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의학석사 학위논문

생애 초기 항생제 노출과 *IL-13* 다형성의 상호작용이 조기지속성 아토피피부염에 미친 영향

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이 논문을 의학석사 학위 논문으로
제출함

2021년 2월

울 산 대 학 교 대 학 원
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박민지의 의학석사학위 논문을
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국문요약

생애 초기 항생제 노출과 *IL-13* 다형성의 상호작용이 조기지속성 아토피피부염에 미친 영향

박민지

울산대학교 대학원 의학과 전공

아토피피부염은 특정 유전자 변이와 관련이 있지만 아토피피부염의 유병률이 빠르게 증가하는 것은 변화된 환경적 요인이 질병 발생에 기여한다는 점을 시사한다. 최근 질병의 발생기전을 이해하는데 위생가설이 제기되고 있는데 그 중에서도 항생제가 미생물을 통해 아토피피부염의 발생에 영향을 미친다는 보고가 있었다. 하지만, 여러 연구들 간에 어린 시기의 항생제 노출과 아토피피부염 발병 사이의 연관성에 대해서는 아직까지도 논란이 많고, 이러한 노출이 아토피피부염의 자연경과 및 중증도에 어떠한 영향을 주는지 아직까지 밝혀진 바가 없다. 따라서 본 연구를 통해 생애 초기 6 개월 이내의 항생제의 노출이 아토피피부염의 발생과 아토피피부염 표현형 및 중증도에 미치는 영향을 항생제 횟수를 고려하여 살펴보고자 한다.

또한, 아토피피부염의 발생에는 Th2 면역반응이 중요한데, 인터루킨 *(IL)-4, -13*과 같은 Th2 사이토카인이 생산되어 염증반응을 일으킨다. 최근 *IL-13*의 promoter 부위의 유전자 다형성과 아토피피부염의 발생에 대한 다양한 연구가 보고되었고 그 중 아시아인종에서는 rs20541 과 아토피피부염 간의 관련성에 대해 더 많은 보고가 있었다. 따라서 위의 사항들을 고려하여, 항생제 노출이 아토피피부염의 발병 및 자연경과에 영향을 미치고 이러한 관련성이 *IL-13* (rs20541) 유전자 다형성에 의해 수정될 수 있다고 가설을 세웠다.

우리나라에서 알레르기질환 연구를 목표로 시작된 병원과 지역사회 기반 출생 코호트인 Cohort for Childhood Origin of Asthma and allergic diseases (COCOA)를 통해 1,637명의 아동을 대상으로 소아 알레르기 분과 전문의사들이 6개월, 1세 이후 매년 설문지와 함께 매 방문마다 아토피피부염 유무를 평가하고, 3일 이상 항생제를 사용하였는지 여부에 대해 조사하였다. *IL-13* (rs20541) 유전자 다형성은 TaqMan PCR기법을 이용하여 확인하였다. 성별, 엄마의 교육수준, 알레르기질환 가족력, 수유방법, 분만 방법에 의한 영향을 보정하여 항생제 및 *IL-13* (rs20541) 유전자 다형성, 그리고 이 두 요소의 상호작용의 아토피피부염 발병에 대한 교차비 (odds

ratio; OR)를 계산하기 위하여 로지스틱 분석을 이용하였다. 아토피피부염의 중증도는 SCORAD를 사용하여 아토피피부염 병변의 범위(extent), 정도(intensity)에 대한 진찰을 시행하였으며, 항생제 노출 및 *IL-13* (rs20541) 유전자 다형성, 그리고 이 두 요소의 상호작용이 중증도에 미치는 영향을 분석하였다. 또한 아토피피부염의 표현형을 구분하기 위해 생후 6개월에서 2세 미만, 2세에서 5세미만으로 나누어 조기 발병성, 후기 발병성, 조기 지속성, 그리고 아토피피부염이 아닌 그룹, 총 4그룹으로 분류하였고, 생애 초기 항생제 노출 및 유전자 다형성, 그리고 이 둘의 상호작용이 아토피피부염 표현형에 미치는 영향을 조사하기 위해 다항 로지스틱 회귀 모델을 이용하였다.

생후 6개월 이내의 항생제 노출은 생후 3년 이내의 아토피피부염의 발생 증가와 관련이 있었으며 (aOR=1.40, 95% CI 1.09-1.81) 항생제 사용 빈도가 증가함에 따라 1-3세의 아토피피부염의 발생률이 통계적으로 유의하게 증가하였다. 생후 6개월 이내에 항생제에 2회 이상 노출된 영아는 항생제를 전혀 사용하지 않는 영아에 비해 조기 지속성 아토피피부염 발생 위험도가 2.5배 증가했다 (aOR=2.50, 95% CI 1.35-4.63). 중증도에 대해 분석을 하였을 때, 항생제 노출이 없는 경우 1세 때 경증 아토피피부염은 82.5%로 대다수를 차지한 반면, 항생제에 노출이 있는 경우 66.3%의 아이들이 경증 아토피피부염으로 파악되어, 항생제에 대한 노출이 있을수록 중증도가 증가하는 것을 볼 수 있었다.

또한 *IL-13* (rs20541) GA+AA 유전자형을 가진 영아는 GG 유전자형을 가진 영아에 비해 1세때 아토피피부염이 발생할 위험도가 더 높았고 (aOR=1.27, 95% CI 0.99-1.62) *IL-13* (rs20541) GA+AA 유전자형을 가진 어린이가 생후 6개월 이내에 2회 이상 항생제에 노출될 경우, GG 유전자형을 가진 어린이가 항생제에 노출되지 않는 경우 보다 조기 지속성 아토피피부염으로 발현될 위험이 높았다 (aOR=4.73, 95% CI 2.01-11.14).

결론적으로, 생후 6개월 이내 항생제에 노출될 경우 1-3세의 아토피피부염의 발병을 용량 의존적으로 증가시켰을 뿐만 아니라 조기 지속성 아토피피부염의 위험을 높였고, 이 효과는 *IL-13* (rs20541) GA+AA 유전자형에 의해 두드러짐을 보였다

중심 단어: 아토피피부염, 항생제, 표현형; *IL-13* 유전자 다형성

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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)] Moreover, it was found that antibiotics are prescribed to toddlers at a prescription rate that is 7.5-fold higher than that in Norway.[6)] 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 [7)] and antibiotic administration aggravates clinical signs in a mouse model of AD[8)]. 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*.[9)] Previous studies have identified that polymorphisms in the *IL-13* promoters are associated with the development of AD,[10, 11)] and among them, many studies have focused on rs20541, especially in terms of its relationship with AD, in the existing Asian population[12-14)]. 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.

Materials and methods

1) Study population

The COCOA (COhort for Childhood Origin of Asthma and Allergic diseases) was composed of the general Korean population after recruiting healthy pregnant women who delivered at five hospitals in a metropolitan city (Seoul) from December 2007 to investigate the causal contribution of genetics, perinatal environment, maternal lifestyle, and psychosocial stress of mother and child on pediatric susceptibility to allergic diseases. The delivery mode and other prenatal variables were extracted from the maternal and neonatal medical records shortly after delivery. Regular follow-up visits for clinical examination by the physician and assessment using self-report questionnaires about indoor environment were conducted as scheduled, starting from 36 weeks of gestation, at birth, at the ages of 6 months, 12 months of age, and regularly once a year thereafter. The mother completed a modified version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. From annual follow-ups conducted by pediatric allergists, we collected information on the allergic symptoms, diagnosis, and given treatment at that time.

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.

2) Ethics Statement

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.

3) 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.

4) Physician's assessment of AD and antibiotics 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.

5) 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]

6) 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

1) 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).

Table 1. Demographic characteristics of the study population

		AD incidence at 1 year		P-value
		No (n=1089)	Yes (n=369)	
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

2) Association between antibiotics 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. 2).

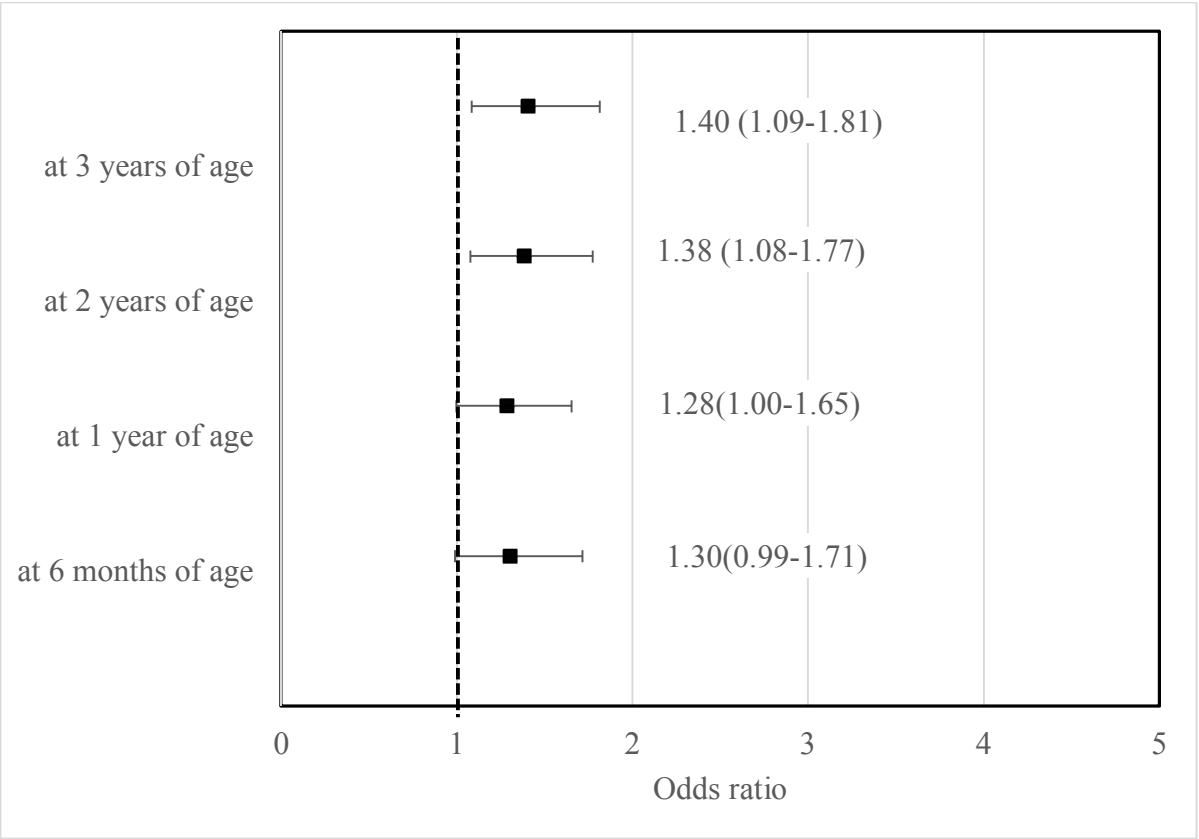


Figure 1. Forest plot showing the adjusted odds ratio (95% confidence interval) for association of incidence of atopic dermatitis at the age of 6 months and 1 to 3 years with antibiotic exposure within the first 6 months of age.

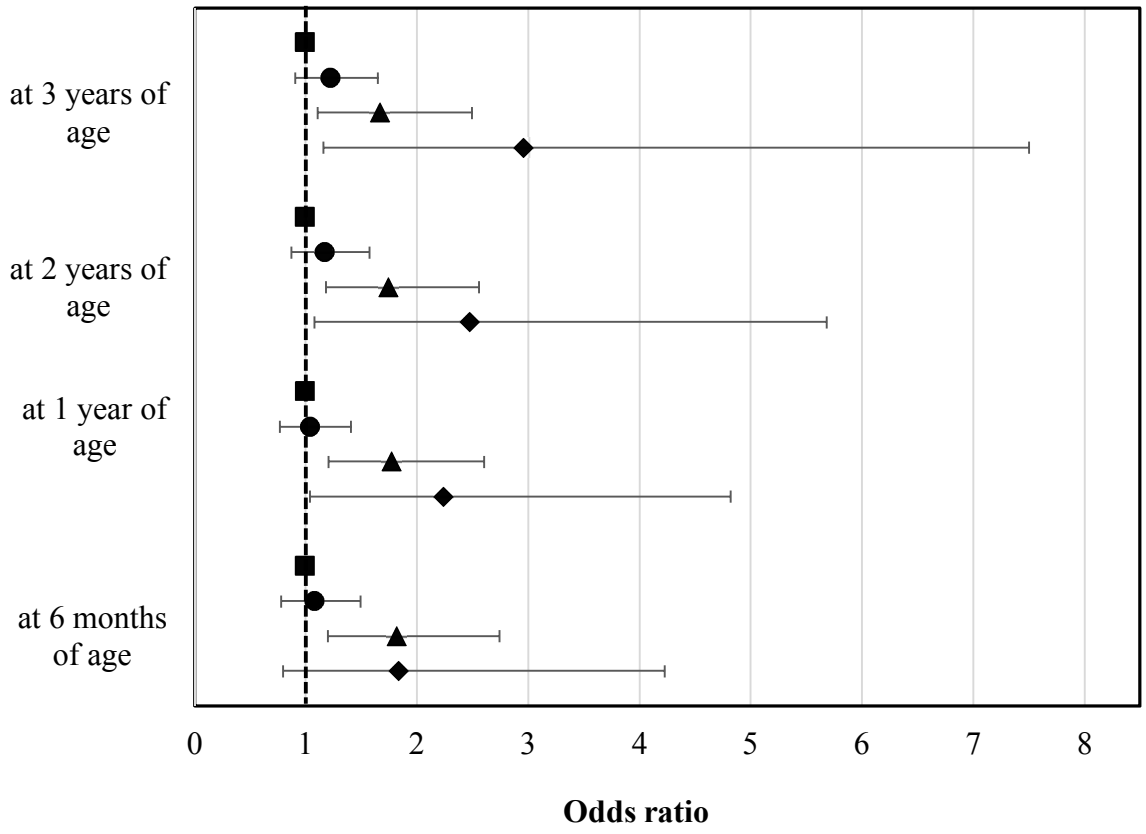


Figure 2. Forest plot showing the adjusted odds ratio for association of incidence of atopic dermatitis with antibiotic dose–response effect within 6 months of age. Children who were never exposed to antibiotics are represented by square symbols (Ref.), those who were exposed to antibiotics once are represented by circle symbols, those who were exposed to antibiotics twice to thrice are represented by triangle symbols, and those who were exposed to antibiotics more than four times are represented by diamond symbols.

3) 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.

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 group: Non-AD)								
	Non-AD (ref.) *vs.* Early transient			Non-AD (ref.) *vs.* Late onset			Non-AD (ref.) *vs.* Early persistent		
	aOR	95% CI		aOR	95% CI		aOR	95% CI	
No	1			1			1		
Yes	0.97	0.55	1.7	1.16	0.62	2.19	1.84	1.18	2.86
No	1			1			1		
Once	0.97	0.51	1.85	1.25	0.61	2.53	1.51	0.9	2.55
≥2 times	0.98	0.41	2.32	0.97	0.34	2.75	2.50	1.35	4.63

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.

4) 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 ($p=0.02$).

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

Variables		Antibiotic exposure within 6 months of age		p-value
		Yes (n=566)	No (n=1071)	
AD at 6 months	Number	77	110	
	Mean ± SD	12.56±10.94	14.24±12.13	0.33
	Mild(<15)	49 (63.6)	71 (64.5)	0.90
	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	0.02
	Mild (<15)	55(66.3)	118 (82.5)	<0.01
	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	0.11
	Mild (<15)	77(74.0)	123(82.6)	0.10
	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	0.21
	Mild (<15)	72(82.8)	123(86.6)	0.43
	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

5) Association between *IL-13* gene polymorphism and AD

The genetic polymorphism *IL-13* (rs20541) themselves also significantly influence the risk of AD in childhood. In multivariable regression analyses, *IL-13* (rs20541) GA+AA genotypes significantly increased the odds of AD in young children (Table 4, at 6 months: aOR=1.41, 95% CI 1.07–1.86, at 1 year: aOR=1.27, 95% CI 0.99–1.62; at 2 years: aOR=1.30, 95% CI 1.03–1.64; at 3 years: aOR=1.27, 95% CI 1.01–1.60).

Table 4. Influence of *IL-13* (rs20541) genetic variation on the development of atopic dermatitis.

IL-13 genotype	AD ever at 6 months			AD ever at 1 year			AD ever at 2 year			AD ever at 3 year		
	aOR	95% CI		aOR	95% CI		aOR	95% CI		aOR	95% CI	
GG	1			1			1			1		
GA+AA	1.41	1.07	1.86	1.27	0.99	1.62	1.30	1.03	1.64	1.27	1.01	1.60

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

$P<0.05$ calculated by multivariable logistic regression analysis.

6) Association between *IL-13* gene polymorphism and phenotypes and severity of AD.

IL-13 (rs20541) GA+AA genotypes was associated with early-persistent AD (aOR=2.02, 95% CI 1.38–2.95), and no association with other phenotypes (Table 5). For the severity, there was no statistically significant difference in the SCORAD according to *IL-13* (rs20541) genotypes. (Table 6)

Table 5. Multinomial logistic regression model for the association of *IL-13* (rs20541) genetic variation with the atopic dermatitis phenotypes.

IL-13	Non-AD (ref) *vs.* Early-transient			Non-AD (ref) *vs.* Late onset			Non-AD (ref) *vs.* Early-persistent		
	aOR	95% CI		aOR	95% CI		aOR	95% CI	
GG	1.00			1.00			1.00		
GA+AA	1.28	0.83	1.99	1.06	0.62	1.81	2.02	1.38	2.95

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.

Table 6. SCORAD score for atopic dermatitis patients at each time point based on the *IL-13* (rs20541) polymorphism.

	Variables	*IL-13* (rs20541) genotype		p-value
		GG genotype	GA+AA genotype	
AD at 6 months	Number	108	149	
	Mean ± SD	14.02±14.43	12.83±8.12	0.48
	Mild (<15)	57(64)	81(63.8)	>0.999
	Moderate-severe (≥15)	32(36.0)	46(36.2)	
AD at 1 year	Number	158	199	
	Mean ± SD	7.29±9.27	7.80±9.63	0.65
	Mild (<15)	103(79.8)	110(75.3)	0.39
	Moderate-severe (≥15)	26(20.2)	36(24.7)	
AD at 2 year	Number	198	249	
	Mean ± SD	7.29±10.85	7.90±10.05	0.60
	Mild (<15)	109(77.3)	143(75.7)	0.79
	Moderate-severe (≥15)	32(22.7)	46(24.3)	
AD at 3 year	Number	225	272	
	Mean ± SD	6.56±11.09	5.19±9.27	0.25
	Mild (<15)	104(78.2)	157(86.3)	0.07
	Moderate-severe (≥15)	29(21.8)	25(13.7)	

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

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

The effect of GA+AA genotypes on the occurrence of AD was even more pronounced when exposed to antibiotics within first 6 months of age. 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 7, p for interaction=0.06, at 6 months: aOR=1.66, 95% CI 1.07–2.57; at 1 year: aOR=1.55, 95% CI 1.05–2.29; at 2 years: aOR=1.85, 95% CI 1.26–2.70; at 3 years: aOR=1.83, 95% CI 1.25–2.69).
Regarding antibiotics, the dose–response relationship was particularly notable in children with *IL-13* (rs20541) GA+AA genotype (Fig. 3). 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)

Table 7. Combined effects of *IL-13* (rs20541) genetic variation and exposure to antibiotics within 6 months of age on the development of atopic dermatitis.

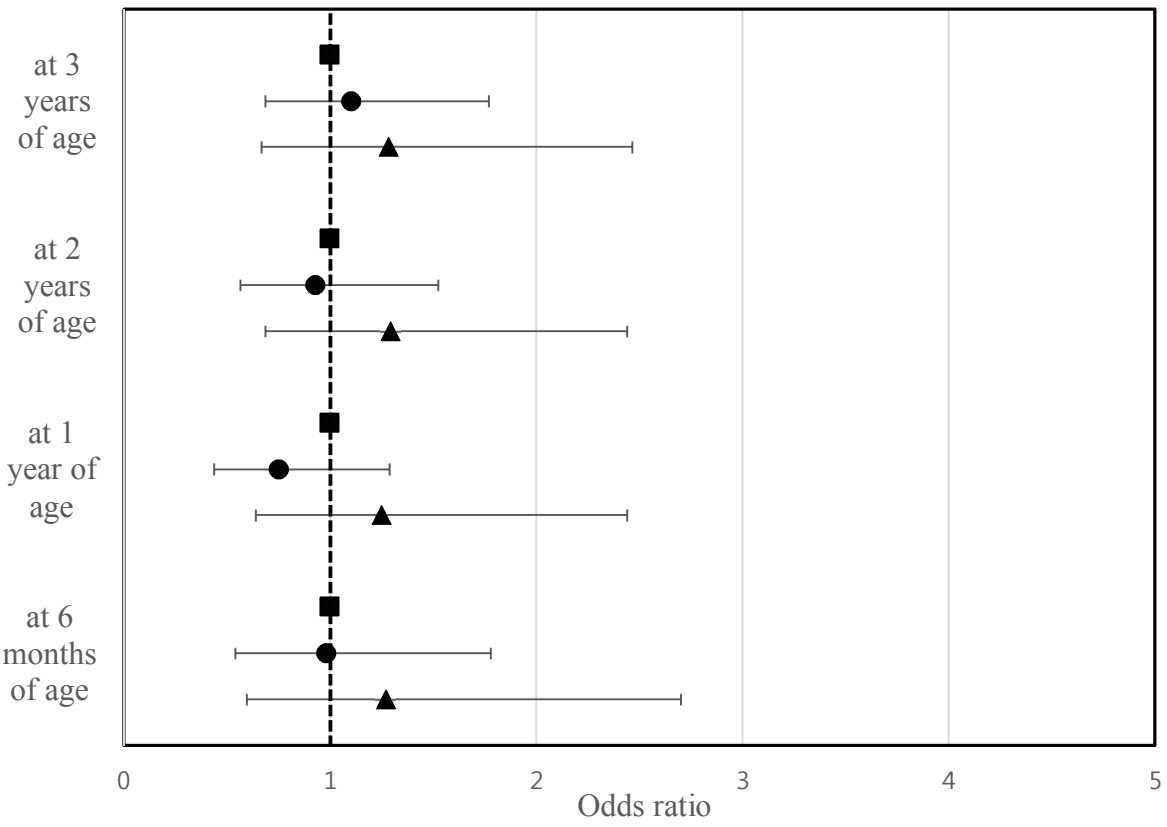
IL-13 genotype	Antibiotic exposure	AD ever at 6 months			AD ever at 1 year			AD ever at 2 year			AD ever at 3 year		
		aOR	95% CI		aOR	95% CI		aOR	95% CI		aOR	95% CI	
GG	No	1			1			1			1		
GG	Yes	1.09	0.66	1.80	0.90	0.58	1.41	1.04	0.68	1.58	1.16	0.77	1.74
GA+AA	No	1.27	0.85	1.90	1.00	0.70	1.43	1.11	0.80	1.56	1.09	0.78	1.52
GA+AA	Yes	1.66	1.07	2.57	1.55	1.05	2.29	1.85	1.26	2.70	1.83	1.25	2.69
Interaction p[a]		0.48			0.06			0.08			0.17		

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

(A)


at 3 years of age
at 2 years of age
at 1 year of age
at 6 months of age
0
1
2
3
4
5
Odds ratio

(B)

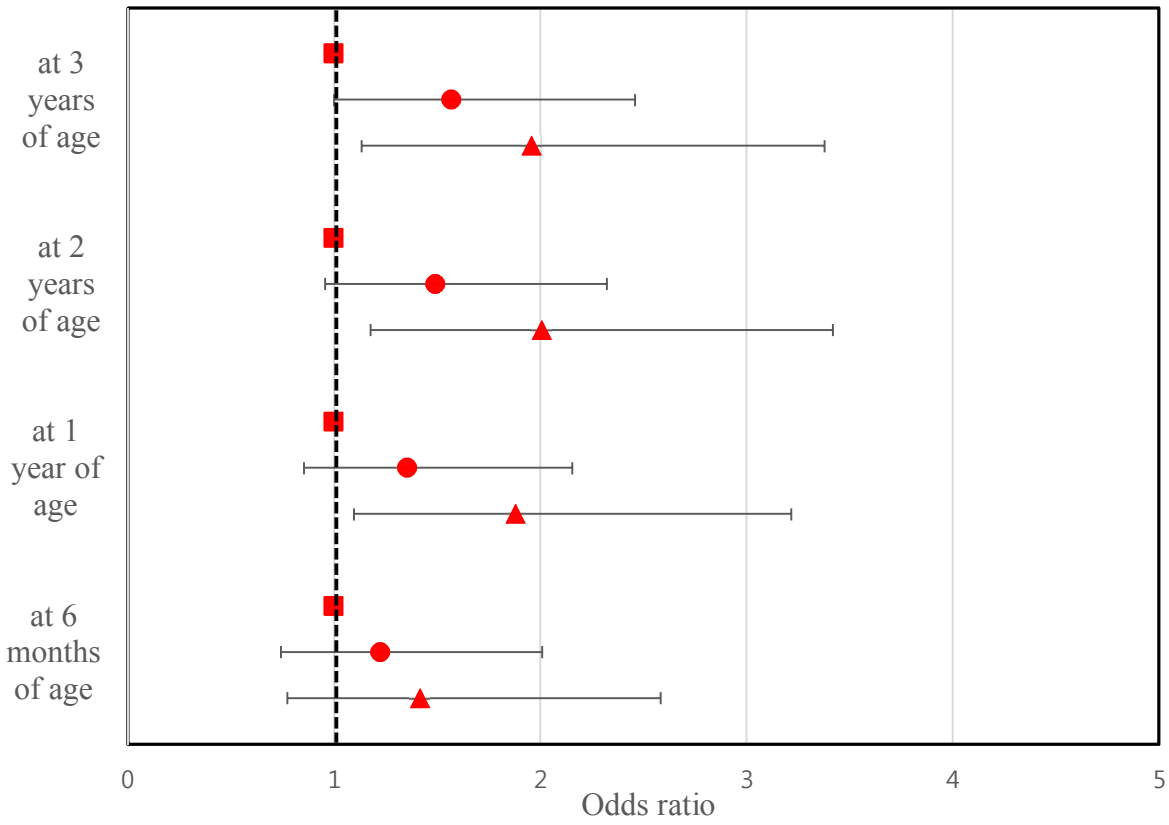


Figure 3. Adjusted odds ratio (95% confidence interval) of the development of atopic dermatitis in children with (A) *IL-13 (rs20541)* GG genotype or (B) GA+AA genotype according to the frequency of antibiotic exposure. Children who were never exposed to antibiotics are represented by square symbols (Ref.), those who were exposed to antibiotics once are represented by circle symbols, and those who were exposed to antibiotics more than twice are represented by triangle symbols.

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

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).

Table 8. 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.* Late onset			Non-AD (ref) *vs.* Early persistent		
		aOR	95% CI		aOR	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	3.68
	≥2 times	1.11	0.33	3.74	0.80	0.16	3.95	1.62	0.55	4.77
GA/AA	Never	1.19	0.62	2.29	0.92	0.41	2.04	**2.06**	**1.13**	**3.77**
	Once	1.30	0.55	3.07	0.81	0.27	2.43	**2.99**	**1.44**	**6.22**
	≥2 times	0.81	0.21	3.09	1.21	0.31	4.82	**4.73**	**2.01**	**11.14**

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.

Association between combined effects of *IL-13* gene polymorphism and antibiotic exposure within 6 months of age and severity of AD

We investigated whether there is a combined effect of *IL-13* gene polymorphism and antibiotic exposure within the first 6 months of age on the severity of AD. Although there is no statistically significant association between gene-environment interactions and the severity of AD, children who exposed to antibiotics and had the *IL-13* (rs20541) GA+AA genotypes were more likely to be in the moderate to severe group than in other cases at AD at 1 year (Table 9).

Table 9. SCORAD score for atopic dermatitis patients at each time point based on the antibiotic exposure and *IL-13* (rs20541) polymorphism.

	IL-13 (rs20541) genotype	GG	GG	GA+AA	GA+AA	p-value
	Antibiotic exposure	No	Yes	No	Yes	
AD at 6 months	Number	53	28	63	47	0.46
	Mean ± SD	15.58±15.24	11.37±14.82	12.53±8.24	12.73±9.20	
	Mild (<15)	26 (60.5)	17(73.9)	34(68.0)	27 (64.3)	0.71
	Moderate-severe (≥15)	17 (39.5)	6(26.1)	16(32.0)	15(35.7)	
AD at 1 year	Number	81	36	80	64	0.32
	Mean ± SD	7.09±9.87	8.12±9.45	6.32±9.15	9.85±10.41	
	Mild (<15)	54 (83.1)	20(74.1)	46(82.1)	28(63.6)	0.08
	Moderate-severe (≥15)	11(16.9)	7(25.9)	10(17.9)	16(36.4)	
AD at 2 years	Number	94	48	100	79	0.41
	Mean ± SD	5.94±10.46	8.20±10.74	5.87±8.15	8.16±10.38	
	Mild (<15)	52(82.5)	22(68.8)	56(83.6)	44(73.3)	0.23
	Moderate-severe (≥15)	11(17.5)	10(31.3)	11(16.4)	16(26.7)	
AD at 3 years	Number	108	57	108	88	0.17
	Mean ± SD	4.75±8.29	8.85±16.09	3.81±7.97	4.76±8.86	
	Mild (<15)	52(81.3)	19(76.0)	60(90.9)	49(86.0)	0.25
	Moderate-severe (≥15)	12(18.8)	6(24.0)	6(9.1)	8(14.0)	

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

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 incidence of AD seems to be due to environmental factors, such as air pollution[25] and inhalant allergens (e.g., animal hair, house dust mite, and pollen),[26] and changes in lifestyles such as longer duration of bathing,[27] regular use of soap,[28] and exposure to cold wind by air conditioner.[29] Also, 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 in this COCOA study population was higher than other studies. [21, 30] 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.[31-33] 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.[8)] Also modification of gut flora early in life in mice treated with antibiotics favors the production of Th2 cytokines and increase susceptibility to allergy.[34)] Another pathogenetic mechnism is that gut epithelial barrier destruction through the disturbance of microbiota after antibiotic administration may lead to tissue damage and allergic sensitization.[35)] These 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[10, 36)] 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.[12, 37)] 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[38)]. Since skin microbiome modulates skin barrier function[39)], exposure to antibiotics, which decreases the bacterial density and alters innate immune responses in the skin, predisposes the infants to cutaneous disease[8, 40)]. 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,[14)] and this may have an additional role in aggravating the barrier function of the skin. Further studies regarding skin barrier disturbance, microbiome and metabolites 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.[41, 42)] 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. In Korea, a region with higher resistance rates, healthcare providers are more inclined to prescribe antibiotics with broader coverage than other countries.[43] 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.”[44] This can significantly reduce a person’s quality of life and cause anxiety and depression.[45] 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. This study assesses how antibiotic exposure during first 6 months of life itself, and how antibiotic exposure and its interaction with the IL-13 risk alleles affect the development and persistency of AD in childhood. Attention needs to be paid to unwarranted prescription of antibiotics, especially in susceptible children receiving primary care.

Conclusion

In conclusion, our birth cohort study suggested that antibiotic exposure early in life was at risk to develop AD in young children and the risk increased as the number of prescriptions of antibiotic rose. It not only related to increased risk of persistent AD phenotype but also to the occurrence of moderate to severe AD at the age of 1 year. We also demonstrated that genetic polymorphisms *IL-13 (rs20541)* significantly influence the risk of AD and the interactions between *IL-13 (rs20541)* and antibiotic exposure in early life with dose-response relation may play a role in development of AD in early childhood, especially on early-persistent AD phenotype. Further studies are needed to confirm causality and proven the mechanism by which antibiotic exposure at young age changes in skin or gut microbiome and lead to the development of AD. 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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Abstract

Effect of Early-Life Antibiotic Exposure and IL-13 Polymorphism (rs20541) on Atopic Dermatitis Phenotype

Although atopic dermatitis (AD) is associated with certain gene variants, the rapidly increasing incidence of AD suggests that environmental factors contribute to disease development To identify the cause of AD, and to effectively prevent and manage it, lots of epidemiologic studies were conducted as a concept of hygiene hypothesis. As part of this concept, there is growing evidence that the increased prevalence of allergic diseases can be attributed to increased exposure to antibiotics. Despite antibiotic exposure in early life being a plausible risk factor for the development of AD in children, controversy remains about whether early life antibiotic exposure is a risk factor in their development of AD.

No other studies have analyzed the severity and natural course of AD according to the frequency of early life antibiotic exposure in children. Thus, in this prospective birth cohort study, we focused on the relationship between development of AD and antibiotic exposure within 6 months, a critical period in the development of microbiome and immune system, and further examined whether the frequency of antibiotic exposure is differently associated with severity as well as 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 alteration of the skin microbiome, deterioration of the skin barrier function, and may be a more important mediator for the TH_2 response in the skin than *IL-4*. Previous studies had identified that polymorphisms in the *IL-13* promoters are associated with the development of AD and among them, rs20541 has been discussed a lot about the relationship with AD in existing Asian papers. Following on from the above considerations, we hypothesized that there is influence on AD phenotype by antibiotic exposure and this relationship can be modified by *IL-13* polymorphism.

This study conducted within the Cohort for Childhood Origin of Asthma and Allergic diseases (COCOA) included 1,637 children. Pediatric allergists assessed the presence of AD at each visit and obtained the information about antibiotic exposure for more than 3 days. AD phenotype was divided into 4 groups according to the onset and persistence of AD. the early-transient phenotype, with onset of AD within 2 years old and no further symptoms after then; the early-persistent phenotype, with onset within 2 years old and improvement of AD symptom less than 2 years old; the late phenotype, with onset of AD after 2 years old; and the never phenotype. *IL-13* (rs20541) polymorphism was

genotyped by the TaqMan method. The multivariable logistic regression models were used to estimate adjusted odd ratios (aOR) and corresponding 95% confidence intervals (95% CI) for comparison of AD occurrence risk by relevant covariates and frequency of antibiotics or *IL-13* (rs20541) polymorphism. To identify the effect of antibiotic exposure within 6 months of age and combined effect of exposure to antibiotics and *IL-13* genetic variations on AD phenotypes, subjects were divided into 4 groups based on environmental factors (antibiotic exposure within 6 months for more than 3 days) and genetic background (genotypes) and multinomial logistic regression models were used after adjusting for potential confounders: gender, maternal education level, family history of allergic diseases, history of breast milk feeding, and mode of delivery.

The effects of antibiotic exposure, *IL-13* (rs20541) polymorphism, and the interaction of these two factors on severity of AD evaluated as SCORing Atopic Dermatitis (SCORAD)were analyzed.

Antibiotics exposure within 6 months of age was associated with increased risk of AD within the first 3 years of life (aOR=1.40, 95% CI 1.09-1.81). The cumulative incidence of AD within the first 3 years of life increased as the frequency of antibiotic exposure increased. Infants who used antibiotics more than 2 times within 6 months of age had an increased risk of developing early-persistent AD compared with those who never exposed to antibiotics (aOR=2.50, 95% CI 1.35-4.63). In addition, infants with GA+ AA genotypes of *IL-13* (rs20541) had a higher risk of AD at 1 year (aOR=1.27, 95% CI 0.99-1.62) and we found weak interaction between genetic polymorphisms and environmental factors on the development of AD (p for interaction=0.06). In those with GA+AA genotypes of *IL-13* (rs20541), there was an increased risk of AD at 1 year when exposed to antibiotics (aOR=1.55, 95% CI 1.05-2.29). If infants carrying *IL-13 (rs20541)* GA+ AA genotype exposed to antibiotics more than twice within 6 months, there is a higher risk of being early persistent AD phenotype than children with *IL-13 (rs20541)* GG genotype and no antibiotics (aOR=4.73, 95% CI 2.01-11.14). As a result of analysis of the severity, the severity increased with exposure to antibiotics that mild AD at 1 year old accounted for the majority when the antibiotics were not exposed (82.5%), whereas 66.3% of children were identified as mild AD when exposed to antibiotics.

Antibiotics exposure within 6 months was related to the early-persistent AD as well as the dose-dependent increase in incidence of AD in childhood, which effect was modified by *IL-13* (rs20541) genotype. This study will improve our understanding of influence of genetic and environmental causes and their interactions on childhood AD, and provide comprehensive insights of pathogenesis of AD, therefore enable improved prevention.

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