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ORIGINAL ARTICLE

Gene-environment interaction between Toll-like receptor 4 and mold exposure in the development of atopic dermatitis in preschool children

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Purpose: Genetic factors and environmental exposures are recognized as important risk factors for atopic dermatitis (AD) in children. Inflammatory responses by molds can be mediated via Toll-like receptor 4 (TLR4). The aims of this study were to investigate mold as risk factor of AD and gene-environment interaction on AD in preschool children.

Methods: We undertook a cross-sectional survey with 986 preschool children. We investigated five mold exposure measures (dampness stain, dampness damage, visible mold, mold odor, and house repair). The TLR4 polymorphism (rs1927911) was genotyped by TaqMan assay.

Results: The prevalence of AD was as follows: AD diagnosis by questionnaire, 35.1%; current AD (lifetime diagnosis together with symptoms in the last 12 months), 21.5%. When children with parental history of AD were exposed to mold odor during infancy and house repair during the last 12 months, the risk for current AD (adjusted odds ratio [aOR], 6.826; 95% confidence interval [CI], 2.511 to 18.554 vs. aOR, 6.143; 95% CI, 2.348 to 16.074) was further increased than only with parental history of AD. In children with the CC genotype of TLR4 polymorphism, the risk of AD was increased by mold exposure.

Conclusion: This investigation identified that mold exposure is potential risk factor for AD in preschool children. Parental history of AD and mold exposure during infancy and the last 12 months had synergistic effect on high prevalence of AD. We identified that mold exposure and TLR4 polymorphism have an effect on the development of atopic dermatitis. *(Allergy Asthma Respir Dis 2013;1:129-137)*

Keywords: Atopic dermatitis, Mold, Risk factor, Toll-like receptor 4, Polymorphism

INTRODUCTION

Atopic dermatitis (AD) is a genetically determined, chronic, relapsing and highly pruritic inflammatory skin disease frequently associated with the development of allergic rhinitis or asthma.[1)] The prevalence of AD is increasing in developed countries, including Korea, probably due to changes in lifestyle and the environment.[2-5)]

The etiology of AD is thought to be associated with diverse factors including personal, genetic and environmental factors. The rapid increase in the prevalence of AD over the last 30 to 40 years suggests that environmental factors rather than genetic effects are responsible for this increased prevalence, although interactions between genetic and environmental factors may also be important.[6-8)] Recent clinical research has therefore focused on identifying gene-environment interactions that contribute to the development of allergic diseases including AD.[4,8-13)]

Household moisture and mold are associated with adverse health effects in children,[14,15)] and mold is considered as a key indoor environmental exposure.[16)] Previously we reported early life exposure to mold was important risk factor for the development of AD especially in susceptible children.[4)] Some studies have shown that markers of indoor dampness, such as visible mold and water

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damage, were associated with increased risk of AD in childhood,[17,18] but this association was not found in all studies.[6] The relative importance of mold exposure for the development of AD in preschool children remains unexplored.

Immune responsiveness to many microbial pathogens depends on a family of pattern recognition receptors known as Toll-like receptors, which are the major innate recognition system for microbial invaders in vertebrates.[19] Toll-like receptor 4 (TLR4) recognizes, and transduces intracellular signals in response to bacterial endotoxin, a major component of the bacterial cell wall of gram-negative bacteria.[20] Several studies have shown that TLR4 were related to fungal infection.[21,22] Several studies showed the associations that inflammatory responses by molds are mediated via TLR4.[23,24] However, it is not known whether interactions between TLR4 and mold have an impact on the development of allergic diseases, including AD.

The purpose of the present study was to investigate mold as risk factor of AD and gene-environment interaction on AD in preschool children.

MATERIALS AND METHODS

1. Subjects and study design

Between July and August 2010, this study recruited 986 preschool children aged 3 to 7 years from 16 kindergartens randomly selected from Seoul, Ilsan, and Gwacheon in Korea. A modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire[25] was used to determine the prevalence of symptoms and diagnosis of allergic diseases. Cases in which gender was not reported were excluded, and responses from a total of 933 preschool children and their parents were included in the analyses. Among 933 preschooler, 901 children answered the questions related to current AD. A total of 919 preschool children and their parents responded to the study questionnaires, which represents an overall response rate of 98.5%.

The mean age was 4.87 ± 1.05 years in preschool children included in this survey. The sample included 480 boys and 453 girls (male:female, 51.4:48.6). The prevalence of a parental history of allergic disease (including AD, asthma, and allergic rhinitis) that had been previously diagnosed by a doctor was 49.0% in preschool children. The prevalences of mold exposure in the house during infancy and in the current house were 50.1% and 46.1%, respectively (Table 1).

Table 1. Subject characteristics (n = 933)

Characteristic	Value
Responded to questionnaire	919 (98.5)
Age (yr)	4.87 ± 1.05
Sex (male/female)	480/453
Body mass index (kg/m^2)	15.90 ± 1.65
Parental history of allergic diseases	449/916 (49.0)
Parental history of asthma	46/914 (5.0)
Parental history of allergic rhinitis	403/915 (44.0)
Parental history of atopic dermatitis	105/915 (11.5)
Environmental tobacco smoking	105/914 (11.5)
Educational level of mother	
≤ High school graduate	240/905 (26.5)
≥ University graduate	665/905 (73.5)
Economic state (monthly income)	
Low (< 3 million Won)	328/890 (36.9)
Middle (3–5 million Won)	341/890 (38.3)
High (≥ 5 million Won)	221/890 (24.8)
Any measures of mold exposure*	
During infancy	427/852 (50.1)
During the last 12 months	395/857 (46.1)
Biomarker	
Eosinophil (%)	4.17 ± 3.20
Total immunoglobulin E (IU/mL)	78.31 ± 1.78

Values are presented as number (%) or mean ± standard deviation.
*Five measures: 1) dampness stain, 2) dampness damage, 3) visible mold, 4) mold odor, and 5) house repair due to mold.

This study was approved by the Institutional Review Board of Asan Medical Center. Written consent was obtained from all parents and guardians following a detailed explanation of the study.

2. ISAAC questionnaire and SCORing atopic dermatitis index

The modified Korean version of ISAAC was previously validated as a tool for assessing allergic symptoms and diagnosis in Korean children.[26] The questionnaire consists of three main sections: 1) general characteristics including names, sex, date of birth, height, and weight; 2) a history of symptoms related to asthma, allergic rhinitis, AD, allergic conjunctivitis and food allergy; and 3) exposure to environmental factors associated with allergic disease. The Korean version of the ISSAC questionnaire was completed by parents or guardians of the preschool children. Association between mold exposure and the development of AD was identified in our previous study.[4] So the questionnaires related to mold exposure are subclassified into 5 categories (dampness stain, dampness damage, visible mold, mold odor and house repair due to mold) in this

survey in order to identify more correctly the impact of mold exposure.[27]

3. White blood cells, eosinophils, total IgE, and specific IgE

White blood cells and the percentage of blood eosinophils were measured. Serum total IgE and specific IgE were measured by fluorescent enzyme immunoassay using the ImmunoCAP system (Phadia AB, Uppsala, Sweden). The specific IgE was measured for seven major inhaled allergens: *Dermatophagoides farinae*, cat, dog, cockroach, *alternaria*, mugwort, and alder. The specific IgE was considered to be positive if it was over 0.35 kUA/L.

4. Genotyping

The genotyping of the TLR4 (rs1927911) polymorphism was conducted using a TaqMan assay (ABI, Foster City, CA, USA). The final volume of polymerase chain reaction (PCR) was 5 μL, containing 10 ng of genomic DNA and 2.5 μL TaqMan Universal PCR Master Mix, with 0.13 μL of 40X Assay Mix (Assay ID C_11722141_10) in a 384-well plate.

5. Statistical analysis

Statistic analyses were performed using the IBM SPSS ver. 18.0 (IBM Co., Armonk, NY, USA). Prevalence rates are presented with their 95% confidence interval (CI). Chi-square test and logistic regression analyses were conducted to identify potential risk factors for AD. Age, sex, and body mass index (BMI) were adjusted as personal factors, parental history of AD as a familial factor, the degree of maternal education as a socioeconomic factor, and sensitization of house dust mite as a confounding factor in multivariate analysis. Sensitization of house dust mite means the positivity on skin prick test or ImmunoCAP of house dust mite. For all analyses P value < 0.05 was regarded as statistically significant.

RESULTS

1. Prevalence of AD

The prevalence of AD was as follows: lifetime symptoms, 28.0%; symptoms in the last 12 months, 28.7%; lifetime diagnosis by questionnaire, 35.1%; treatment in the last 12 months, 16.6%; current AD (which was defined as lifetime diagnosis by questionnaire together with symptoms in the last 12 months), 21.5% (Table 2). In consideration of natural course of AD in children, current AD can be regarded as proper item for evaluating exactly children with AD.

Table 2. Prevalence of atopic dermatitis (AD)

	Value
AD symptoms in the lifetime	255/911 (28.0)
AD symptoms in the last 12 months	259/904 (28.7)
AD diagnosis by questionnaire	319/910 (35.1)
AD treatment in the last 12 months	150/905 (16.6)
Current AD*	194/901 (21.5)

Values are presented as number (%).
*Lifetime AD diagnosis by questionnaire together with AD symptoms in the last 12 months.

So, we used "current AD" in further statistical analysis.

2. Risk factors for AD

By multivariate logistic regression analyses, possible risk factors for current AD were identified as a parental history of AD and some measures related to mold exposure. In preschool children with current AD, a parental history of AD (adjusted odds ratio [aOR], 3.313; 95% CI, 2.107 to 5.208), three mold measures during infancy (dampness damage [aOR, 1.873; 95% CI, 1.223 to 2.869], mold odor [aOR, 1.740; 95% CI, 1.148 to 2.639], house repair due to mold [aOR, 1.611; 95% CI, 1.060 to 2.448]) and four mold measures during the last 12 months (dampness stain [aOR, 1.579; 95% CI, 1.054 to 2.367], dampness damage [aOR, 1.722; 95% CI, 1.065 to 2.784], mold odor [aOR, 1.733; 95% CI, 1.118 to 2.684], house repair due to mold [aOR, 1.758; 95% CI, 1.128 to 2.742]) were verified as potential risk factors (Table 3). In brief, parental history of AD and mold exposure were potential risk factors for AD in preschool children.

3. Interaction between family history of AD and mold exposure

Analyses were conducted to identify whether an interaction between a hereditary factor (parental history of AD) and environmental exposure (five measures related to mold exposure: dampness stain, dampness damage, visible mold, mold odor, and house repair due to mold in the house) was associated with the occurrence of AD. The study group was divided into four subgroups according to the presence or absence of the environmental and hereditary factors (parental history of AD and the measures of mold exposure).

For a parental history of AD and four mold measures (dampness stain, visible mold, mold odor, and house repair) during infancy, the aOR of current AD increased up to 4.360 (95% CI, 1.949 to 9.755), 4.996 (95% CI, 2.294 to 10.884), 6.826 (95% CI, 2.511 to

Table 3. Risk factors for atopic dermatitis in preschool children

Risk factor	Current AD[§] (194/901, 21.5%)			
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Demographic factors				
Male sex	0.782 (0.569–1.075)	0.130	0.822 (0.586–1.154)*	0.258
Body mass index	1.029 (0.933–1.134)	0.571	1.027 (0.928–1.137)*	0.601
Maternal education (<university graduate)	0.758 (0.520–1.106)	0.150	0.738 (0.493–1.104)*	0.140
Genetic factors				
Parental history of AD or AR or asthma	1.619 (1.173–2.234)	0.003	1.463 (1.045–2.046)†	0.026
Parental history of AD	3.353 (2.304–5.418)	<0.001	3.313 (2.107–5.208)†	<0.001
Paternal history of AD	3.545 (1.995–6.300)	<0.001	3.387 (2.021–6.728)†	<0.001
Maternal history of AD	3.292 (1.926–5.628)	<0.001	2.985 (1.679–5.307)†	<0.001
Parental history of AR	1.404 (1.020–1.932)	0.037	1.131 (0.803–1.594)*	0.482
Parental history of asthma	1.511 (0.777–2.938)	0.224	1.210 (0.602–2.429)*	0.593
Environmental factors				
Environmental tobacco smoking	0.934 (0.678–1.286)	0.675	1.330 (0.946–1.869)*	0.101
Pet ownership in the last 12 mo	0.492 (0.146–1.662)	0.254	0.628 (0.182–2.164)*	0.461
Pet ownership in infancy	0.543 (0.227–1.303)	0.172	0.578 (0.236–1.417)*	0.231
Factors related to mold				
During infancy				
Dampness stain	1.570 (1.131–2.180)	0.007	1.392 (0.937–2.069)‡	0.102
Dampness damage	1.907 (1.337–2.718)	<0.001	1.873 (1.223–2.869)‡	0.004
Visible mold	1.479 (1.071–2.043)	0.018	1.465 (0.991–2.164)‡	0.055
Mold odor	1.638 (1.158–2.317)	0.005	1.740 (1.148–2.639)‡	0.009
House repair due to mold	1.534 (1.084–2.172)	0.016	1.611 (1.060–2.448)‡	0.026
During the last 12 mo				
Dampness stain	1.529 (1.090–2.143)	0.014	1.579 (1.054–2.367)‡	0.027
Dampness damage	1.527 (1.000–2.333)	0.050	1.722 (1.065–2.784)‡	0.027
Visible mold	1.220 (0.879–1.692)	0.234	1.383 (0.933–2.051)‡	0.107
Mold odor	1.582 (1.090–2.296)	0.016	1.733 (1.118–2.684)‡	0.014
House repair due to mold	1.538 (1.057–2.237)	0.024	1.758 (1.128–2.742)‡	0.013
Biomarkers				
Eosinophil 4th quartile (>5.5%)	1.760 (1.164–2.661)	0.007	1.895 (1.236–2.904)*	0.003
Total IgE 4th quartile (>187 IU/mL)	1.831 (1.232–2.722)	0.003	1.707 (1.098–2.653)*	0.017

Data were calculated by logistic regression multivariate analysis.
OR, odds ratio; AD, atopic dermatitis; BMI, body mass index; AR, allergic rhinitis; IgE, immunoglobulin E.
*OR=adjusted by age, sex, BMI, parental history of atopic dermatitis, and degree of maternal education. †OR=adjusted by age, sex, BMI, and degree of maternal education. ‡OR=adjusted by age, sex, BMI, parental history of atopic dermatitis, degree of maternal education, and sensitization of house dust mite. §Lifetime AD diagnosis by questionnaire together with AD symptoms in the last 12 months.

18.554) and 4.571 (95% CI, 1.814 to 11.515), respectively (Table 4). For a parental history of AD and five mold measures (dampness stain, dampness damage, visible mold, mold odor, and house repair due to mold) during the last 12 months, the aOR of current AD increased up to 4.622 (95% CI, 1.894 to 11.280), 4.104 (95% CI, 1.144 to 14.717), 3.817 (95% CI, 1.747 to 8.339), 5.512 (95% CI, 2.073 to 14.656) and 6.143 (95% CI, 2.348 to 16.074), respectively (Table 4).

4. Gene–environment interaction between TLR4 (rs1927911) polymorphism and mold exposure

We also identified a relationship between a specific gene and environment effects that is associated with the development of AD. Children with only TLR4 polymorphism have no risk on the development of AD (Table 5). When children with the CC genotype, however, were exposed to mold (dampness damage) during infancy, the development of current AD was increased (aOR, 3.163; 95% CI, 1.485 to 6.738) (Table 6). For the children with the CC genotype

Table 4. Interactions between parental history of atopic dermatitis and mold exposure on the development of current atopic dermatitis

		Current AD* (during infancy)			Current AD* (during the last 12 mo)		
		No.	OR† (95% CI)	*P*-value	No.	OR† (95% CI)	*P*-value
Parental AD	Dampness stain						
No	No	437	1 (ref)		489	1 (ref)	
No	Yes	263	1.367 (0.883–2.116)	0.161	225	1.569 (1.011–2.435)	0.045
Yes	No	57	2.873 (1.497–5.512)	0.002	61	2.824 (1.510–5.281)	0.001
Yes	Yes	32	4.360 (1.949–9.755)	<0.001	26	4.622 (1.894–11.280)	0.001
Parental AD	Dampness damage						
No	No	576	1 (ref)		627	1 (ref)	
No	Yes	159	2.149 (1.349–3.425)	0.001	110	1.808 (1.085–3.012)	0.023
Yes	No	66	3.628 (2.014–6.535)	<0.001	82	3.330 (1.947–5.696)	<0.001
Yes	Yes	25	3.544 (1.421–8.835)	0.007	10	4.104 (1.144–14.717)	0.030
Parental AD	Visible mold						
No	No	461	1 (ref)		462	1 (ref)	
No	Yes	276	1.415 (0.921–2.174)	0.113	272	1.452 (0.941–2.239)	0.092
Yes	No	56	2.896 (1.518–5.523)	0.001	56	3.450 (1.825–6.521)	<0.001
Yes	Yes	35	4.996 (2.294–10.884)	<0.001	36	3.817 (1.747–8.339)	0.001
Parental AD	Mold odor						
No	No	551	1 (ref)		582	1 (ref)	
No	Yes	145	1.652 (1.051–2.597)	0.030	151	1.724 (1.069–2.780)	0.026
Yes	No	69	2.898 (1.625–5.169)	<0.001	72	3.097 (1.748–5.488)	<0.001
Yes	Yes	18	6.826 (2.511–18.554)	<0.001	20	5.512 (2.073–14.656)	0.001
Parental AD	House repair						
No	No	546	1 (ref)		581	1 (ref)	
No	Yes	188	1.656 (1.048–2.617)	0.031	149	1.695 (1.040–2.764)	0.034
Yes	No	69	3.266 (1.824–5.851)	<0.001	69	2.926 (1.628–5.259)	<0.001
Yes	Yes	23	4.571 (1.814–11.515)	0.001	21	6.143 (2.348–16.074)	<0.001

Data were calculated by logistic regression multivariate analysis.
OR, odds ratio; AD, atopic dermatitis.
*Lifetime AD diagnosis by questionnaire together with AD symptoms in the last 12 months. †OR=adjusted by age, sex, body mass index, degree of maternal education, and sensitization of house dust mite.

Table 5. Association analysis between TLR4 (rs1927911) and current atopic dermatitis

TLR4 polymorphism	Genotypes	Current AD* (-)	Current AD* (+)	aOR (95% CI)	*P*-value
rs1927911	CC	164	45	1.000	
	CT+TT	327	105	1.023 (0.678–1.543)	0.914

Data were calculated by logistic regression multivariate analysis. Data were adjusted by age, sex, body mass index, parental history of atopic dermatitis and degree of maternal education.
TLR4, Toll-like receptor 4; AD, atopic dermatitis; aOR, adjusted odds ratio; CI, confidence interval.
*Lifetime AD diagnosis by questionnaire together with AD symptoms in the last 12 months.

and three mold measures (dampness stain, dampness damage, and visible mold) during the last 12 months, the aOR of current AD increased up to 2.372 (95% CI, 1.144 to 4.919), 2.939 (95% CI, 1.250 to 6.910) and 3.561 (95% CI, 1.727 to 7.343), respectively (Table 6).

DISCUSSION

This was the first representative cross-sectional survey identifying the association between AD and mold exposure in preschool children in metropolitan area of Korea.

In the present study, we identified the fact that mold exposure could be significantly relevant to the development of AD. Our results show that children with a hereditary susceptibility to AD, defined as a parental history of AD, were more likely to develop AD when they had a history of exposure to mold. The coexistence of a parental history of AD and factors related to mold exposure, such

Table 6. Interactions between TLR4 polymorphism (rs1927911) and mold exposure on the development of current atopic dermatitis

		Current AD* (during infancy)			Current AD* (during the last 12 mo)		
		No.	OR† (95% CI)	*P*-value	No.	OR† (95% CI)	*P*-value
TLR4	Dampness stain						
CC	No	117	1 (ref)		130	1 (ref)	
CC	Yes	82	1.836 (0.905–3.726)	0.093	65	2.372 (1.144–4.919)	0.020
CT+TT	No	231	1.295 (0.724–2.317)	0.384	255	1.273 (0.724–2.239)	0.401
CT+TT	Yes	162	1.444 (0.781–2.669)	0.241	145	1.747 (0.949–3.213)	0.073
TLR4	Dampness damage						
CC	No	157	1 (ref)		174	1 (ref)	
CC	Yes	48	3.163 (1.485–6.738)	0.003	32	2.939 (1.250–6.910)	0.013
CT+TT	No	312	1.423 (0.845–2.397)	0.184	340	1.287 (0.796–2.082)	0.304
CT+TT	Yes	99	1.906 (1.012–3.590)	0.046	73	1.734 (0.891–3.375)	0.105
TLR4	Visible mold						
CC	No	127	1 (ref)		133	1 (ref)	
CC	Yes	77	1.724 (0.848–3.506)	0.133	71	3.561 (1.727–7.343)	0.001
CT+TT	No	245	1.236 (0.704–2.169)	0.461	237	1.862 (1.033–3.355)	0.039
CT+TT	Yes	169	1.570 (0.871–2.830)	0.133	176	1.704 (0.913–3.181)	0.094
TLR4	Mold odor						
CC	No	150	1 (ref)		164	1 (ref)	
CC	Yes	55	1.417 (0.649–3.097)	0.382	41	2.082 (0.927–4.673)	0.076
CT+TT	No	291	1.009 (0.607–1.677)	0.972	311	1.116 (0.678–1.837)	0.666
CT+TT	Yes	123	1.760 (0.987–3.138)	0.056	102	1.777 (0.972–3.247)	0.062
TLR4	House repair						
CC	No	151	1 (ref)		157	1 (ref)	
CC	Yes	54	2.124 (0.997–4.523)	0.051	45	2.021 (0.897–4.553)	0.089
CT+TT	No	295	1.241 (0.739–2.081)	0.414	314	1.148 (0.691–1.907)	0.595
CT+TT	Yes	119	1.638 (0.896–2.995)	0.109	96	1.903 (1.021–3.547)	0.043

Data were calculated by logistic regression multivariate analysis.
OR, odds ratio; AD, atopic dermatitis; TLR, Toll-like receptor.
*Lifetime AD diagnosis by questionnaire together with AD symptoms in the last 12 months. †OR = adjusted by age, sex, body mass index, parental history of atopic dermatitis, degree of maternal education, and sensitization of house dust mite.

as mold odor during infancy and house repair during the last 12 months, was synergistically associated with occurence of current AD (aOR, 6.826; 95% CI, 2.511 to 18.554 and aOR, 6.143; 95% CI, 2.348 to 16.074, respectively). It is thought that mold exposure during infancy is very important for the development of AD in susceptible children. Mold exposure during in preschool age, on the other hand, can be a possible cause for occurrence or aggravation of AD in susceptible children.

Some studies show that TLR4 polymorphisms may have a role in the development of Th2-dominant allergic inflammation.[28,29] In this study, there was no significant association between the TLR4 (rs1927911) polymorphism and development of AD. However, the risk of AD was increased by mold exposure in children with the CC genotype of TLR4 polymorphism. Even for the relationship between the nonrisk genotype and mold exposure, the present study shows that the risk of developing AD is increased in children who have the specific genotype. It will be important to elucidate the basis of the genetic interaction with mold exposure in future studies. The reason why the effect of TLR4 polymorphism is less than familial history of AD as a hereditary factor: presumably, unknown factors such as other genes except TLR4 or other common environment are regarded as possible explanations.

It was well known that a family history of allergic diseases is associated with the prevalence of AD, suggesting that genetic factors play a central role in the development of childhood AD.[4,8,16,30] In the present study, parental history of AD was the only significant familial risk factor for AD in preschool children; parental histories of other allergic diseases such as asthma and allergic rhinitis were not significant.

Molds are important indoor allergens for asthma and allergic

rhinitis, and indoor exposure to certain fungal genera such as *Aspergillus* and *Penicillium* has been identified as a risk factor for asthma, atopy and respiratory symptoms in children.[31] One study reported that molds were unrelated to the prevalence of AD,[32] whereas several other studies have reported an increased risk of developing AD associated with mold as home environments.[33-36] The mechanisms underlying the association between mold and AD are not known. The skin is an active immunological organ that functions as a primary defense and biosensor to the external environment.[37] Skin barrier dysfunction caused by environmental proteases has emerged as a critical driving force in the initiation and exacerbation of AD and the "atopic march" in allergic diseases.[38] Mycotoxins are secondary metabolites produced by mold as they grow. Many studies have shown that inhalation of molds and their products (e.g., conidia and mycotoxins) leads to adverse health effects such as allergic and respiratory diseases.[39,40] The skin barrier of patients with AD is damaged, which facilitates penetration of allergens such as molds.[11] The findings that mold exposure as potential environmental risk factor could interact with hereditary factors and act on the development of AD were verified in this investigation. However, it is still not known exactly how interaction between TLR4 polymorphism and mold affects the development of allergic diseases like AD.

Our study has some limitations. First, this is a cross-sectional study, which does not allow us to establish a true causal relationship and therefore the questionnaire about mold exposure cannot explain entirely whether mold exposure can act on a cause of the first development of AD or a mere aggravation factor of AD in preschool age. So, it is reasonable to assume that mold exposure during the last 12 months may be a aggravation factor rather than a major cause for the development of current AD. Second, the exposure levels of factors related to mold were not quantitatively measured but were based on a questionnaire. It is likely that the questionnaire-based assessment is usual situation in epidemiologic studies and only partially reflects true mold exposure, but there is very rare data of the real measurement of fungus by culture or metagenomics regarding to allergic diseases because of expensive cost. However, by investigating five measures related to mold exposure in detail, we tried to maximize the accuracy of the questionnaire for assessing mold exposure. And the results of each individual questionnaire based measures of mold exposure was consistent effect on the development of AD in this study. Third, the number of children included in this study was relatively small. However, the number of children was greater than 900 and the response rate was comparatively high (98.5%). Fourth, there may be some recall bias in the study. However, in comparison to studies investigating older children, the possibility of recall bias may be low because the subjects were preschool-aged. Fifth, there may be many possibilities of nonmeasured important variables and confounding factors. Although all of variables and confounding factors are not considered in this study, we tried to investigate the fact that mold exposure could affect the development of AD by adjusting the sensitization of house dust mite as representative confounding factor.

The main strengths of the present study include the in-depth study examination of various factors related to mold exposure and the replication of our previous study[4] related to gene-environment interactions on the development of AD. These detailed questionnaire based measurements of indoor mold exposure may be used in the future epidemiologic study for the evaluation of environmental factors.

In conclusion, the main focus of the present study was to analyze interactions between genetic and environmental risk factors in the development of AD, especially in susceptible children. Additional experimental studies, such as the direct measurement of mold exposure in a cohort study or an animal study of mold exposure, will be needed to better understand these observations. In addition, we identified a possible gene-environment interaction between TLR4 and mold exposure. These findings suggest that early avoidance of mold exposure during infancy is important to prevent the development of AD, especially in susceptible children.

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