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Effect of Mold Exposure during Pregnancy on the Development of Offspring's Atopic Dermatitis

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Abstract

Atopic dermatitis is one of the most common chronic skin diseases. Infantile atopic dermatitis may be caused, in particular, by exposure to indoor environmental factors during gestation and infancy; however, the underlying mechanism is unknown. A total of 2,609 healthy newborns who were enrolled in the COCOA study (COCOA) from 2008 to 2015 were surveyed for indoor environmental exposure to fungi during gestation and then diagnosed postnatally for atopic dermatitis. Fungal microorganisms were analyzed in house dust samples collected during gestation and their relationship was investigated. A total of 2,609 respondents were surveyed (52.8% male and 47.2% female) Children, 1, 2, and 3 years old diagnosed with atopic dermatitis comprised 15.2%, 15.7%, and 14.1% of the respondents, respectively. The prevalence of exposure to mold during gestation was 1.46 (95% CI, 1.05-2.04) and 1.52 (95% CI, 0.95-2.43), in the first and third years after birth, respectively. One-year-old children with atopic dermatitis and no fungal markers detected in the bathroom environment during gestation accounted for less than 5% (aOR, 1.51; 95% CI, 0.96-2.38). Exposure to indoor fungi during gestation and infancy is associated with the development of atopic dermatitis in children. Future research will be necessary to establish the underlying mechanisms.

Keywords: Atopic dermatitis; Indoor environment; Mold; Pregnancy; Child

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Introduction

Atopy dermatitis is known to be the most common occurrence of chronic recurrent dermatitis in children in childhood, and it is a disease that can be carried out with asthma, allergic rhinitis and chronic care [1]. According to the epidemiological studies of childhood asthma and allergic diseases in children (International Study of Asthma and Allergies in Childhood, ISAAC), the results of a recent survey of atopic dermatitis in the 12 months of childhood (6~7 years old) are steadily increasing trend in the latest 12 months. Domestic children's atopic dermatitis also rose to 17 percent in 2000 and 27 percent in 2010 [2,3].

Major causes of occurrence of atopic dermatitis are not known specifically, but are thought to be caused by interactions between genetic factors and environmental factors. With one parent's atopic dermatitis at least one child, the occurrence of a single parent is very high at 41.7 percent. In the event of parental dermatitis in parents, the birth of a child affects the mother's atopic dermatitis in her mother (30.7%) and father (22.2%) [4]. Genetic factors cause that the mutations in the skin of the skin, which are genetically related to the skin barrier, are responsible for atopic dermatitis [5]. Recent trend in the atopic dermatitis in atopic dermatitis has caused a major obstacle to the formation of a normal skin barrier due to the deterioration of the protein in the outer layer of the skin and the function of the protein [6]. Influenced by environmental effects, the effects of changes in chemicals and living conditions (Especially, foods like food, formaldehyde, volatile organic

chemicals, indirect smoking, mold, house dust mites, cockroach, house dust mites, etc.) from nearby factories contribute to the occurrence of atopic dermatitis [7,8]. Among various environmental factors, mold exposure is thought to be the cause of the occurrence of atopic dermatitis [9]. Global warming has caused temperatures worldwide to rise 0.1°C over the next ten years, and the humidity is increasing by about 2% to 5%. The occurrence of fungal growth on this planet is reported to be growing, and the occurrence of fungal growth may be linked to increased occurrence of atopic dermatitis [10]. Effects of microbial reproduction and development on indoor and outdoor air include temperature, humidity, and various nutrients. Among them, humidity is an important factor that directly effects the density of the fungi in the air [11]. In the case of increased humidity in the home, the reproduction of fungus on walls and ceilings can be enhanced, resulting in an increase in the density of the airborne fungus due to increased concentrations of airborne tissue [12].

The results of the analysis of mold strain and fungal exposure caused by the influence of air are as follows. There is a lot of environmental assessment for households in determining the scope and types of microbial growth. It was conducted at random to assess the water damage by selecting 112 houses. Mold analysis collected 20 bedrooms and 10 roof tiles, using a badge (polycarbonate filters, SKC, Eighty-four, PA, USA) to confirm the specimen with a microscope (bacteria). As a result, the main fungus is *Aspergillus* and *Penicillium*. There was a higher concentration of water than water damage where there was no water damage. The concentrations of toxins and β-glucan exceeded the criteria for health effects [13]. In other journals, indoor exposure to moisture and mold was associated with the atopic dermatitis in the child's atopic dermatitis [14,15]. We detected mold fouling on the walls of our homes through an infrared camera (IR: Infrared Radiation). As a result, the risk of fungal and bacterial concentrations and symptoms of atopic dermatitis in the air increased fifteen fold [16].

Fungal exposure affects the cells (Langerhans cells, keratinocytes, T lymphocytes) and factors (cytokines and Immunoglobulin (Ig), IgE) involved in the development of atopic dermatitis. Increased CD4 and CD8+ T cell ratios, (Dupuy 1994) CD18, Th2-mediated cytokines Interleukin (IL) -4, IL-5, IL-10 and IgE promote the immune response and increase the risk of atopic dermatitis [17,18]. In the development of atopic dermatitis, changes in the immune response due to exposure of microorganisms and fungi in the indoor and outdoor air are mainly caused during infancy [19]. According to a recent UK study, fungal concentrations were measured using quantitative analytical methods (Polymerase Chain Reaction (PCR)). Ascomycota, Basidiomycota, and *Malassezia* were found to have a health effect at high concentrations in molds that affect water damage in domestic baths. Exposure to fungal species in the home has been a major cause of deterioration of immune function [20].

As a result, there are many data on fungus exposure cases reported abroad, but studies (cross-sectional study) on fungi mainly consist of questionnaires and observations.

There are very few studies on domestic atopic dermatitis and fungi. As a result of small - scale studies and short-term measurements and analyzes, there is little analysis of the basic data of early pregnancy. It was confirmed that exposure to fungi in early pregnancy had a negative effect on weight and growth of infants. Other influences on infant development have been proven by causality, such as temperature, humidity, and seasonality [21,22]. As a prospective study, there is little research into the risk of fungal exposure after pregnancy. Among them, no data were available to assess the risk level according to the indoor mold exposure results of the family. Exposure to mold during pregnancy and infancy can be considered as an important risk factor for the development of atopic dermatitis [11,23-25]. It is necessary to investigate the characteristics of fungal exposure in the environment which is one of the increasing factors of atopic dermatitis. Therefore, the purpose of this study is to determine whether exposure of fungi during pregnancy is related to the development of atopic dermatitis in infants and young children. Second, we analyzed the relationship between fungal species measured in indoor dust during pregnancy and infantile atopic dermatitis.

Materials and Methods

Subjects and study design

The subjects of this study were collected from the cohort (COCOA: Childhood Asthma and allergic Diseases Cohort Study) of cause identification of atopic asthma in the Korea Centers for Disease Control and Prevention since 2008. The study was conducted on 2,609 children who participated until December 2015. Among them, there were 86 withdrawal consent, 3 tracking loss, 89 twins at birth, 123 withdrawal victims, 123 withdrawal consent, 25 tracking loss, 429 under 1 year old, 50 twins at 1 year old, withdrawal of consent 180. The study was conducted by excluding 13 cases of tracing loss, 493 cases of under 3 years old, and 25 cases of twins at 3 years of age. The result is the remaining 1,064 (Figure 1). The subjects were surveyed 36 weeks of pregnancy and 6 months, 1, 2, and 3 years after birth, focusing on the basic data of dust samples and questionnaires. The ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire was used to confirm symptoms and diagnosis of atopic dermatitis.

Research method overview

The environmental factors were divided into indoor dust analysis results and survey questionnaire. The diagnosis and symptoms of atopic dermatitis that appeared early after birth were investigated. The results of fungal exposure and allergic diseases were analyzed for causality and relevance to confirm environmental factors.

Questionnaire survey

The study was approved by the Institutional Review Board (IRB) at each hospital. This study was first conducted at Seoul Asan Hospital (IRB number 2008-0616), Samsung Seoul Hospital (IRB number 2009-02-021), Yonsei University Hospital (IRB number 2008-0588) and CHA Gangnam Medical Center Hospital (IRB number 2010-010) in December 2008, and Seoul National University Hospital (IRB number H-1401-086-550) began recruiting at COCOA in 2014. Consent was obtained from the mother. The interviews were carried out based on environmental factors. Acceptance of consent was made in accordance with the principles and recommendations of the Declaration of Helsinki. The environmental questionnaire is as follows. The environmental questionnaire surveyed bed and living room, kitchen, bathroom, and other conditions in the residence period, mold exposure, exposure to wet space, and mold space. Among the main data, the severity of fungus exposure was examined by separating the fungi into 'invisible' and 'visible (as 5%, 5~30%, 30% more)'. Here, the 5% criterion was determined as adult hand size.

Diagnosis of allergic diseases

The questionnaire for atopic dermatitis was used in ISAAS questionnaires. Periodic follow-up was performed, parents' diagnosis

Table 1: Subject characteristics.

Children's age	1- year	2-year	3-year
Variables	**N (%), means ± SDs**	**N (%), means ± SDs**	**N (%), means ± SDs**
Number	1750	1288	1064
Maternal age (years)	32.7 ± 3.4	32.6 ± 3.5	32.5 ± 3.5
Maternal history of allergic diseases			
No	1160 (70.2)	869 (70.3)	724 (70.8)
Yes	492 (29.8)	367 (29.7)	299 (29.2)
Paternal history of allergic diseases			
No	1099 (72.0)	869 (73.3)	721 (73.1)
Yes	428 (28.0)	317 (26.7)	266 (26.9)
Household income (10,000 won)			
≤ 299	303 (19.1)	241 (20.2)	211 (21.3)
300-499	661 (41.7)	509 (42.7)	418 (42.6)
≥ 500	622 (39.2)	441 (37.0)	359 (36.1)
Maternal educational level			
≤ High school	99 (5.8)	81 (6.4)	70 (6.7)
≤ University	1243 (73.3)	912 (72.0)	738 (70.3)
≥ Graduate school	355 (20.9)	273 (21.6)	242 (23.0)
Child's sex			
Boy	907 (52.0)	681 (52.9)	571 (53.7)
Girl	839 (48.0)	607 (47.1)	493 (46.3)
Gestational age (week)	39.2 ± 1.2	39.2 ± 1.2	39.1 ± 1.2

AR: Allergic Rhinitis; AD: Atopic Dermatitis

questionnaires were used at 6 months, 1, 2, and 3 years after the birth of the subjects diagnosed with atopic dermatitis during the observation period. It is estimated that allergic physicians diagnosed directly at 5 hospitals. "Atopic dermatitis was defined as" when an itchy skin rash (fever or atopic dermatitis) has been present for at least 6 months and has been diagnosed as "eczema" (fever or atopic dermatitis) by a doctor since birth.

Indoor mold exposure survey

Dust collection was conducted in 40 out of 2609 children living in Seoul and Gyeonggi-do from 2008 to December 2015. Twenty of the normal subjects and twenty of the atopic dermatitis were collected in the space where the infants live in the bedroom. The collection method was collected for 20 min through a professional vacuum cleaner (Electrolux), and the collected dust was separated and stored in the freezer (Figure 2). The microorganism DNA present in the house dust was separated using FastDNA® SPIN Kit for Soil (Qbiogene, MP Biomedicals, Illkirch, France). The isolated DNA was PCR amplified using the fusion primer containing the barcode and the Internal Transcribed Spacer (ITS) region. The sequences of the PCR products were confirmed using Illumina's MiSeq platform. The sequence of the microorganisms was analyzed by CL community (Chunlab, Seoul, korea) program.

Statistical analysis

In this study, dependent variables (atopic dermatitis at 6 months, 1, 2, and 3 years) such as the correlation with fungal environmental factors were analyzed using SAS (SAS Institute Inc., Cary, NC, USA) version 9.3. Frequency analysis was used to show the general characteristics (percentage) of subjects (infants during pregnancy).

Table 2: Prevalence of atopic dermatitis.

	N (%), means ± SDs
Parent-reported, doctor diagnosed atopic dermatitis	
1 count	229/1139 (20.1%)
2 count	171/852 (20.2%)
3 count	109/600 (18.2%)
diagnosis of atopic dermatitis by physician	
1 count	200/1314 (15.2%)
2 count	152/966 (15.7%)
3 count	104/737 (14.1%)

The diagnosis of atopic dermatitis due to general characteristics is considered to affect children, parents, and environmental questionnaires. The association between parental allergic disease history and chi-square test and t-test was used to determine the difference in fungal exposure. The effects of fungi on children's atopic dermatitis were analyzed by adjusting the exposure period according to the factors of children, socioeconomic factors of parents, and parental allergy history. To determine the effect of indoor dust collection on atopic dermatitis, multiple logistic regression analyzes were used to calculate the difference in ratios (Odds ratio, OR) and the 95% confidence interval (95% Confidence Interval, 95% CI).

Results

Characteristics of the study population

The subjects were 1 (N=1750), 2 (N=1288), and 3 (N=1064) year old, the average age of the mother was 32 (32.6 ± 3.5) years old. The allergic disease history of mothers was 1 (N=492, 29.8%), 2 (N=367, 29.7%), and 3 (N=299, 29.2%) years old, and the father's allergy history was similar to 1 (N=428, 28.0) and 2 (N=317, 26.7%), 3 (N=266, 26.9%) years old. The monthly income of households (300-499 million won) is distributed in the order of 1 (N=661, 41.7%), 2 (N=509, 42.7%), and 3 (N=418, 42.6%) years old, and it is distributed in the order of 1 (N=1243, 73.3%), 2 (N=912, 72.0%), and 3 (N=738, 70.3%) years old even in mother's academic ability. University graduates tend to be very high here (Table 1).

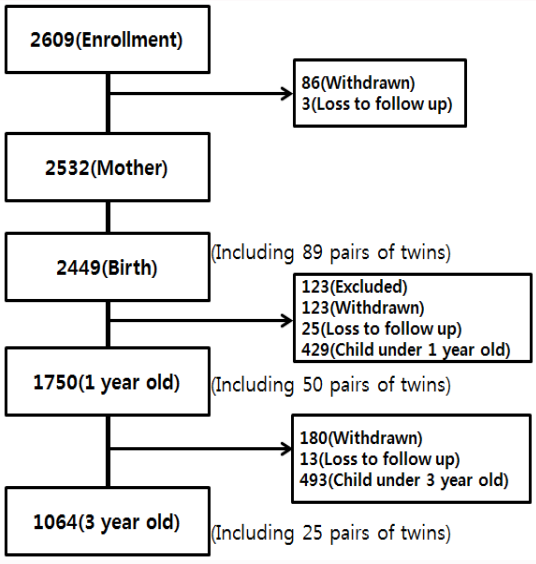

Figure 1. Flow chart of participant.

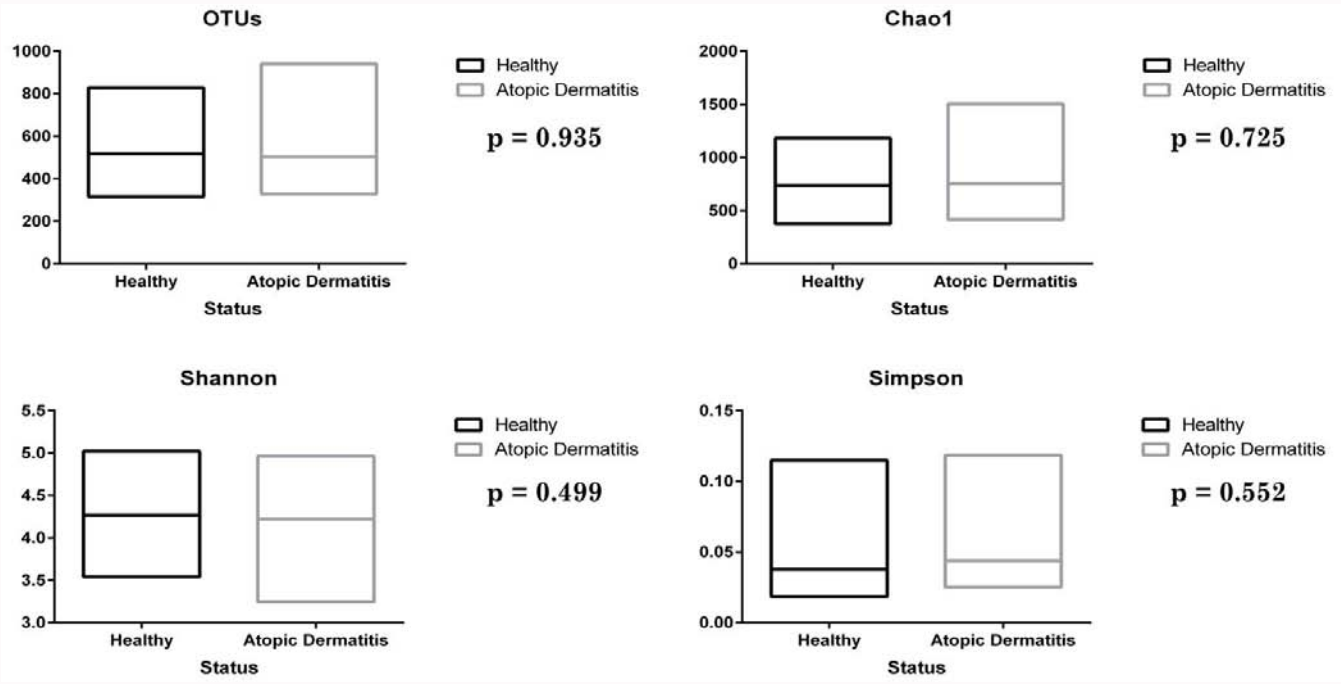

Figure 2: Comparison of OTUs, Chao1, Shannon, and Simpson values from house dust sample in normal group and atopic dermatitis group.

Table 3: Association analysis between mold exposure by spot (bathroom and other site except bathroom) or water leaks during pregnancy and risk of atopic dermatitis by parental report***.

Variable (mold exposure)		Parental report of a doctor's diagnosis of atopic dermatitis																	
		N (%)			1 years AD*			N (%)			2 years AD*			N (%)			3 years AD*		
		Yes	No	%	OR* (95% CI)			Yes	No	%	OR* (95% CI)			Yes	No	%	OR* (95% CI)		
Spot (bathroom)	Invisible (0%)	126	534	(19.1)	1			98	404	(19.5)	1			62	310	(16.7)	1		
	Under 5%	32	94	(25.4)	1.51	(0.96	2.38)	17	72	(19.1)	0.97	(0.54	1.74)	7	45	(13.5)	0.83	(0.35	1.96)
	Under 5~30%	10	21	(32.3)	2.21	(1.00	4.89)	7	20	(25.9)	1.51	(0.61	3.74)	4	17	(19)	1.1	(0.35	3.47)
	More than 30%	1	11	(8.3)	0.36	(0.05	2.86)	2	5	(28.6)	1.43	(0.26	7.96)	3	3	(50)	9.48	(1.42	63.13)
Water leaks or get wet	Degree of moistness	16	99	(13.9)	1			18	68	(16.7)	1			13	53	(16.7)	1		
	Variable	31	102	(23.3)	1.99	(1.00	3.96)	18	84	(20.3)	0.74	(0.34	1.61)	8	66	(20.3)	0.39	(0.14	1.14)
Spot (etc)	Invisible (0%)	122	465	(20.8)	1			87	359	(21.4)	1			53	267	(21.4)	1		
	Under 5%	27	101	(21.1)	0.99	(0.61	1.59)	22	73	(31.1)	1.16	(0.68	2.00)	15	51	(31.1)	1.3	(0.66	2.53)
	Under 5~30%	15	69	(17.9)	0.78	(0.43	1.42)	12	49	(17.5)	1.01	(0.51	2.00)	6	39	(17.5)	0.75	(0.30	1.91)
	More than 30%	5	25	(16.7)	0.76	(0.28	2.06)	3	20	(10.5)	0.59	(0.17	2.10)	2	18	(10.5)	0.55	(0.12	2.50)
Water leaks or get wet	NO	138	531	(20.6)	1	98	395	(25.3)	1	62	284	(25.3)	1						
	YES	43	174	(19.8)	0.96	(0.65	1.41)	31	129	(19.1)	0.92	(0.58	1.45)	18	98	(19.1)	0.76	(0.42	1.36)

* AD: Atopic Dermatitis

** Diagnosis of atopic dermatitis was assessed by parental report of a physician's diagnosis at 1, 2, and 3 years of age

*** Multivariate analysis was performed by logistic regression analysis, and adjustments were made for pregnant mother age, child's sex, maternal body mass index, maternal educational level, gestational age, and parental history of allergic diseases including atopic dermatitis, allergic rhinitis, and asthma

Prevalence of atopic dermatitis

The prevalence of atopic dermatitis was 20.1% (N=229/1,139) at 1 year, 20.2% (N=171/852) at 2 years and 18.2% (N=109/600) at 3 years. The prevalence rate of diagnosis of atopic dermatitis was 15.2% (N=200/1,314) at 1 year, 15.7% (N=152/966) at 2 years and 14.1% (N=104/737) at 3 years (Table 2).

The relationship between indoor fungal exposure and the development of atopic dermatitis during pregnancy

One-year old atopic dermatitis increased the risk of 1.51 (95% CI, 0.96-2.38) and 2.21 (95% CI, 1.00-4.89) in less than 5% of total area and less than 5% to 30%. The results show that the risk increases with the degree of fungal exposure. On the other hand, 3-year-old atopic dermatitis was at risk 9.48 (95% CI, 1.42-63.13) in subjects whose exposure was less than 1 year old but whose fungal exposure was 30% or more of the total area. One-year old atopic dermatitis was more dangerous than water leakage and wet place (1.99, 95% CI, 1.00-3.96). However, two-aged atopic dermatitis, the greater the area, the greater the risk. But it was not statistically significant. On the other hand, 1.08 (95% CI, 0.59-1.99), 1.82 (95% CI, 0.69-4.82), and 3.45 (95% CI, 0.82-14.58) in 2-year-old atopic dermatitis and 1.33 (95% CI, 0.61-2.92), 1.77 (95% CI, 0.56-5.58), and 1.94 (95% CI, 0.21-17.97) in 3-year-

Table 4: Association analysis between mold exposure by spot (bathroom and other site except bathroom) or water leaks during pregnancy and risk of atopic dermatitis by physician***.

Variable (mold exposure)		Diagnosis of atopic dermatitis																	
		N (%)			1 years AD*			N (%)			2 years AD*			N (%)			3 years AD*		
		Yes	No	%	OR* (95% CI)			Yes	No	%	OR* (95% CI)			Yes	No	%	OR* (95% CI)		
spot (bathroom)	Invisible (0%)	95	517	(17)	1			75	440	(17)	1			45	365	(17)	1		
	Under 5%	15	98	(30.9)	0.89	(0.49	1.62)	15	81	(30.9)	1.08	(0.59	1.99)	9	58	(30.9)	1.33	(0.61	2.92)
	Under 5~30%	8	21	(31.3)	2.3	(0.96	5.50)	6	19	(31.3)	1.82	(0.69	4.82)	4	18	(31.3)	1.77	(0.56	5.58)
	More than 30%	2	10	0	1.37	(0.29	6.61)	3	6	(0.0)	3.45	(0.82	14.58)	1	5	(0.0)	1.94	(0.21	17.97)
Water leaks or get wet	Degree of moistness	14	93	(16.7)	1			12	72	(16.7)	1			8	66	(16.7)	1		
	Variable	23	101	(20.3)	1.59	(0.74	3.40)	17	87	(20.3)	1.17	(0.50	2.71)	5	79	(20.3)	0.51	(0.15	1.71)
Spot (etc)	Invisible (0%)	88	462	(16)	1			66	397	(14.3)	1			42	322	(11.5)	1		
	Under 5%	12	103	(10.4)	0.57	(0.30	1.09)	13	85	(13.3)	0.87	(0.46	1.66)	11	63	(14.9)	1.21	(0.59	2.50)
	Under 5~30%	13	63	(17.1)	1.01	(0.53	1.93)	14	45	(23.7)	1.81	(0.93	3.51)	4	45	(8.2)	0.61	(0.21	1.79)
	More than 30%	7	18	(28)	2.69	(1.04	6.97)	6	19	(24)	2.15	(0.81	5.71)	2	16	(11.1)	1.05	(0.23	4.81)
Water leaks or get wet	NO	98	520	(15.9)	1	76	432	-15	1	52	330	-13.6	1						
	YES	34	167	(16.9)	1.08	(0.70	1.66)	27	135	(16.7)	1.08	(0.67	1.76)	10	121	(7.6)	0.5	(0.25	1.03)

* AD: atopic dermatitis

** Diagnosis of atopic dermatitis was assessed by parental report of a physician's diagnosis at 1, 2, and 3 years of age

** Multivariate analysis was performed by logistic regression analysis, and adjustments were made for pregnant mother age, child's sex, maternal body mass index, maternal educational level, gestational age, and parental history of allergic diseases including atopic dermatitis, allergic rhinitis, and asthma

Table 5: Demographic characteristics of the analysis.

	Normal control	AD
N	20	20
Sex (Male/Female)	10/10	12/8
Mother age (years)	32.90 ± 3.58	33.55 ± 3.66
Gestational age (weeks)	39.17 ± 1.21	39.49 ± 1.08
Birth weight (gram)	2925.79 ± 753.74	3043.58 ± 849.40

Table 6: Library coverage estimations and sequence diversity of fungal ITS genes pyrosequencing.

ID	Valid reads	OTUs	Chao1	Shannon	Simpson
Control 1	9475	474	769.5882	3.619653	0.114784
Control 2	8588	456	567.125	4.541252	0.02198
Control 3	10169	611	731.3051	4.629529	0.024318
Control 4	9719	377	455.9153	3.690041	0.090025
Control 5	7111	315	404.7581	3.541059	0.09324
Control 6	7899	439	757.3333	3.991715	0.051364
Control 7	9467	343	574.6667	3.859098	0.05143
Control 8	10273	520	775.6081	4.314013	0.038003
Control 9	8788	382	518.1224	4.288241	0.030933
Control 10	11603	576	952.9615	4.045346	0.052539
Control 11	9386	517	740.9474	4.419917	0.037477
Control 12	11197	469	599.013	4.253351	0.033662
Control 13	11167	530	693.4	4.24386	0.047244
Control 14	12620	725	1126.857	4.347734	0.035431
Control 15	11089	828	1181.979	4.64697	0.035575
Control 16	12210	764	1144.971	4.907156	0.02687
Control 17	10215	570	733.8298	4.774492	0.021033
Control 18	6413	317	375.2632	3.910256	0.058516
Control 19	12008	638	949.3542	4.230534	0.070634
Control 20	4753	544	746.4118	5.021128	0.018621
Case 1	7282	329	416.3061	4.209385	0.030832
Case 2	9011	608	787.1	4.68377	0.025285
Case 3	10195	410	549.2642	3.913959	0.060137
Case 4	10885	366	425.7143	4.232292	0.035282
Case 5	11250	573	778.8875	4.588823	0.026887
Case 6	8323	341	639.5294	3.248262	0.102067
Case 7	10255	504	647.3544	4.15016	0.049551
Case 8	11210	940	1505.511	4.965526	0.03022
Case 9	10900	578	787	3.604678	0.118101
Case 10	11160	688	1004.469	4.548595	0.0311
Case 11	11206	492	711.6133	3.816188	0.086481
Case 12	11982	562	863.8889	4.367747	0.030685
Case 13	11885	496	632.2805	4.114662	0.045829
Case 14	8604	438	726.0159	3.686982	0.075458
Case 15	9653	503	786.5156	4.473125	0.025739
Case 16	10485	580	815.6915	4.305426	0.03932
Case 17	11607	633	941.9326	4.389317	0.04185
Case 18	10267	528	823.5882	4.297914	0.050246
Case 19	10219	480	665.2442	4.017626	0.053966
Case 20	10677	421	560.2344	3.541324	0.118609
Total	401206	20865			

*Control: Healthy

**Case: Atopic Dermatitis

old atopic dermatitis increase the risk as the area increases. But it was not statistically significant. In the case of atopic dermatitis at 1 year, the information on the fungus exposure by the environmental questionnaire was analyzed. As a result, the exposure area of the fungus tended to decrease in 30%, 0.99 (95% CI, 0.61-1.59), less than 5~30%, 0.78 (95% CI, 0.43-1.42) and less than 5% 0.76 (95% CI, 0.28-2.06). There was no statistically significant difference between the two

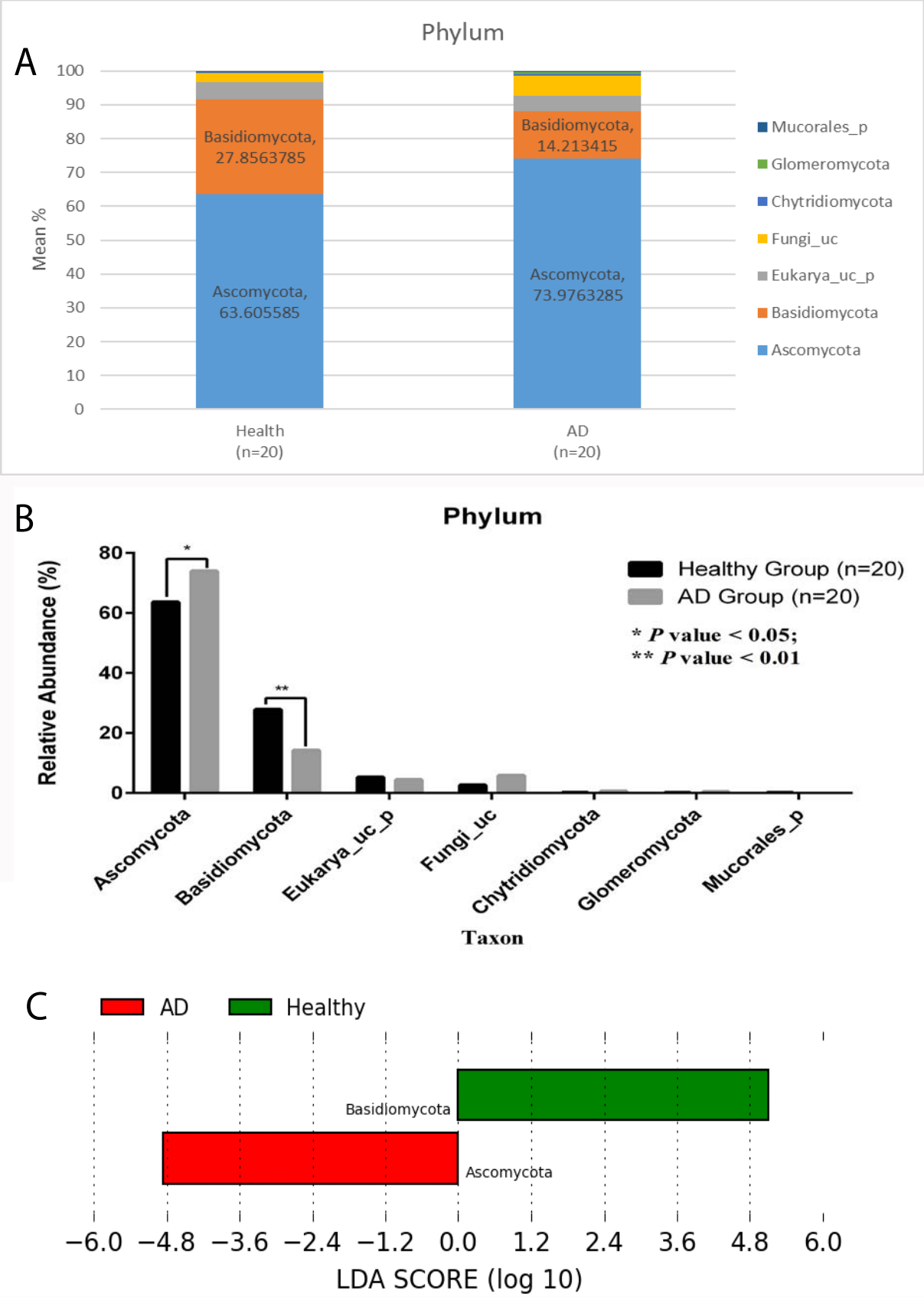

Figure 3: Differences in the fungal communities between house dust samples from healthy and AD groups. Mean relative abundance of fungal community at the phylum level (A), Comparison of the fungal phyla relative abundance between healthy and AD groups (B), taxonomic biomarker compared between two groups by using LEfSe analysis at a log LDA threshold score of 2.0 (C) – the mycobiota in-house dust samples from healthy group(labeled green) and from AD group (labeled red). Symbol (*) represents *P*-values showing the significance of differences between two groups.

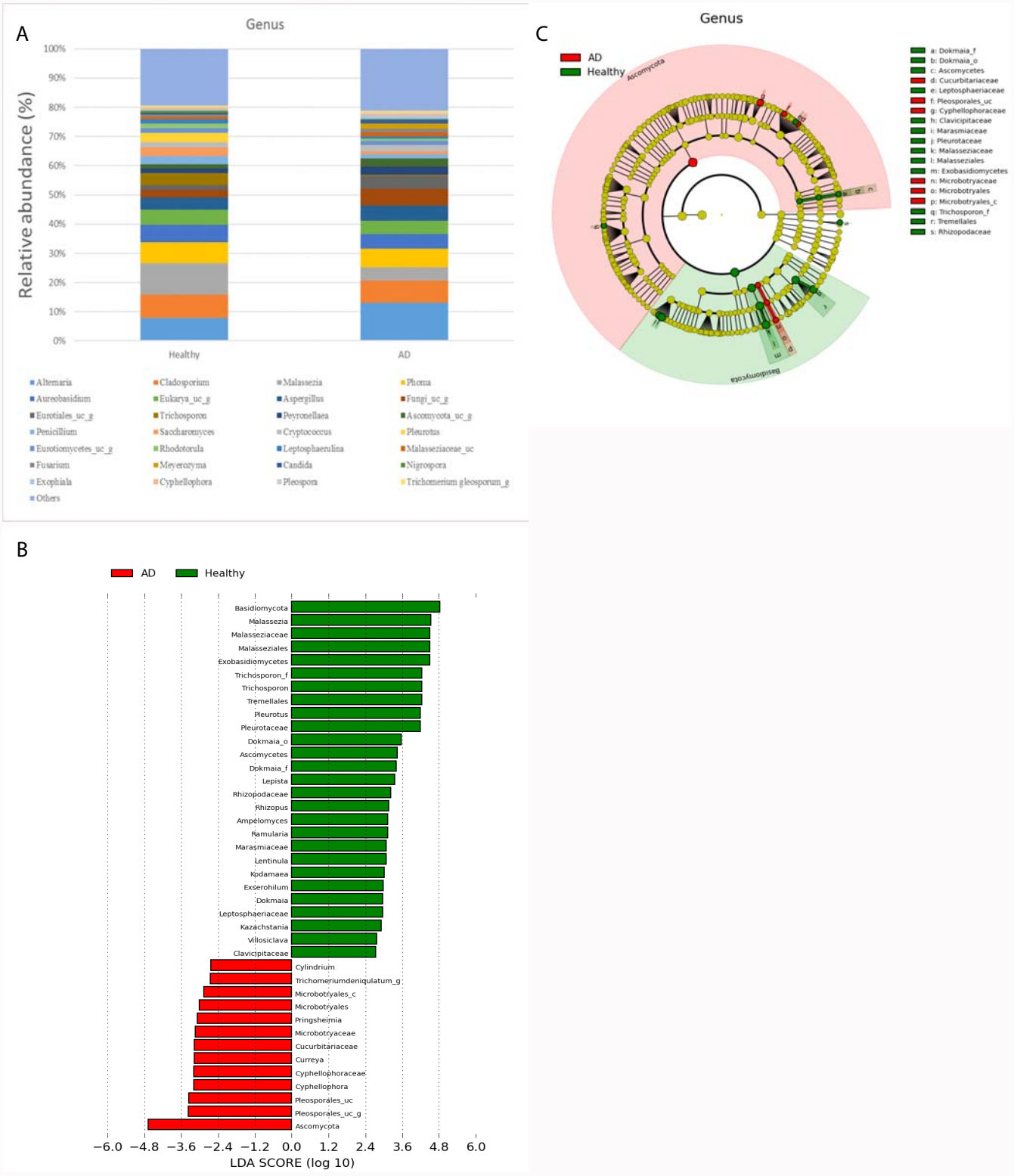

Figure 4: Fungal taxa that were differentially abundant in the house dust mycobiota. Relative abundance at genus level (A), with a log LDA threshold score of 2.0, we found significant difference in the house dust mycobiota between two groups (B), with LEfSe for data analysis and visualization were identified as differentiating between house dust samples from healthy and AD groups.

groups at the age of 2, 3 years (Table 3).

In the environmental questionnaire, 1-year-old atopic dermatitis seemed to increase in less than 5% (0.89: 95% CI, 0.49-1.62) and less than 5% to 30% of area (2.30: 95% CI, 0.96-5.50), and more than 30% (1.37: 95% CI, 0.29 6.61), but not statistically significant, but the risk increased with exposure. In the environmental questionnaire, fungal exposure results show that 2-year-old atopic dermatitis is gradually increasing as the fungus exposure area expands compared

to the group without mold exposure. In atopic dermatitis, the risk of exposure to molds in various places of the room increased from a total area of 30% to 2.69 (95% CI, 1.04-6.97), compared to those without 1 year of age. On the other hand, at 2 years of age, the risk increased to 1.81 (95% CI, 0.93-3.51) at less than 5~30% of the total area compared to the group without mold (Table 4).

Fungal analysis results collected from indoor dust

This study was conducted by visiting a 36-week pregnant home in December 2015 to collect dust from the bedroom. As a result, 20 children with atopic dermatitis and 20 normal children were found to have a parent age of 33.55 years with atopic dermatitis. The gestational age was almost the same (39.17 in the normal group, 39.49 in the atopic dermatitis group), and birth weight was as high as 3043.58 grams in the atopic dermatitis group (Table 5). The total number of 401,206 reads was obtained by sequencing microbial DNA from fungi present in 20 normal and 20 atopic fungi (Table 6). There was no significant difference in OTUs, Chao1, Shannon, and Simpson values between the normal groups and atopic dermatitis groups (Figure 2).

Comparison of fungal distributions diversity

The total number of molds found in the house dust collectors was 10 at the phylum level (Ascomycota, Basidiomycota, Eukarya_uc_p, Fungi_uc, Chytridiomycota, Glomeromycota, Mucorales_p, Mortierellales_p, Blastocladiomycota, Monoblepharidomycota). Among them, Ascomycota was significantly higher in the atopic dermatitis group (73.98 ± 2.96%) than in the normal group (63.61 ± 3.91%) (P = 0.021), and Basidiomycota was lower in the atopic dermatitis group (14.21 ± 1.53%) than in the normal group (27.86 ± 3.87%) (P=0.001) (Figure 3). A total of 1,068 species of fungi were found at the genus level. Among them, there were 4 molds which showed significant difference in the house dust of normal group and atopic dermatitis group (Cyphellophora, Malassezia, Pleurotus, Trichosporon). However, *Alternaria*, *Aspergillus*, *Fusarium*, and *Candida*, which are generally associated with allergic diseases, showed a higher trend in the house dust of the atopic dermatitis group than the normal group (Table 7).

Table 7: List of fungal genera and their relative abundance (%) in house dust samples from two groups.

Taxon		Status		
Phylum	**Genus**	**Health (n=20)**	**AD (n=20)**	***P* Value**
Ascomycota	*Alternaria*	7.9104	12.8797	0.079
Ascomycota	*Aspergillus*	4.1237	5.1118	0.705
Ascomycota	*Aureobasidium*	5.8441	5.0303	0.85
Ascomycota	*Candida*	0.7504	1.0536	0.725
Ascomycota	*Cladosporium*	7.8394	7.6885	0.725
Ascomycota	***Cyphellophora***	**0.5648**	**0.6081**	**0.022**
Ascomycota	*Exophiala*	0.3533	0.8299	0.957
Ascomycota	*Fusarium*	0.6404	1.3015	0.685
Ascomycota	*Leptosphaerulina*	1.4300	0.8881	0.291
Ascomycota	*Meyerozyma*	0.0367	1.7910	0.989
Ascomycota	*Nigrospora*	0.7219	0.5520	0.344
Ascomycota	*Penicillium*	2.7624	1.3681	0.978
Ascomycota	*Peyronellaea*	1.6187	2.7516	0.818
Ascomycota	*Phoma*	7.1393	6.3727	1
Ascomycota	*Pleospora*	0.4702	0.5722	0.487
Ascomycota	*Saccharomyces*	2.9811	1.0517	0.176
Basidiomycota	*Cryptococcus*	1.8277	2.1603	0.725
Basidiomycota	***Malassezia***	**10.8815**	**4.4114**	**0.005**
Basidiomycota	*Malasseziaceae_uc*	0.8520	1.2991	0.626
Basidiomycota	***Pleurotus***	**3.2915**	**0.0260**	**0.005**
Basidiomycota	*Rhodotorula*	1.5704	0.7741	0.725
Basidiomycota	***Trichosporon***	**4.1956**	**0.3016**	**0.003**

Discussion

The results of this study showed that exposure to fungi during pregnancy using environmental questionnaires was a risk factor for the development of atopic dermatitis at 1 and 3 years of age, and increased risk of atopic dermatitis at higher exposure to fungi in bathrooms and other places in the room. The incidence of atopic dermatitis was increased at 2 years of age according to degree of fungus mark. Ascomycota was higher in the atopic dermatitis group (73.98 ± 2.96%) than in the normal group (63.61 ± 3.91%). Analysis of indoor dust during pregnancy showed that Ascomycota was higher in atopic dermatitis than in normal group in molds exposed to mold. These results suggest that exposure of indoor fungi during pregnancy may cause atopic dermatitis and that there is a difference in the types of indoor fungi exposed during pregnancy. There has been no previous study on the sequencing of indoor fungal exposure during pregnancy in Korean infants. In this study, we prospectively investigated the relationship between fungal exposure during pregnancy and atopic dermatitis in pregnant women. In addition, this study was the first study to demonstrate the difference of fungi in indoor dust. Domestic studies have shown that *Malassezia* and *Candida*, which are directly related to atopic dermatitis, are found in blood antibodies [26]. The causal factor of atopic dermatitis is correlated with fungal exposure. In low-income households who remodeled in the past, the fungal concentration was 38% higher than those who did not remodel [27]. Other studies have shown that the risk of exposure to dust mite 94.3%, cockroach 41.5%, and fungus 19.4% increases in children aged 6 to 15 years who were diagnosed with atopic dermatitis [28].

Similar to this study in Japan, fungal exposure in bathrooms and showers was a risk factor for atopic dermatitis [29]. In the United States, the risk factors for fungal exposure in atopic dermatitis patients are reported to affect young adults and adults as well as the prevalence rate in children under 6 years of age [5,30]. As a result of this study, the risk of atopic dermatitis was increased to 1.46 and 1.52 times in 1 year and 3-year-old children. In case of 1-year old atopic dermatitis, the degree of fungus exposure in the bathroom was less than 5% the risk of atopic dermatitis was increased according to the degree of exposure, which was 1.51- and 2.21-times higher risk than the group without fungus exposure.

Direct measurement of mold in dust, the phylum level of mold was higher in indoor dust mildew during pregnancy than Ascomycota in atopic dermatitis group (P=0.021). Basidiomycota was lower in *Malassezia*, *Pleurotus* and *Trichosporon* in atopic dermatitis group (P=0.001). At the genus level, the fungi that showed statistically significant differences were *Cyphellophora*, *Malassezia*, *Pleurotus* and *Trichosporon*. According to foreign data, the result of using the rRNA clone library for fungal microorganisms in 9 patients with atopic dermatitis and 10 patients with normal group. *Candida albicans*, *Cryptococcus diffluens*, and *Cryptococcus liquefaciens* were found to

be a new type of skin in atopic dermatitis patients [31]. Species as a cause of deterioration of atopic dermatitis and in some adult studies, molecular DNA classification and *Malassezia* yeast were found in 75% to 98% of healthy adults in the normal mass of the skin and normal skin [32]. Patients with atopic dermatitis are very susceptible to mold because their skin is dry and damages the barrier function of the skin. Considering the diversity of indoor fungal concentration (Ventilation, temperature, humidity, etc.), *Malassezia* was found in 3 out of 19 patients, and was found to be an allergic risk factor for increased fungal exposure [33-35].

The effect of fungal exposure on atopic dermatitis was found to affect atopic dermatitis according to the time of exposure, the duration of exposure, and the exposure dose of early childhood (12 months old) during the infancy, which directly affects the production of ROS in the skin. The mechanism of atopic dermatitis of certain fungi exposure is related to the occurrence of atopic dermatitis because the specific fungal toxin component including patulin inhibits the expression of Th1 cytokines such as IFN-γ and IL-12, thereby becoming more prominent in the Th2 response [36]. Previously published studies have shown that certain gene polymorphisms associated with the treatment of reactive oxygen species such as GSTP1 and GSTM1 are associated with the development of atopic dermatitis, which is associated with the development of atopic dermatitis through the production of reactive oxygen species [37,38].

Based on the results of this study, further studies will be needed to determine whether there is any difference in the production of reactive oxygen species depending on the type of fungi [39]. Differences in the fungal cell wall components depending on the type of fungus may affect the immune response, immune system formation, inflammation and gene expression by acting on different cell receptors. In addition, depending on the type of fungus, it is associated with activation of cells to specific antigens. This may cause other immune mediated responses and may be associated with the development of atopic dermatitis. Further studies on this mechanism will be needed in the future.

Limitations of this study include a prospective study of information on the first fungal exposure, but may be inconsistent with the actual exposure as it is based on the survey. However, this questionnaire has been used widely in European studies and has already been applied in the existing epidemiological studies in Korea [40,41]. Second, there is a limit to whether the total number of individuals tested at the molecular level in indoor dust is as low as 40 homes [42]. However, there is no prospectively investigating the exposure of indoor fungi during pregnancy both at home and abroad.

Therefore, I think that it will be a new attempt and I hope that the research will be reproduced in many subjects in the future. Despite these limitations, this study is not only meaningful as a prospective causal relationship between exposure to fungus during pregnancy and early childhood and the development of atopic dermatitis in children through the COCOA study, a birth cohort for the general public in Korea.

In particular, it may be the first study to measure fungi at the molecular level in exposed indoor dust during actual pregnancy. In summary, exposure to mold during pregnancy was associated with the development of atopic dermatitis in infants and young children. Ascomycota was significantly higher in the house dust of subjects with atopic dermatitis than in the normal group, and Basidiomycota was lower in subjects with atopic dermatitis at the phylum level in indoor dust exposed during pregnancy. Among the fungi belonging to Ascomycota at Genus level, *Alternaria*, *Aspergillus*, *Fusarium*, and *Candida* were more common in atopic dermatitis subjects and *Malassezia*, *Pleuotus* and *Trichosporon* were fewer. These results suggest that the fungi of indoor dust exposed during pregnancy may be different. Future research will be necessary.

Conclusion

Indoor mold exposure during pregnancy and infancy is associated with the development of atopic dermatitis in children. At 1 and 3 years old, mold exposure is increased in various places in the bathroom and in the room by doctor's diagnosis of atopic dermatitis. On the other hand, fungal exposure increased at the age of 2 years in the diagnosis of atopic dermatitis. The concentrations of phylum and genus in the indoor house dust molds exposed during pregnancy were different. In the case of 40 households, microbial DNA was isolated and sequenced in indoor dust. Ascomycota was significantly higher in the atopic dermatitis group than in the phylum group, while statistical significance was lower in the atopic dermatitis group in the basidiomycota. These results suggest that maternal exposure to mothers during pregnancy may play an important role in the development of atopic dermatitis in children. According to actual measurement data, the type of fungus exposed to indoor dust during pregnancy is different among normal children and atopic dermatitis among the born children. These results suggest that mothers may be associated with the development of atopic dermatitis in children due to exposure to other types of fungi in indoor environments in the future. More research is needed in the future, as well as how the fungal exposure of the indoor environment is linked to human disease. Studies on the mechanism of fungal exposure related to the development of allergic diseases through animal research and cell research are necessary.

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