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Interaction of the *TLR4* rs1927911 polymorphism with house dust mite sensitization in allergic rhinitis with its prognosis

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Abstract

Background: Sensitization to the house dust mite (HDM) plays important roles in the development of allergic rhinitis (AR). Toll-like receptor 4 (TLR4) is a key initiator of the innate immune system upon exposure to environmental factors.

Objective: The present study investigated the independent and interaction effects of HDM sensitization and *TLR4* rs1927911 polymorphism on AR and its prognosis in children.

Methods: This study included 2,929 children (mean age, 7.8 yrs) from the Children's HEalth and Environmental Research study (CHEER), a prospective study with a 2-year-interval for 4 years. An ISAAC questionnaire was used with skin prick tests in all subjects. TaqMan genotyping was performed for *TLR4* (rs1927911) polymorphism in 1,024 children.

Results: HDM sensitization increased risk of current AR (aOR, 2.50; 95% CI, 1.41-4.41; *P* for interaction = 0.005), current asthma at follow-up (aOR, 4.63; 95% CI, 2.41-8.88; *P* for interaction < 0.001) and allergic march (aOR, 2.57; 95% CI, 1.06-6.22; *P* for interaction = 0.002) by interacting with genotypes of *TLR4* (rs1927911). HDM sensitization increased risk of persistence (aOR, 4.17; 95% CI, 1.77-9.83) and new diagnosis of AR (aOR, 2.48; 95% CI, 1.10-5.61), new sensitization to inhalant allergens (aOR, 10.67; 95% CI, 5.83-19.54), and new development of bronchial hyper-responsiveness (aOR, 5.29; 95% CI, 2.29-12.21) in children with CC genotype of *TLR4* rs1927911.

Conclusions: HDM sensitization affects AR and its prognosis by interacting with *TLR4* rs1957911 polymorphism. The preventive and therapeutic strategies for AR in children need to be targeted in accordance with genetic susceptibility with HDM sensitization.

Key words: allergic conjunctivitis; allergic rhinitis; house dust mite; Toll-like receptor 4; polymorphism; sensitization.

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Introduction

Allergic rhinitis (AR) is one of the chronic airway diseases that results from complex interactions between diverse environmental factors and genetic factors.[1] AR, the most common allergic disease, have negative impacts on quality of life, through a reduced capacity for physical activity, poorer school performance, and sleep disturbance.[2] AR can be classified into diverse phenotypes according to the sensitized allergens and genetic susceptibility, which might explain some of the pathophysiologies underlying rhinitis phenotypes.[1] However, phenotype studies on AR in children are relatively lacking compared to other allergic diseases, including atopic dermatitis and asthma, and classifications of AR with consideration of genetic susceptibility are also scarce.

House dust mite (HDM) is the most commonly sensitized allergen in both children and adults.[3] Although sensitization to HDM plays an important role in the development of AR, it is not sufficient alone to cause its onset.[3,4] This indicates that AR development requires other factors, such as genetic susceptibility.[5] HDM allergens can cause AR symptoms,[3] partially through the activation of innate immunity and induction of adaptive Th2 immune responses,[6] which suggests that the effect of HDM sensitization on AR is affected by the types of immune responses in each subject.

Toll-like receptor 4 (TLR4) plays an important role as an innate immune system initiator upon exposure to environmental factors. TLR4 may also be involved in the development and symptom status of AR as antagonists of TLR4 have found to attenuate AR symptoms.[7,8] Single nucleotide polymorphisms (SNPs), the most common variations of human genome, have been widely used to evaluate their association with disease susceptibility in various diseases.[9,10] The previous studies have produced inconclusive results on the associations between genetic variations of *TLR4* and AR.[11-13] Although TLR-mediated pathways may regulate HDM-induced AR,[14] there have been no studies on the interaction effects of *TLR4* polymorphism and HDM sensitization on AR development. In the previous studies, *TLR4* (rs1927911) has been associated with the development of AR in children in the context of gene-environment interactions.[15,16] Therefore, in the present study, we have investigated the independent and interaction effects of HDM sensitization and *TLR4* (rs1927911) polymorphism on AR and its prognosis in children in the prospective follow-up cohort.

Materials and Methods

Study population

The present study enrolled 2,929 children from the Children's HEalth and Environmental Research (CHEER) study, which is a nationwide general population based prospective study starting at a mean age of 7.8 years.[17] At each survey in the CHEER study, an ISAAC questionnaire was used to identify the presence of allergic diseases and skin prick tests (SPTs) were performed on all children. Children sensitized to other inhalant allergens except HDM at the time of enrollment were excluded from the current analyses. The participants had been followed-up for 4 years with a 2-year-interval survey.[17] The present study protocols were reviewed and approved by the institutional review board (IRB) of Ulsan University College of Medicine (IRB no. 2006-0081). Written informed consent was obtained from the parents or guardians of the study participants.

Skin prick tests

SPTs were performed for 13 common inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria alternata*, *Aspergillus fumigatus*, dog dander, cat epithelium, cockroaches, grass pollen mixture, tree mixture, ragweed, mugwort, alder, and oak) at the time of enrollment as well as at each survey.[17] Histamine (10 mg/ml) was used as the positive control and normal saline was used as the negative control. A mean wheal size ≥ 3 mm and wheals caused by histamine when measured after 15 min were considered positive. Sensitization to HDM was defined as positive response to more than one allergen among *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*.

Methacholine bronchial provocation test (MBPT)

MBPTs were performed for all of the participants using a modified 5-breath dosimeter method in accordance with American Thoracic Society guidelines.[18] Normal saline was used as a baseline and was followed by stepwise concentrations of methacholine (0.625 1.25, 2.5, 5, 12.5, and 25 mg/mL). The forced expiratory volume in 1 second (FEV_1) was measured at 30 and 90 seconds after the nebulization was completed, and the next dosing schedule then proceeded within 5 minutes.[19] Bronchial hyperresponsiveness (BHR) was defined as a 20% decrease in the forced expiratory volume in 1 sec caused by a provocative methacholine concentration of less than 8 mg/mL.

Definitions of AR/allergic rhinoconjunctivitis (ARC), current AR/ARC/asthma, and allergic march

AR symptoms was defined as the presence of more than two symptoms among sneezing, runny nose, nasal itching, and nasal stuffiness reported via the ISAAC questionnaire. Current AR was defined as physician diagnosed AR at any point in the child's lifetime with nasal symptoms during the 12 months preceding the questionnaire survey. ARC was defined as nasal symptoms together with itchy or watery eyes. Current ARC was defined as physician-diagnosed ARC at any point in the child's lifetime with ARC symptoms in the prior 12 months. Current asthma was defined as physician-diagnosed asthma at any point in the child's lifetime with asthma symptoms in the prior 12 months reported in response to the question, "Have you ever had symptoms of asthma, such as wheezing or whistling in the chest during the preceding 12 months?" Allergic march was defined as the presence of AR and asthma in children with atopic dermatitis and/or food allergy in their earlier life.

Polymorphism detection

Genotyping of the *TLR4* (rs1927911) polymorphism was performed for 1,024 children using blood samples with consent for genotype analysis by TaqMan assay (ABI, Foster City, California).[16] Duplicate samples and negative controls were included to ensure accuracy.

Statistical analysis

Logistic regression analysis was used to identify factors associated with HDM sensitization, and *TLR4* (rs1927911) polymorphism. The combined effects of HDM sensitization with *TLR4* (rs1927911) polymorphism on diverse outcomes, including AR diagnosis ever and its prognosis, were also identified using logistic regression analysis. The results were expressed as adjusted an odds ratio (aOR) with a 95% confidence interval (95% CI). Adjustments were made for potential confounding factors, including sex, age, living area, income, exposure to environmental tobacco smoke, and a family history of allergic diseases. $P < 0.05$ was considered significant. All statistical analyses were performed using SAS for Windows, version 9.2 (SAS Institute Inc., Cary, NC).

Results

Characteristics of the study population

The characteristics of the study population are indicated in **Table 1**. In the total population, 51.2% of the subjects were male, 29.9% had a family history of any allergic diseases and 20.9% had current AR at the time of enrollment. There were no significant differences in the baseline characteristics of the total cohort other than the family history of allergic diseases and mean age at enrollment and final survey between children with and without information on the *TLR4* (rs1927911) polymorphism.

Factors associated with HDM sensitization

In the total study population, a family history of allergic diseases, higher monthly income levels, and indoor dog ownership in the preceding 12 months increased the risks of HDM sensitization, whereas a female sex was negatively associated with HDM sensitization in the children (**Table 2**). Higher total serum IgE and blood eosinophil (%) levels were also significantly associated with HDM sensitization.

Table 1. Demographics of the study population

Variables, n (%) or mean ± SD	Children with information on the *TLR4* (rs1927911) polymorphism	Children without information on the *TLR4* (rs1927911) polymorphism	*P value*
Number	1024	1905	NA
Age at enrollment (yrs), mean ± SD	7.5 ± 0.7	7.9 ± 1.3	< 0.001
Age at final survey (yrs), mean ± SD	11.6 ± 1.3	11.1 ± 0.5	< 0.001
Gender, male	503/1024 (49.1)	997/1905 (52.3)	0.104
Family history of allergic diseases	396/1009 (39.2)	398/1644 (24.2)	< 0.001
Blood eosinophil (%)	3.6 ± 2.6	3.6 ± 2.9	0.725
Total serum IgE (kU/L)	239.7 ± 498.1	241.9 ± 511.6	0.912
AR diagnosis ever at the time of enrollment	237/973 (24.4)	396/1765 (22.4)	0.256
AR symptoms in the 12 months prior to enrollment	412/974 (42.3)	714/1766 (40.4)	0.351
Current AR at the time of enrollment	215/974 (22.1)	358/1766 (20.3)	0.280
ARC diagnosis ever at the time of enrollment	124/1314 (9.4)	80/718 (11.1)	0.126
ARC symptoms in the 12 months prior to enrollment	262/1142 (22.9)	153/627 (24.4)	0.262
Sensitization to HDM	208/1024 (20.3)	395/1905 (20.7)	0.811
TLR4 (rs1927911) polymorphism	1024 (100.0)	NA	NA
CC	337/1024 (32.9)		
CT+TT	687/1024 (67.1)		

AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis; HDM, house dust mite; NA, not applicable.

Table 2. Factors associated with the sensitization to HDM in the total study population

Variables	No sensitization, n (%)	HDM sensitization, n (%)	*aOR* (95% CI)*	*P* value
Gender, female	1168/2326 (50.2)	261/603 (43.3)	0.76 (0.62-0.93)	< 0.001
Family history of allergic diseases, yes	1168/2326 (50.2)	261/603 (43.3)	1.43 (1.16-1.77)	0.001
Antibiotics taken during the first 1 year	644/1912 (33.7)	168/491 (34.2)	0.97 (0.77-1.20)	0.751
Breastfeeding during the first 6 months	1197/1912 (62.6)	314/492 (63.8)	1.11 (0.89-1.37)	0.372
Bronchiolitis history during the first 24 months	204/1946 (10.5)	51/511 (10.0)	0.83 (0.59-1.16)	0.273
C-section delivery	482/1330 (36.2)	148/345 (42.9)	1.21 (0.93-1.56)	0.156

Table 2. (Continued)

Variables	No sensitization, n (%)	HDM sensitization, n (%)	*aOR* (95% CI)*	*P* value
Number of siblings				
0	494/2129 (23.2)	121/553 (21.9)	Ref.	
1-2	1527/2129 (71.7)	412/553 (74.5)	1.06 (0.81-1.38)	0.691
≥ 3	108/2129 (5.1)	20/553 (3.6)	1.00 (0.58-1.73)	0.994
Indoor dog ownership in the preceding 12 months	124/2118 (5.9)	51/571 (8.9)	1.67 (1.14-2.45)	0.009
Outdoor dog ownership in the preceding 12 months	306/2120 (14.4)	67/572 (11.7)	0.82 (0.60-1.14)	0.239
Monthly income > 300$	706/2188 (32.3)	211/573 (36.8)	1.30 (1.05-1.60)	0.014
Total serum IgE ≥ 100 IU/L	849/2284 (37.2)	448/591 (75.8)	5.46 (4.34-6.87)	< 0.001
Blood eosinophil (%) ≥ 4%	550/2289 (24.0)	351/592 (59.3)	4.42 (3.58-5.47)	< 0.001
CC genotype of *TLR4* (rs1927911)	553/816 (67.8)	134/208 (64.4)	1.16 (0.82-1.64)	0.391

aOR, adjusted odds ratio; AR, allergic rhinitis; ARC, allergic rhino-conjunctivitis; CI, confidence interval; HDM, house dust mite; Ref., reference; SPT, skin prick test.
* adjusted for family history of allergic diseases, sex, region, and monthly income

Association between HDM sensitization and AR/ARC including its prognosis

HDM sensitization at 7 years of age was associated with an increased risk of current AR (aOR, 1.94; 95% CI, 1.54-2.43; **Table 3**) and AR diagnosis ever in the child's lifetime (aOR, 1.83; 95% CI, 1.46-2.28). During the prospective follow-up, children sensitized to HDM showed increased risks of AR symptom persistence (aOR, 3.00; 95% CI, 2.06-4.37), newly diagnosed AR (aOR, 2.22; 95% CI, 1.55-3.18), and new sensitization to inhalant allergens other than HDM (aOR, 5.83; 95% CI, 4.27-7.95). In addition, HDM sensitization was significantly associated with current ARC (aOR, 3.64; 95% CI, 2.59-5.14) with the persistence (aOR, 9.33; 95% CI, 6.12-14.22) and new diagnosis (aOR, 3.31; 95% CI, 1.80-6.91) of ARC during the prospective follow-up period. It was notable however, that the *TLR4* (rs1927911) polymorphism was not associated with current AR/ARC or their prognosis, HDM sensitization, new sensitization to HDM, or inhalant allergens other than HDM during the prospective follow-ups (**Supplementary Table 1**)

Table 3. Association between HDM sensitization and AR/ARC with its prognosis

Outcomes	HDM sensitization (-), n (%)	HDM sensitization (+), n (%)	*aOR* (95% CI)*	*P* value
Current AR	389/2159 (18.0)	184/581 (31.7)	1.94 (1.54-2.43)	< 0.001
AR diagnosis ever	437/2157 (20.3)	196/581 (33.7)	1.83 (1.46-2.28)	< 0.001
Persistence of AR symptoms during the prospective follow-ups	261/642 (40.7)	129/183 (70.5)	3.00 (2.06-4.37)	< 0.001
Newly diagnosed AR during the prospective follow-ups	205/706 (29.0)	85/183 (46.4)	2.22 (1.55-3.18)	< 0.001
Current ARC	210/1354 (15.5)	155/415 (37.3)	3.64 (2.59-5.14)	< 0.001
ARC diagnosis ever	122/1635 (7.5)	82/397 (20.7)	2.97 (2.13-4.14)	< 0.001
Persistence of ARC symptoms during the prospective follow-ups	85/864 (9.8)	94/199 (47.2)	9.33 (6.12-14.22)	< 0.001
Newly diagnosed ARC during the prospective follow-ups	58/837 (6.9)	19/124 (15.3)	3.31 (1.80-6.91)	< 0.001
New sensitization to inhalant allergens other than HDM on SPTs during the prospective follow-ups	172/977 (17.6)	147/276 (53.3)	5.83 (4.27-7.95)	< 0.001

aOR, adjusted odds ratio; AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis; CI, confidence interval; HDM, house dust mite; SPT, skin prick test.
* adjusted for family history of allergic diseases, sex, region, and monthly income

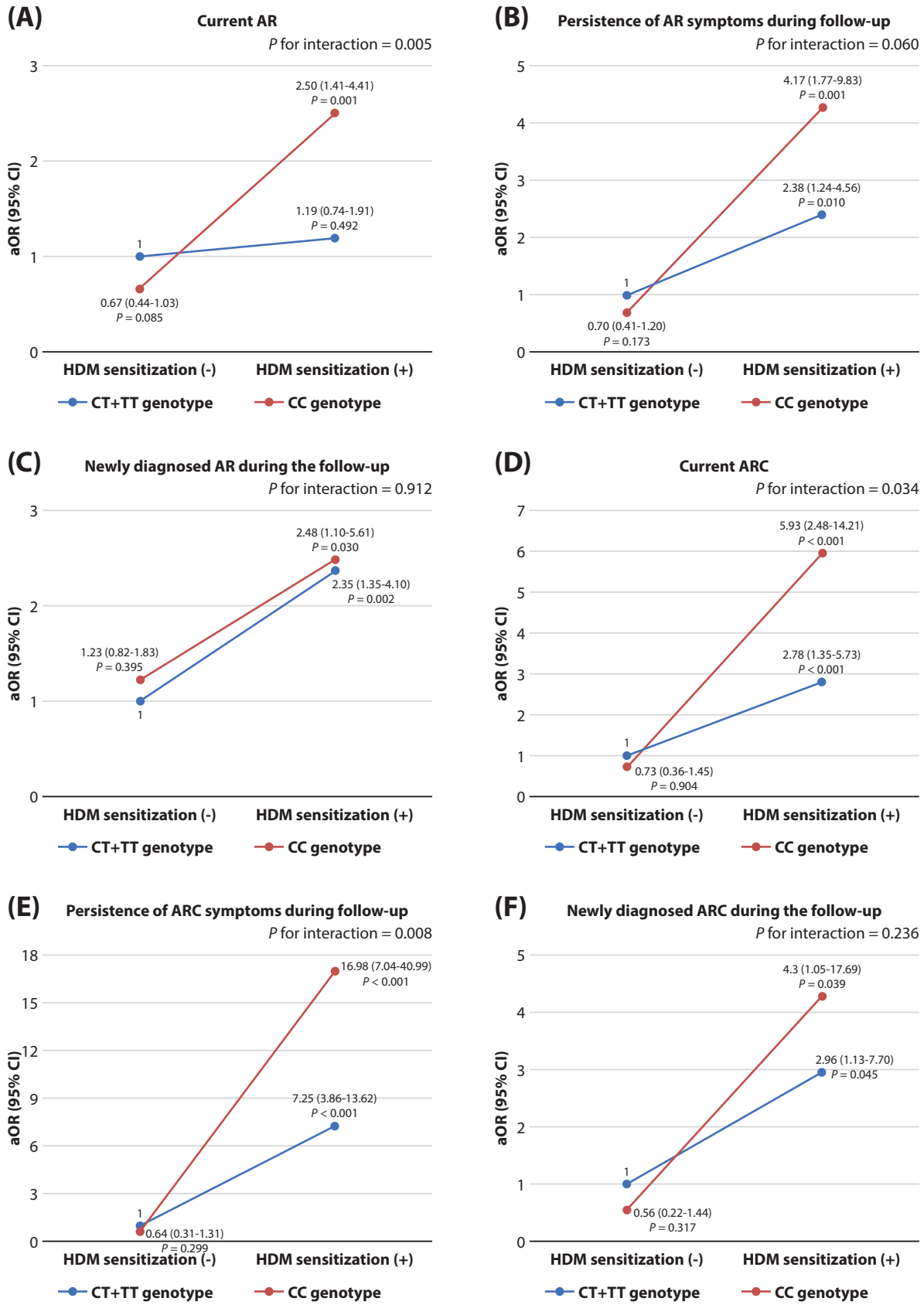

Figure 1. Interaction effect of house dust mite sensitization with *TLR4* (rs1927911) polymorphism on AR in children. (A) Current AR. (B) Persistence of AR symptoms during the prospective follow-up. (C) Newly diagnosed AR during the prospective follow-up. (D) Current ARC. (E) Persistence of ARC symptoms during the prospective follow-up. (F) Newly diagnosed ARC during the prospective follow-up.

Combined effects of sensitization to HDM and TLR4 (rs1927911) polymorphism on AR/ARC and their prognosis in children

Children sensitized to HDM were at increased risk of current AR (aOR, 2.50; 95% CI, 1.41-4.41; *P* for interaction = 0.005; **Figure 1**) and current ARC (aOR, 5.93; 95% CI, 2.48-14.21; *P* for interaction = 0.034), when they had the CC genotype of *TLR4* (rs1927911). During the prospective follow-ups, the persistence (aOR, 4.17; 95% CI, 1.77-9.83) and new diagnosis (aOR, 2.48; 95% CI, 1.10-5.61) of AR were increased in children sensitized to HDM, when they had CC genotype of *TLR4* (rs1927911). However, no significant interaction effects were found. In addition, the risk of persistence (aOR, 16.98; 95% CI, 7.04-40.99; *P* for interaction = 0.008) and a new diagnosis (aOR, 4.30; 95% CI, 1.05-17.69; *P* for interaction = 0.236) of ARC were increased in children sensitized to HDM, when they had CC genotype of *TLR4* (rs1927911).

Associations between a HDM sensitization and TLR4 (rs1927911) polymorphism combinations and prognosis of comorbidities

Children sensitized to HDM at the time of enrollment showed increased risks of new sensitization to inhalant allergens other than HDM (aOR, 10.67; 95% CI, 5.83-19.54; *P* for interaction = 0.822) and to the new development of BHR (aOR, 5.29; 95% CI, 2.29-12.21; *P* for interaction = 0.089) during the prospective follow-ups, when they had CC genotype of *TLR4* (rs1927911) (**Table 4**). The risks of current asthma at the final survey (aOR, 4.63; 95% CI, 2.41-8.88; *P* for interaction < 0.001) and allergic march (aOR, 2.57; 95% CI, 1.06-6.22; *P* for interaction = 0.002) were increased in children sensitized to HDM with significant interactions, when they had CC genotype of *TLR4* (rs1927911).

Table 4. Associations between combinations of HDM sensitization and TLR4 (rs1927911) polymorphism with the prognosis of comorbidities

HDM sensitization	*TLR4* (rs1927911)	Each outcome (-), n (%)	Each outcome (+), n (%)	aOR (95% CIs)	*P* value	Interaction *P*
New sensitization to inhalant allergens on SPTs during the prospective follow-ups						
No	CT+TT	419/687 (61.0)	121/312 (38.8)	Ref.		0.822
No	CC	207/687 (30.1)	52/312 (16.7)	0.91 (0.61-1.35)	0.468	
Yes	CT+TT	39/687 (5.7)	90/312 (28.8)	9.62 (6.11-15.15)	< 0.001	
Yes	CC	22/687 (3.2)	49/312 (15.7)	10.67 (5.83-19.54)	< 0.001	
New development of BHR during the prospective follow-ups						
No	CT+TT	444/784 (56.6)	24/55 (43.6)	Ref.		0.089
No	CC	215/784 (27.4)	11/55 (20.0)	0.85 (0.38-1.91)	0.697	
Yes	CT+TT	81/784 (10.3)	10/55 (18.2)	2.56 (1.15-5.71)	0.022	
Yes	CC	44/784 (5.6)	10/55 (18.2)	5.29 (2.29-12.21)	< 0.001	
Current asthma at the final survey						
No	CT+TT	389/720 (54.0)	8/23 (34.8)	Ref.		< 0.001
No	CC	189/720 (26.3)	8/23 (34.8)	0.47 (0.28-0.78)	0.003	
Yes	CT+TT	92/720 (12.8)	6/23 (26.1)	1.83 (1.12-3.01)	0.016	
Yes	CC	50/720 (6.9)	1/23 (4.3)	4.63 (2.41-8.88)	< 0.001	
Allergic march						
No	CT+TT	108/215 (50.2)	338/640 (52.8)	Ref.		0.002
No	CC	81/215 (37.7)	142/640 (22.2)	0.59 (0.41-0.86)	0.006	
Yes	CT+TT	20/215 (9.3)	102/640 (15.9)	1.59 (0.92-2.76)	0.099	
Yes	CC	6/215 (2.8)	58/640 (9.1)	2.57 (1.06-6.22)	0.036	

aOR, adjusted odds ratio; BHR, bronchial hyperresponsiveness; CI, confidence interval; HDM, house dust mite; ref., reference group; SPT, skin prick test.
* adjusted for family history of allergic diseases, sex, region, and monthly income.

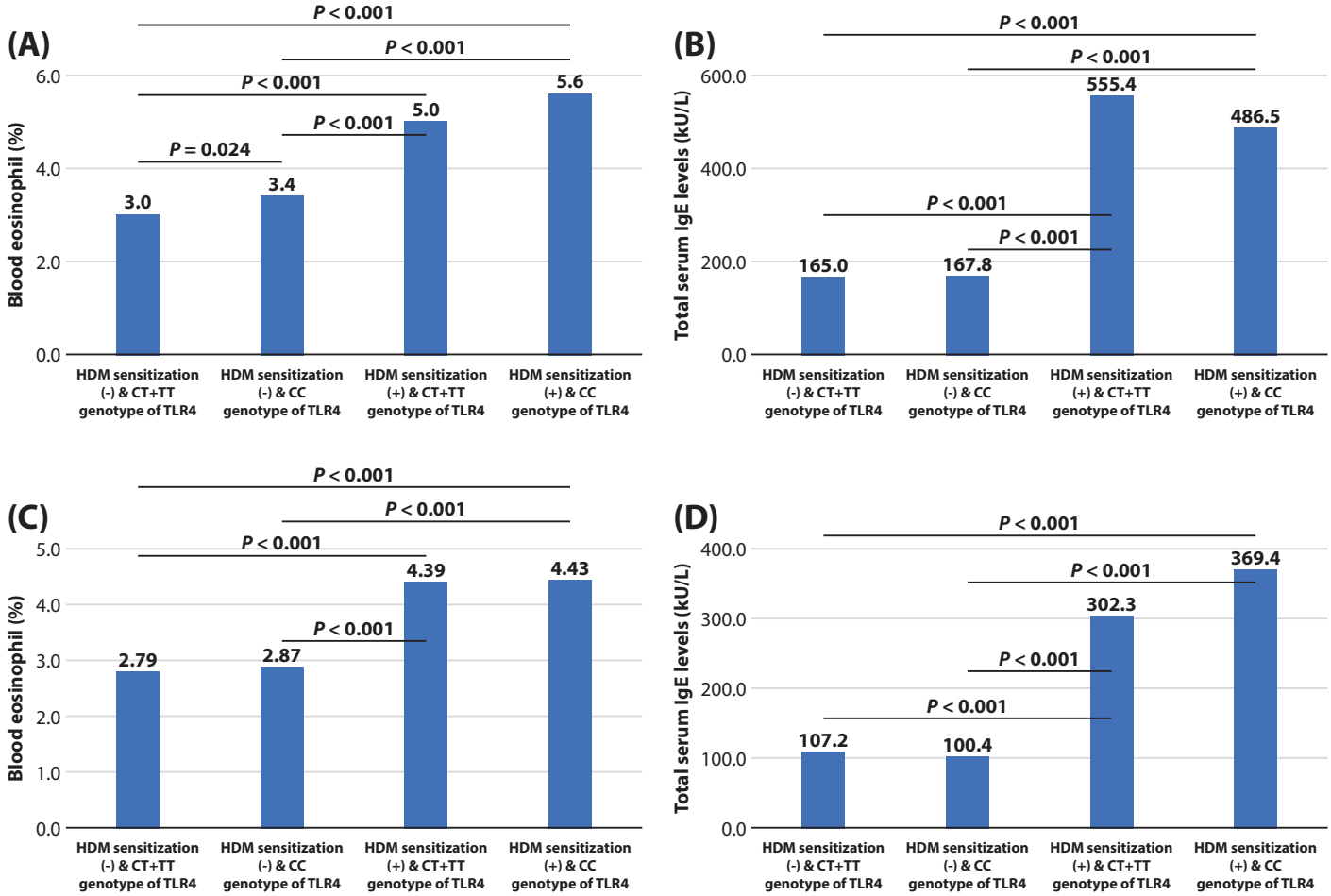

Figure 2. Comparisons of (A) blood eosinophil (%) and (B) total serum IgE levels at the time of enrollment and (C) blood eosinophil (%) and (D) total serum IgE levels at the time of follow-up according to the combination of HDM sensitization and *TLR4* (rs1927911) polymorphism.

Blood eosinophil (%) and total serum IgE levels according to HDM sensitization and TLR4 (rs1927911) polymorphism

The blood eosinophil (%) levels at the time of enrollment and at follow-up were highest in the children sensitized to HDM with the CC genotype of *TLR4* (rs1927911), followed by those sensitized to HDM with a CT+TT genotype of *TLR4* (rs1927911), those with the CC genotype who were not sensitized to HDM, and those with a CT+TT genotype who were not sensitized to HDM (**Figure 2**). The total serum IgE levels were higher in children sensitized to HDM, regardless of *TLR4* (rs1927911) polymorphism, both at the time of enrollment and at follow-up.

Discussion

We have identified that HDM sensitization affects current AR/ARC by significantly interacting with the *TLR4* (rs1927911) polymorphism in children. Although there were no significant interactions, the combination of HDM sensitization with the *TLR4* (rs1927911) polymorphism was associated with the prognosis of AR and ARC in relation to the persistence and new development of them during the prospective follow-up. This combination also increased the risk of new sensitization to inhalant allergens other than HDM and the new development of BHR during the follow-up period. Furthermore, sensitization to HDM increased the risk of current asthma at the final survey and also allergic march with significant interactions with *TLR4* (rs1927911) polymorphism in children. The results of the present study suggest that HDM sensitization affects the development of AR/ARC and their prognosis in combination with the *TLR4* (rs1927911) polymorphism. Hence, therapeutic and preventive strategies for AR/ARC require a consideration of genetic susceptibility combined with sensitization patterns.

Although the prevalence of AR is mostly high among diverse allergic diseases, few studies to date have reported on the natural course of AR in children.[4,20,21] In addition, these reports have focused only on the trends in AR prevalence during the study period and so that information is lacking on specific prognostic factors, particularly genetic susceptibility, which may be one of the most significant factors in AR development and its prognosis.[4] One previous study has reported that AR in preschool children tends to be persistent compared to nonallergic rhinitis, which suggests that sensitization to inhalant allergens in AR is a risk factor for its persistence.[4] However, that report did not consider genetic susceptibility, sensitization patterns, or diverse aspects of prognosis, including new development in AR.[4] On the other hand, we have identified in the present study that sensitization to HDM

can be a risk factor for the development and persistence of AR and ARC, especially in children with specific genetic backgrounds, notably *TLR4* (rs1927911) polymorphism. These findings might be helpful for predicting the development and prognosis of AR and ARC in children according to their genetic susceptibility and sensitization patterns.

More specific and better prediction of the prognosis of AR is necessary to better optimize the therapeutic and preventive plans, particularly in children experiencing significant quality of life impacts due to AR. For children with AR who are predicted to have a poor prognosis based on our present findings, the implementation of more active therapeutic plan at an earlier stage, including allergen immunotherapy, may help alleviate the disease burden and increase the quality of life by improvements to daily functioning.[2,22-24]

Previous studies have reported associations between environmental factors, such as antibiotic usage or mold exposure, and AR with consideration of *TLR4* SNPs.[16] Notably however, there has been no reported evidence from prospective epidemiological studies as to whether SNPs in *TLR* genes contribute to the development and prognosis of AR and ARC when combined with HDM sensitization. Based on previous experimental studies, which reported that the activation of TLR4 during sensitization contributes to the development of allergic diseases,[25] we speculated that TLR4, as one of the diverse pattern recognition receptors, might be involved in the early developmental stage of AR upon exposure to HDM. The association of TLR4 with AR may also be partially linked through the NF-κB pathway,[8] the activation of which is one of the most important pathophysiological mechanisms underlying AR.[26] HDM sensitization in association with TLR4 plays an important role as an initiator or trigger of inflammatory and immune responses in AR, partially in combination with immune-related cytokines.[27-29]

In our present study, we identified that AR, characterized by HDM sensitization in children with the CC genotype of *TLR4* (rs1927911), is significantly associated with allergic march as well as BHR with current asthma at follow-up.[30] Allergen sensitization in children with skin barrier dysfunction may be a critical component of the development of allergic march.[31-33] It has also been proposed that allergen sensitization and skin barrier dysfunction may share an altered immunologic environment with regard to both innate and adaptive immunity, both of which are affected by genetic factors.[34] The results of our current study suggest that specific sensitization combined with genetic polymorphisms in innate immunity-related factors is one of the diverse pathways underlying allergic march and helps to explain the mechanisms underlying allergic march.[33]

Previous studies have identified an association between *TLR4* polymorphisms, such as rs1927911 genotypes, with susceptibility to diverse diseases, including allergic disease.[35,36] However, no prior report has focused on the functions of *TLR4* polymorphisms, particularly with respect to the increased risk of disease susceptibility. Future functional studies of the association of *TLR4* polymorphisms with disease susceptibility are therefore warranted to further develop the preventive and therapeutic strategies for affected patients.

A notable strength of our present study was its prospective, general population-based design and high follow-up rate (> 80%).[1,17,37] We have thereby newly identified diverse aspects of different allergic diseases, including BHR and allergic march with the prognosis of AR, during our prospective follow-ups. In addition, to improve the reliability of our current findings, we investigated the associations between HDM sensitization and *TLR4* (rs1927911) polymorphism with ARC, and found similar patterns in cases of AR. Our current study had limitations however that are noteworthy. First, the ISAAC questionnaire was used to identify the presence of allergic diseases, including AR and ARC, but has been most commonly and widely used in the epidemiologic studies of allergic diseases.[4,38] Genotyping of *TLR4* (rs1927911) was performed in 35.0% of the total study population as the genetic analysis could be possible in children with blood samples as well as their parents' agreement for genetic analysis. However, the two groups classified by the presence of information on *TLR4* (rs1927911) showed no significant differences in their baseline characteristics except for age at enrollment and final survey, and family history of allergic diseases. More long-term follow-up studies are warranted to validate the association between *TLR4* (rs1927911) polymorphism with HDM sensitization and AR over the lifetime of the affected patients. There were limitations to our investigation of the sensitization to inhalant allergens other than HDM at the time of enrollment due to limited number of children sensitized to other inhalant allergens at 7 years of age. Hence, we could investigate only the effect of HDM sensitization with *TLR4* (rs1927911) polymorphism in AR. We also only investigated one SNP (rs1927911) among the diverse *TLR4* polymorphisms, as this is the most commonly studied in association with allergic diseases. Future studies of the associations between sensitization patterns, diverse *TLR4* polymorphisms, and allergic diseases would be necessary to fully elucidate the underlying pathophysiologies of AR.

In conclusion, HDM sensitization affects the development of AR in children by interacting with *TLR4* (rs1927911) polymorphism. Furthermore, the combination of HDM sensitization with *TLR4* (rs1927911) polymorphism affects the development and prognosis of AR, including the new development and persistence of AR and ARC with new sensitization to inhalant allergens other than HDM in children. This result indicates the need for application of more active therapeutics, such as allergen immunotherapy, and preventive strategies to prevent the development of comorbidities of AR and improve the prognosis in children with susceptible genetic background and sensitization to HDM. The targeted therapeutic and preventive strategies are inevitable to effectively decrease the disease burden of AR and its comorbidities in children.

Conflicts of interest

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

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Statement of contributions

Eun Lee designed and wrote the manuscript, and performed the analyses. All authors participated in the collection, analysis, and interpretation of the data. Soo-Jong Hong supervised the study.

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Supplementary Table 1. Associations between the *TLR4* (rs1927911) polymorphism and prognosis of AR

Outcome	aOR* (95% CI)	*P* value
HDM sensitization at the time of enrollment	1.16 (0.82-1.63)	0.398
Current AR	0.95 (0.67-1.34)	0.750
AR diagnosis ever in lifetime	0.92 (0.66-1.29)	0.645
Persistence of AR symptoms during the prospective follow-up	1.11 (0.74-1.67)	0.619
Newly diagnosed AR during the prospective follow-up	1.16 (0.81-1.67)	0.417
Current ARC	0.96 (0.56-1.65)	0.894
ARC diagnosis ever in lifetime	0.84 (0.49-1.42)	0.514
Persistence of ARC symptoms during the prospective follow-up	1.02 (0.61-1.70)	0.951
Newly diagnosed ARC during the prospective follow-up	0.73 (0.33-1.61)	0.428
New sensitization to inhalant allergens other than HDM on SPTs during the prospective follow-up	1.05 (0.79-1.39)	0.727

Logistic regression was performed when CT+TT genotype of *TLR4* (rs1927911) was considered as a reference.
aOR, adjusted odds ratio; AR, allergic rhinitis; ARC, allergic rhinoconjunctivitis; 95% CI, 95% confidence interval; HDM, house dust mite.