and altered innate or adaptive immune responses.(24) The recent increase in the incidence of AD seems to be due to changes in lifestyles and environmental factors. For example, there is evidence that increasing antibiotic exposure in early life contributes to increased AD susceptibility in children. Note that the exposure to at least one antibiotic between 0-6 months and 0-1 year occurred in 34.3% and 67.4% of the study population, which comprised the COCOA study population (data not shown); this rate was higher than those reported in other studies.(21, 25) Therefore, much attention should be paid to various antibiotic exposure-related factors influencing human health.

The hypothesized mechanism was supported by findings of studies showing antibiotic-induced killing of commensal bacteria is important for the normal development of immune function, which in turn, leads to gut dysbiosis of the microbial community in infants. This increases the risk of developing allergic conditions later in life. In mouse model studies, antibiotics administration exacerbated clinical signs of AD and caused gut dysbiosis such as increased levels of Th2 cytokine *IL-4* with significantly suppressed short-chain fatty acid levels, which influence Treg cell induction and enhance barrier function.(7) To identify potentially relevant gut microbiome changes in the early life with regard to antibiotics use and AD outcomes in children, we additionally analyzed the fecal samples of 235 Korean infants who were enrolled in COCOA study (See "**Gut microbiome analysis** " in the Online Repository). The relative abundance of Firmicutes was significantly lower and Fusobacteria composition was significantly higher in AD subjects with antibiotic exposure compared with those without antibiotic exposure (Fig. E3A, E3B), which is consistent with other studies.(26, 27) Recent evidence indicates that a disproportionate representation-such as a decrease or increase in the composition-of Firmicutes and Fusobacteria can impact the CD4 T cell function and development of immune pathology in intestine.(26, 27) Therefore, antibiotic exposure in early life may affect immune development through changes in gut microbiomes, which attribute to increase the incidence of AD.

Another pathogenetic mechanism is that gut epithelial barrier destruction through the disturbance of microbiota after antibiotic administration may lead to tissue damage and allergic sensitization.(28) The aforementioned studies suggest that gut epithelial inflammation resulting from antibiotic-induced dysbiosis may play a decisive role in the development, persistence, or aggravation of AD.

Several studies have indicated that *IL-13 (rs20541)* is associated with the risk of AD(9, 29) and there may be gene–environment interactions between *IL-13* polymorphisms and antibiotic exposure in early life, which affects the clinical features of allergic diseases.(11) Since studies regarding the association of AD phenotypes with these factors are lacking, this study focused on the effect of the interaction between risk genes and environmental risk factors and demonstrated that while antibiotic exposure in early life itself may influence the development of AD, especially early-persistent AD, this trend was particularly notable in infants carrying the *IL-13 (rs20541)* variant. However, we should note that we had limited power to detect such an interaction.

Key factors of AD are defects in the skin barrier function, abnormality of the skin immunologic barrier, and dysbiosis, which may aggravate each other. Gut dysbiosis induced by antibiotics was associated with reduction in mucosal CD4+T cells expressing IFN-γ which play a role in maintaining the barrier function of the skin,(27) therefore predisposes the infants to cutaneous disease. In addition, when considering the complex nature of AD along with genetic and environmental risk factors,*IL-13* genetic polymorphism increases *IL-13* production, which affects the expression level of the skin barrier protein,(13) and this may have an additional role in aggravating the barrier function of the skin. Further studies regarding skin barrier disturbance, microbiome and metabolite\souts induced during early life antibiotic exposure are needed to support a plausible biological pathway.

The strength of our study is the use of a large general population-based birth cohort and the measurement of potential confounders included in the analysis. We found the results for AD to be similar to those in previous studies, suggesting that antibiotic exposure in early life was associated with the risk of childhood AD.(30, 31) Our study is also strengthened by the use of pediatric allergist's medical report of doctor-diagnosed AD as the outcome variable. In addition, this study assessed the effects of antibiotic exposure in the first 6 months of life, focusing on not only the occurrence but also the severity and persistency of AD in childhood, raising awareness about the prescription of antibiotics that can be abuse.

This study also had some limitations. First, our cohort study could not determine the exact dose, total duration, and types of antibiotics that could influence the effect of antibiotics on immune responses. As broad-spectrum antibiotics were found to have stronger effects than the narrow-spectrum one,(23) further study by utilization of the National Health Insurance data is needed to confirm these specific effects of individual antibiotics on AD. Another limitation is the increased incidence of skin infections in children with AD, which makes it difficult to identify a genuine causal association, as children are more likely to receive antibiotics. Additional studies are needed to confirm the causality and prove the mechanism by which antibiotic exposure at a young age causes changes in the skin or gut microbiome and leads to the development of AD. Moreover, acute bronchiolitis, one of the main causes of antibiotic exposure in early life, is a long-term risk factor for asthma; hence, frequent bronchiolitis may be related to predisposition to allergy. However, there was no significant difference in the history of acute bronchiolitis within the first 6 months of life according to the diagnosis of AD. More studies are still needed on maternal antibiotic administration during pregnancy, antibiotic exposure after 6 months after birth, and confounding factors caused by maternal and child infections.

Although AD is not a life-threatening disease, more than 60% of children with AD are predisposed to develop one or more atopic comorbidities, such as food allergy, asthma, or allergic rhinitis, which is so-called "atopic march."(32) Therefore, it is important to define the exact role of early-life antibiotic exposure to evaluate its association with the development and clinical course of AD and progression to other allergic diseases to make successful strategies in allergy prevention. Our present study suggested that children with antibiotic exposure early in life were at risk of developing early-persistent AD, and a dose–response relationship was observed in young children, particularly in infants carrying *IL-13* (rs20541) genetic susceptibility alleles. Attention needs to be paid to unwarranted prescription of antibiotics, especially in susceptible children receiving primary care. This study will improve our understanding on the influence of genetic and environmental causes and their interactions on childhood AD and provide comprehensive insights into the pathogenesis and phenotype of AD and therefore enable improved prevention.

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Table 1. Demographic characteristics of the study population

		AD incidence at 1 year No (n=1089)	AD incidence at 1 year Yes (n=369)	P-val
History of the parent's allergic disease	Yes	571/1087(52.5)	208/367(56.7)	0.169
Sex	Boys	451/904 (49.9)	172/295(58.3)	0.012
Exposure to ETS (pregnancy)	Yes	568/1023(55.5)	179/346(51.7)	0.221
Delivery method	CS	416/1084(38.4)	127/368(34.5)	0.185
Highest education level of the mother	High school	56/1626(3.9)	13/289(3.5)	0.077
	University/college	814/1626(75.1)	265/289(71.8)	
	Graduate school	214/1626(19.7)	91/289(24.7)	
Breastfeeding at 6 months	Yes	853/1042(81.9)	294/358(82.1)	0.912
Exposure to antibiotics during pregnancy	Yes	147/1029(14.3)	48/346(13.9)	0.849
Acute bronchiolitis history within 6 months	Yes	100/933(10.7)	44/325(13.5)	0.169
Respiratory disease within 6 months	Yes	105/934(11.2)	49/325(15.1)	0.069
AOM within 6 months	Yes	129/1089(11.8)	44/369(11.9)	0.968

AD, Atopic dermatitis; ETS, Environmental tobacco smoke; URI, Upper respiratory infection; AOM, acute

otitis media; CS, Cesarean section

Data are reported as number (%).

Chi-square test for proportions

Table 2. Multinomial logistic regression model for the association of antibiotic exposure within 6 months of age with the atopic dermatitis phenotypes.

Antibiotic exposure within the first 6 months of life	Atopic dermatitis phenotypes between 6 months to 5 years (reference
	Non-AD (ref.) *vs.* Early transient aOR
No	1
Yes	0.97
No	1
Once	0.97
2 times	0.98

AD, Atopic dermatitis; aOR, adjusted odds ratio; CI, confidence interval

Values are presented as aOR (95% CI) from multinomial logistic regression models, related to non-AD.

Adjusted by sex, maternal education, family history of allergic disease, breastfeeding, mode of delivery

P <0.05 calculated by multinomial logistic regression analysis.

Table 3. SCORAD score for atopic dermatitis patients at each time point based on antibiotic exposure within 6 months of age.

Variables	Variables	Antibiotic exposure within 6 months of age	Antibiotic exposure within 6 mo
		Yes (n=566)	No (n=1071)
AD at 6 months	Number	77	110
	Mean ± SD	12.56±10.94	14.24±12.13
	Mild(<15)	49 (63.6)	71 (64.5)
	Moderate-severe([?]15)	28(36.4)	39(35.5)
AD at 1 year	Number	83	143
	Mean ± SD	9.42±10.00	6.38±9.26
	Mild (<15)	55(66.3)	118 (82.5)
	Moderate-severe ([?]15)	28(33.7)	25(17.5)
AD at 2 years	Number	104	149
	Mean ± SD	7.67±10.40	5.71±9.07
	Mild (<15)	77(74.0)	123(82.6)
	Moderate-severe ([?]15)	27(26.0)	26(17.4)
AD at 3 years	Number	87	142
	Mean ± SD	5.85±11.38	4.09±7.89
	Mild (<15)	72(82.8)	123(86.6)
	Moderate-severe ([?]15)	15(17.2)	19(13.4)

AD, Atopic dermatitis; SCORAD, SCORing*atopic dermatitis; SD,*standard deviation

Data were expressed as number and means with standard deviation (SD) of the estimated mean.

Student's t test for means, as appropriate.

mild AD: Score <15; moderate to severe AD: score [?]15

Table 4. Combined effects of *IL-13* (rs20541) genetic variation and exposure to antibiotics within 6 months of age on the development of atopic dermatitis.

IL-13 genotype	Antibiotic exposure	AD ever at 6 months aOR	AD ever at 6 months 95% CI	AD ever at 6 months 95% CI	AD ever at 1 year aOR	AD ever at 1 year 95% CI	AD ever at 1 year 95% CI	AD ever at 2 year aOR	AD ever at 2 year 95% CI	AD ever at 2 year 95% CI	AD ever at 3 year aOR	A e 3 9 C
GG	No	1			1			1			1	
GG	Yes	1.09	0.66	1.80	0.90	0.58	1.41	1.04	0.68	1.58	1.16	0
GA+AA	No	1.27	0.85	1.90	1.00	0.70	1.43	1.11	0.80	1.56	1.09	0
GA+AA	Yes	**1.66**	1.07	2.57	1.55	1.05	2.29	1.85	1.26	2.70	1.83	1
Interaction p[a]		0.48	0.48	0.48	0.06	0.06	0.06	0.08	0.08	0.08	0.17	0

AD, Atopic dermatitis; IL, interleukin; aOR, adjusted odds ratio; CI, confidence interval

Adjusted by sex, maternal education, family history of allergic disease, breastfeeding, mode of delivery

P< 0.05, Statistically significant differences were determined using a two-way analysis of variance to examine the effects of*IL-13* genetic variation and/or antibiotics within 6 months of age and/or interaction

Table 5. Multinomial logistic regression model for the association of antibiotic exposure within 6 months of age and *IL-13* (rs20541) genetic variation with the atopic dermatitis phenotypes.

IL-13	No. of antibiotic courses within the first 6 months of life	Non-AD (ref) *vs.* Early transient	Non-AD (ref) *vs.* Early transient	Non-AD (ref) *vs.* Early transient	Non-AD (ref) *vs.* Late onset	Non-AD (ref) *vs.* Late onset	Non-AD (ref) *vs.* Late onset	Non-AD (ref) *vs.* Early persistent	Non-AD (ref) *vs.* Early persistent
		aOR	95% CI	95% CI	aOR	95% CI	95% CI	aOR	95% CI
GG	Never	1.00			1.00			1.00	
	Once	0.64	0.20	2.04	2.05	0.79	5.29	1.52	0.63
	[?]2 times	1.11	0.33	3.74	0.80	0.16	3.95	1.62	0.55
GA/AA	Never	1.19	0.62	2.29	0.92	0.41	2.04	2.06	1.13
	Once	1.30	0.55	3.07	0.81	0.27	2.43	2.99	1.44
	[?]2 times	0.81	0.21	3.09	1.21	0.31	4.82	4.73	2.01

AD, Atopic dermatitis; IL, interleukin; aOR, adjusted odds ratio; CI, confidence interval

Values are presented as aOR (95% CI) from multinomial logistic regression models, related to Non-AD.

Adjusted by sex, maternal education, family history of allergic disease, breast feeding, mode of delivery

P <0.05 calculated by multinomial logistic regression analysis.

Figure legends

Fig. 1. Forest plot showing the adjusted odds ratio (95% confidence interval) for association of incidence of atopic dermatitis at the age of 6 months and 1 to 3 years with antibiotic exposure within the first 6 months of age.

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`Figure.pdf` available at https://authorea.com/users/357972/articles/502357-effect-of-early-life-antibiotic-exposure-and-il-13-polymorphism-on-atopic-dermatitis-phenotype