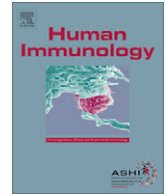




Contents lists available at SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

GSDMB/ORMDL3 variants contribute to asthma susceptibility and eosinophil-mediated bronchial hyperresponsiveness

Mi-Jin Kang^a, Ho-Sung Yu^a, Ju-Hee Seo^b, Hyung-Young Kim^c, Young-Ho Jung^c, Young-Joon Kim^a, Ha-Jung Kim^a, So-Yeon Lee^{d,*}, Soo-Jong Hong^{c,*}

^a Asan Institute for Life Science, Asan Medical Center, Seoul, South Korea

^b Department of Pediatrics, Korea Cancer Center Hospital, Seoul, South Korea

^c Department of Pediatrics, Childhood Asthma Atopy Center, Research Center for Standardization of Allergic Disease, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea

^d Department of Pediatrics, Hallym University College of Medicine, Sacred Heart Hospital, Anyang, South Korea

ARTICLE INFO

Article history:

Received 20 February 2012

Accepted 13 June 2012

Available online 23 June 2012

ABSTRACT

In 2007, a genome-wide association study identified associations between variants involved in the regulation of *ORMDL3* expression and asthma. These observations were subsequently replicated in case-control studies in several ethnic groups. We investigated the possible contribution of *GSDMB/ORMDL3* variants to asthma susceptibility and intermediate asthma phenotypes in Korean children. The polymorphisms rs7216389, rs4794820, rs4065275, and rs11650680 were genotyped using the TaqMan assay in 931 asthmatics and 480 normal controls in a case-control study, and in 1907 elementary school children in a general population study. Each subject also underwent peripheral blood analysis of immunoglobulin E levels, eosinophil cationic protein (ECP) levels, and eosinophil percentage. Pulmonary function testing (FEV₁ and MMEF) and a methacholine provocation test (PC₂₀) were also performed. The case-control study revealed a significant association between a linkage disequilibrium block, including rs7216389, rs4794820, and rs4065275, and susceptibility to asthma and atopic asthma. The CT and TT genotypes of rs11650680 were associated with lower logECP levels than the CC genotype in asthmatics, while the GA and AA genotypes of rs4794820 were associated with higher logPC₂₀ values than the GG genotype in atopic asthmatics. The haplotype (CAA) of rs7216389, rs4794820 and rs4065275 was associated with a lower risk of asthma susceptibility and a higher logPC₂₀. In the general population study, rs11650680 was significantly associated with a diagnosis of asthma. Moreover, the GA and AA genotypes of rs4794820 were associated with higher logPC₂₀ values and lower eosinophil percentages than the GG genotype in subjects who had been diagnosed with asthma, or showed bronchial hyperresponsiveness (PC₂₀ ≤ 16). The *GSDMB/ORMDL3* gene block, which includes rs7216389, rs4065275, rs4794820, and rs11650680, may be associated with asthma susceptibility in Korean children because it promotes eosinophilic inflammation, which induces bronchial hyperresponsiveness.

© 2012 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.

1. Introduction

Since chronic inflammatory diseases such as asthma have a strong genetic component, genome-wide linkage studies within families and pedigrees might help to delineate the critical region(s)

of the gene(s) that cause these diseases. Candidate gene studies have been carried out to corroborate results from linkage studies in both ethnically similar and different populations. The findings from these studies, however, have been poorly replicated, and they have done little to advance our understanding of asthma susceptibility genes, or of the means by which they may induce asthma. In recent years, advances in technology have allowed genome-wide association studies (GWASs) to replace candidate gene studies for complex diseases, and Moffatt et al. identified the first susceptibility locus for asthma in 2007 [1]. This locus was 17q12–21.1, which contains the *gasdermin B* (*GSDMB*) and *ORM1-like 3* (*ORMDL3*) genes. Functional analyses of 17q12–q21 recently revealed that the *ORMDL3* protein binds to and inhibits the sarco-endoplasmic reticulum Ca²⁺ pump (SERCA). This reduces the Ca²⁺ concentration

* Corresponding authors. Addresses: Department of Pediatrics, College of Medicine Hallym University, 896 Pyongchon-dong, Dongan-gu, Anyang, Gyeonggi-do 120-752, Korea. Fax: +82 2 3010 6978 (S.-Y. Lee), Department of Pediatrics, Childhood Asthma Atopy Center, Research Center for Standardization of Allergic Disease, University of Ulsan College of Medicine, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, Korea. Fax: +82 31 380 3733 (S.-J. Hong).

E-mail addresses: imipenem@hanmail.net (S.-Y. Lee), sjhong@amc.seoul.kr (S.-J. Hong).

¹ These authors contributed equally to this manuscript.

in the endoplasmic reticulum (ER) and increases the unfolded-protein response (UPR) [2], which is believed to be a factor in the development of chronic inflammatory diseases such as asthma [3]. Thus, the importance of the 17q12–q21 locus in asthma has been clearly established. However, the functions of the *GSDMB* and *ORMDL3* genes, and how they participate in the development of asthma, remain poorly understood.

Several studies have associated polymorphisms in the *GSDMB* and *ORMDL3* genes with asthma in several ethnic groups. Tavendale et al. showed that a variant of *ORMDL3* is associated with both increased susceptibility to asthma and decreased effectiveness of oral steroids against asthma in children and young adults [4]. Bouzigon et al. showed that 17q21 variants are associated with a higher risk of early-onset asthma and that this risk is increased by exposure to environmental tobacco smoke in early life [5]. Galanter et al. confirmed that the *ORMDL3* locus is a risk factor for asthma in three populations [6], while a longitudinal study by Bisgaard et al. showed that the 17q12–q21 locus is also associated with recurrent wheezing, asthma, asthma exacerbation, and bronchial hyperresponsiveness (BHR) in children who were followed from early infancy through school age [7]. With regard to Asian populations, rs7216389 is associated with atopic asthma in a Japanese population [8], while rs11650680 is associated with asthma and atopy in a Chinese population [9].

The aim of the current study was to examine whether asthma, atopic asthma, and intermediate phenotypes of asthma in Korean children associate significantly with genetic variants of the 17q12–q21 locus. Therefore, we analyzed both case and control subjects from the Childhood Asthma Atopy Center. We also assessed whether associations detected by the case-control study were also observed in a general population study of elementary school children.

2. Materials and methods

2.1. Subjects

In total, 1411 cases (asthmatics) and controls were enrolled from the Pediatrics Department of Asan Medical Center, of whom 480 were normal controls and 931 were diagnosed with asthma. A subgroup of the latter group was also identified; namely, those diagnosed with atopic asthma ($N = 774$). The criteria for atopic asthma diagnosis, or for classification as normal controls, have been described previously [10]. For the general population study, 1907 elementary school children were recruited from two regions in Korea (Seoul and Jeongseup) for an epidemiological study of allergic disease. Parents completed a questionnaire asking about their child's history. This questionnaire was created by us based on the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. Subjects who met the following criteria were excluded: chronic or immunological diseases or use of inhaled or oral corticosteroids or antihistamines within the preceding four weeks. Two characteristics were used to divide the general population subjects into two groups. First, whether asthma had ever been diagnosed in the child's lifetime was determined by questionnaires, allowing classification of the children into "Asthma Diagnosis" (–) and (+) groups [11]. Asthma diagnosis (+) was defined as a positive response to the question, "Has your child ever had doctor-diagnosed asthma?" Second, subjects were classified into $PC_{20} \leq 16$ and $PC_{20} > 16$ groups according to whether the value of the methacholine provocation test (PC_{20}) was above or below 16. Asthma was defined as $PC_{20} \leq 16$ because PC_{20} values below 16 represent BHR [12,13]. Peripheral blood was taken from all subjects for DNA isolation. The case-control study was approved by the human ethics committee of the Asan Medical Center Institu-

tional Review Board, while approval for the general population study was obtained from the human ethics committee of Hallym University and from the principals of the schools attended by the children. Written informed consent was obtained from the parents of all subjects.

2.2. Determination of immunoglobulin E (IgE) levels, total eosinophil counts (TEC), and eosinophil cationic protein (ECP) levels

The methods used to identify the intermediate phenotypes of asthma have been described previously [10]. Briefly, total serum IgE levels were measured using the UniCAP fluoroenzyme immunoassay (Pharmacia Diagnostics, Uppsala, Sweden). The total number of blood eosinophils was determined using an automated hematology analyzer (Coulter Counter STKS; Beckman Coulter, Fullerton, CA). ECP levels were measured by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden).

2.3. Pulmonary function and bronchial hyperresponsiveness challenge tests

Pulmonary function was tested using a VMAX22 system (Sensor Medics, Anaheim, CA). The bronchial hyperresponsiveness challenge test was performed using a dosimeter and methacholine in concentrations ranging from 0.625 to 25 mg/ml.

2.4. Selection and genotyping of single nucleotide polymorphisms (SNPs)

SNPs in *GSDMB/ORMDL3* were identified from previous studies [1,4–9,14,15] and 10 were chosen: rs7216389, rs8069176, rs12936231, rs11650680, rs4378650, rs12603332, rs8067378, rs2305480, rs4795400, and rs4065275. The Tagger-pairwise-Tagging algorithm from the international HapMap project revealed that the TagSNP in eight of the SNPs (rs7216389, rs8069176, rs12936231, rs4378650, rs12603332, rs8067378, rs2305480, and rs4795400) in the Japanese population was rs4794820. Therefore, rs4794820, rs11650680, and rs4065275 were selected for further study. The rs7216389 SNP was also included because of its association with asthma in many studies. Genotyping was performed using the TaqMan assay (ABI, Foster City, CA, USA). The assay IDs of SNPs rs4794820, rs11650680, rs4065275, and rs7216389 were C_29609729_20, C_1318895_10, C_1385765_10, and C_29062108_10, respectively.

2.5. Statistical analysis

Statistical analyses were performed using SPSS version 19 (SPSS, Inc., Chicago, IL, USA). Multiple logistic regression analysis was used to compare genotype frequency in the case-control, study, and general populations, and mean values were compared using a independent t-test. The linkage disequilibrium (LD) between the four SNPs and a haplotype of three SNPs was calculated using the HapAnalyzer program in the National Genome Research Institute of Korea. The power, calculated by the Genetic Power Calculator, was 0.9962 and 0.9135 for case-control and general population studies, respectively. A P -value of <0.05 was considered statistically significant.

3. Results

3.1. Population characteristics and linkage disequilibrium

The case-control study comprised 480 normal control subjects and 931 asthmatics. A subgroup of the latter group, atopic asth-

matic patients, was also identified ($N = 774$). In the general population study, 162 of 1797 subjects were defined as asthma diagnosis (+), while 286 of 1841 subjects showed BHR, as defined by $PC_{20} \leq 16$. The characteristics of the study populations are presented in Table 1. The distribution of the four *GSDMB/ORMDL3* polymorphisms was in Hardy–Weinberg equilibrium. As expected, in the case-control study, rs4794820 and rs7216389 were located within the same LD block ($D' = 0.974$). Unexpectedly, rs4794820 and rs4065275 were also located together in a single LD block ($D' = 0.975$; Supplementary Table 1). This means that, essentially, rs7216389, rs4794820, and rs4065275 show almost complete LD.

3.2. Association between asthma and atopic asthma and the *GSDMB/ORMDL3* SNPs in the case-control study

After adjusting for age and gender, two SNPs, rs7216389 and rs4065275, were significantly associated with susceptibility to asthma and atopic asthma, while rs4794820 was significantly and specifically associated with atopic asthma (Table 2). The rs11650680 did not associate significantly with either susceptibility to asthma or to atopic asthma. Susceptibility to non-atopic asthma was also not influenced by these four SNPs (data not shown). Analysis of the intermediate phenotypes of asthmatic, atopic asthmatic and non-atopic asthmatic subjects also revealed associations between logECP and log PC_{20} and two of the SNPs (rs4794820 and rs11650680). Both atopic asthmatic subjects with the GA and AA genotypes of rs4794820 and non-atopic asthmatic subjects with the CT and TT genotypes of rs11650680 showed higher log PC_{20} values than those with the GG and CC genotypes ($P = 0.044$ and $P = 0.007$; Fig. 1 and Supplementary Fig. 1). This was not observed when comparing eosinophil% (Fig. 1). Moreover, asthmatic subjects with the CT and TT genotypes of rs11650680 showed lower logECP values ($P = 0.009$); this was also observed for subjects with atopic asthma ($P = 0.008$) (Supplementary Fig. 1).

Haplotypes for rs7216389, rs4794820, and rs4065275 were then characterized and tested for association with asthma susceptibility and intermediate phenotypes. Haplotypes with estimated frequencies below 0.05 were not included in either analysis. Supplementary Table 2 shows the odds ratios and 95% confidence intervals for asthma and atopic asthma in three genetic models (co-dominant, dominant, and recessive). The TGG haplotype increased the risk of developing asthma and atopic asthma in all three genetic models. By contrast, a protective relationship between the CAA haplotype and asthma and atopic asthma, were observed in the co-dominant and dominant models. Intermediate

phenotype analysis revealed that homozygosity of the CAA haplotype (CAA/CAA) was positively associated with log PC_{20} in asthmatic subjects compared with $-/-$ (Supplementary Fig. 2). In atopic asthma, heterozygosity of CAA ($-/CAA$) was associated with increased log PC_{20} values compared with $-/-$ (Supplementary Fig. 2).

To further explore the association between *GSDMB/ORMDL3* and asthma, combination tests were used to identify synergistic effects of the polymorphisms on asthma or atopic asthma susceptibility. A combination effect was observed for rs4794820 and rs11650680 because of the complete LD of the three SNPs, rs7216389, rs4065275 and rs4794820. However, this effect did not reach statistical significance (Table 3). None of the four combinations of the two SNPs correlated with intermediate phenotypes (data not shown).

3.3. Associations between the *GSDMB/ORMDL3* SNPs and asthma in the general population study

The general population subjects were genotyped for the two SNPs, rs4794820 and rs11650680 that were associated with asthma in the case-control study. A diagnosis of asthma was significantly associated with rs11650680, but not with rs4794820 (Table 4). Testing for a possible association between the two SNPs and the intermediate phenotypes revealed that, in asthma diagnosis (+) subjects, the GA + AA genotypes of rs4794820 were associated with higher log PC_{20} values ($P = 0.040$; Fig. 2A). This was not the case for the asthma diagnosis ($-$) subjects. Comparison of the bronchial hyperresponsive ($PC_{20} \leq 16$) and $PC_{20} > 16$ subjects failed to identify any significant association with either of these two SNPs (Supplementary Table 3). However, in the $PC_{20} \leq 16$ subjects, but not the $PC_{20} > 16$ subjects, the GA and AA genotypes of rs4794820 were associated with significantly lower eosinophil % values than the GG genotype ($P = 0.042$; Fig. 2B). The rs4794820 and rs11650680 SNPs did not exhibit any synergistic effects on the development of asthma in the general population (data not shown).

4. Discussion

Previous studies show that susceptibility to asthma [4,6,8,9,16], atopy [9,10], responsiveness to steroids [4], environmental exposure to smoking [5], and intermediate phenotypes, including BHR [7,10] and IgE [10], are associated with the 17q12–21 locus. The primary purpose of the present study was to determine whether

Table 1
Characteristics of the study subjects.

Characteristics	Case-control				General population			
	Normal control ($N = 480$)	Asthma ($N = 931$)	Atopic asthma ($N = 774$)	Non-atopic asthma ($N = 157$)	Asthma Dx ($-$) ($N = 1635$)	Asthma Dx (+) ($N = 162$)	$PC_{20} > 16$ ($N = 1555$)	$PC_{20} \leq 16$ ($N = 286$)
Age (y)	9.63 $\pm$ 2.25	8.98 $\pm$ 2.83	9.13 $\pm$ 2.86	8.28 $\pm$ 2.60	10.35 $\pm$ 1.64	10.19 $\pm$ 1.72	10.47 $\pm$ 1.59	9.54 $\pm$ 1.78
Male (%)	51.9	63.5	65.1	55.4	49.7	62.1	51.6	49.5
LogECP (μ g/L)	1.21 $\pm$ 0.44	1.55 $\pm$ 0.45	1.59 $\pm$ 0.44	1.31 $\pm$ 0.43	NA	NA	NA	NA
Eosinophil (%)	2.54 $\pm$ 1.63	6.45 $\pm$ 4.40	7.00 $\pm$ 4.42	3.73 $\pm$ 3.15	3.56 $\pm$ 2.54	4.79 $\pm$ 3.73	3.44 $\pm$ 2.47	4.73 $\pm$ 3.09
LogIgE (KU/L)	1.49 $\pm$ 0.49	2.36 $\pm$ 0.58	2.49 $\pm$ 0.50	1.72 $\pm$ 0.55	1.88 $\pm$ 0.61	2.08 $\pm$ 0.63	1.86 $\pm$ 0.61	2.08 $\pm$ 0.64
FEV ₁ (%)	98.29 $\pm$ 13.40	86.62 $\pm$ 14.52	86.00 $\pm$ 14.18	89.70 $\pm$ 15.80	99.40 $\pm$ 11.23	96.67 $\pm$ 11.59	99.84 $\pm$ 11.11	95.49 $\pm$ 11.44
MMEF (%)	99.52 $\pm$ 21.45	81.60 $\pm$ 24.97	80.56 $\pm$ 24.96	86.77 $\pm$ 24.49	98.24 $\pm$ 20.57	89.77 $\pm$ 19.97	98.67 $\pm$ 20.20	90.07 $\pm$ 20.54
Log PC_{20} (mg/mL)	1.39 $\pm$ 0.07	0.41 $\pm$ 0.54	0.38 $\pm$ 0.54	0.53 $\pm$ 0.52	1.31 $\pm$ 0.24	1.23 $\pm$ 0.33	1.39 $\pm$ 0.02	0.81 $\pm$ 0.34

Asthma Dx, a current or preceding diagnosis of asthma in child lifetime.

ECP, eosinophilic cationic protein.

FEV₁, forced expiratory volume in 1 s.

MMEF, maximum midexpiratory flow.

PC_{20} , concentration of methacholine that provokes a 20% fall in FEV₁.

NA, not applicable.

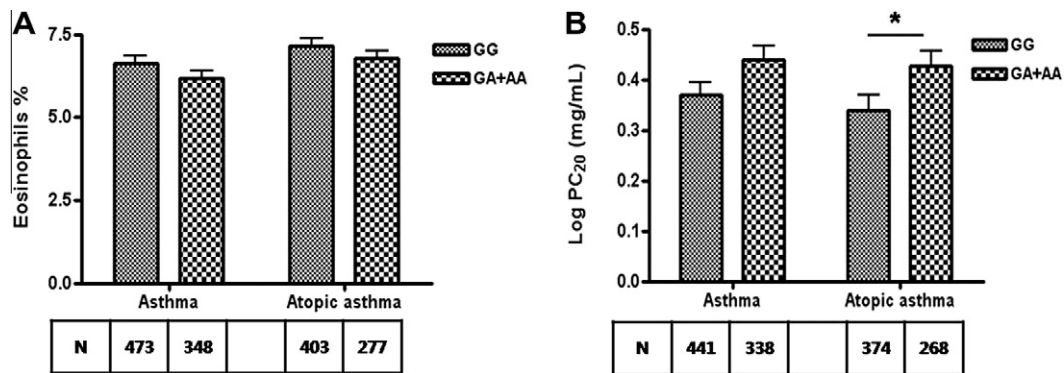
Data are expressed as the mean $\pm$ SD.

Table 2Association of *GSDMB/ORMDL3* polymorphisms with asthma or atopic asthma in the case-control study.

Polymorphism	Genotypes	Control N	Asthma N	aOR (95% CI)	P-value	Atopic asthma N	aOR (95% CI)	P-value
rs7216389	TT	232	466	1.000	0.007	385	1.000	0.004
	TC + CC	219	309	0.722 (0.569–0.915)		246	0.694 (0.542–0.889)	
rs4794820	GG	235	474	1.000	0.060	404	1.000	0.018
	GA + AA	221	349	0.880 (0.634–1.009)		278	0.746 (0.586–0.950)	
rs4065275	GG	240	526	1.000	0.023	445	1.000	0.011
	GA + AA	233	384	0.770 (0.615–0.965)		311	0.738 (0.584–0.933)	
rs11650680	CC	340	602	1.000	0.225	501	1.000	0.255
	CT + TT	118	244	1.175 (0.906–1.524)		201	1.169 (0.893–1.530)	

aOR, adjusted Odds Ratio (adjusted by age and sex).

Logistic regression analysis was performed.

**Fig. 1.** Association of rs4794820 with asthmatic phenotypes in the case-control study. The eosinophil% (A) and logPC₂₀ values (B) of children with asthma or atopic asthma who bear the GG or GA + AA genotypes of rs4794820 are shown. Data represent the mean ± SE. *P = 0.044.**Table 3**Effect of combining the *GSDMB/ORMDL3* polymorphisms rs4794820 and rs11650680 on asthma or atopic asthma in the case-control study.

rs4794820/rs11650680	Control	Asthma	aOR (95% CI)	P-value	Atopic asthma	aOR (95% CI)	P-value
GG/CC	175	316	1.000		267	1.000	
GG/CT + TT	56	132	1.299 (0.900–1.876)	0.162	112	1.321 (0.906–1.927)	0.148
GA + AA/CC	157	235	0.839 (0.636–1.107)	0.839	188	0.796 (0.597–1.062)	0.121
GA + AA/CT + TT	57	99	0.988 (0.676–1.443)	0.988	78	0.926 (0.623–1.376)	0.703

aOR, adjusted Odds Ratio (adjusted by age and sex).

Logistic regression analysis was performed.

Table 4Association of *GSDMB/ORMDL3* polymorphisms with a previous or current asthma diagnosis in the general population

Polymorphisms	Genotypes	Asthma Dx (–)		Asthma Dx (+)		aOR (95% CI)	P-value
		N	%	N	%		
rs4794820	GG	895	55.9	95	59.0	1.000	0.317
	GA + AA	705	44.1	66	41.0	0.843 (0.604–1.177)	
rs11650680	CC	938	74.1	114	83.2	1.000	0.010
	CT + TT	328	25.9	23	16.8	0.531 (0.327–0.861)	

Asthma Dx, a current or preceding diagnosis of asthma in child lifetime.

aOR, adjusted Odds Ratio (adjusted by age and sex).

Logistic regression analysis was performed.

the polymorphisms identified in previous studies are also associated with the development of asthma in Korean children. This is necessary because the results of genetic studies in European subjects can often not be replicated in Asians. These disparities may reflect differences in the criteria being used to diagnose asthma, subject characteristics, or ethnicity. The present study confirmed the association between asthma and *GSDMB/ORMDL3* gene polymorphisms in Korea children. This, together with the studies

described above, suggests that the 17q12–21 locus, which includes the *GSDMB/ORMDL3* genes, is an asthma-susceptibility locus irrespective of ethnicity.

The airway inflammation that characterizes asthma mainly results from an allergic reaction to an inhaled allergen [17]. Asthmatic airways are characterized by eosinophil infiltration, which develops into eosinophilic inflammation [18]. This accumulation and activation of eosinophils in bronchial tissues contributes to

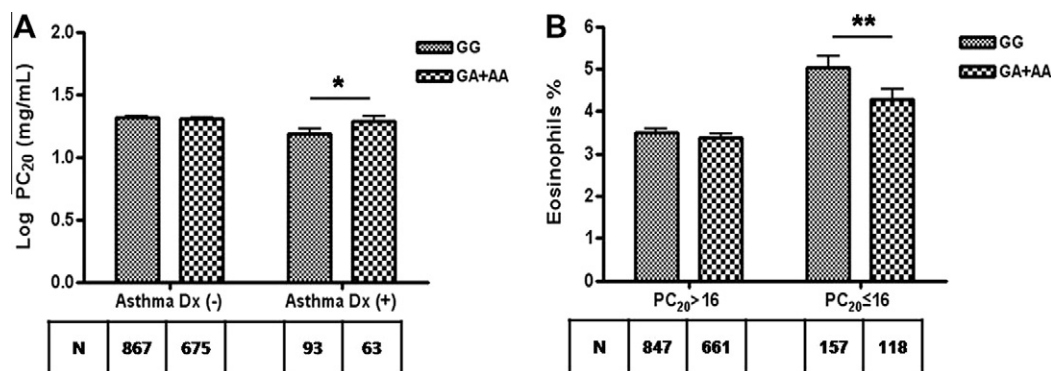


Fig. 2. Association of rs4794820 with asthmatic phenotypes in the general population. (A) LogPC₂₀ values of subjects diagnosed with asthma [designated as Asthma Dx (+)] and subjects who had never been diagnosed with asthma [Asthma Dx (-)] when they had the GG or GA + AA genotypes of rs4794820. (B) The eosinophil% of subjects whose PC₂₀ values exceeded 16 (PC₂₀ > 16) or were below 16 (PC₂₀ ≤ 16) when they had the GG or GA + AA genotypes of rs4794820. Data represent the mean ± SE. **P* = 0.040, ***P* = 0.042.

the airway remodeling and airway hyperresponsiveness that is seen in chronic asthma [19]. In the present case-control study, the T allele of rs11650680 was significantly associated with decreased logECP values, and the A allele of rs4794820 was significantly associated with increased logPC₂₀ values. Moreover, in the general population study, the A allele of rs4794820 was associated with logPC₂₀ in asthma diagnosis (+) subjects and with eosinophil% in PC₂₀ ≤ 16 subjects. These results, together with those of other recent association studies, show that *GSDMB/ORMDL3* variations are associated with BHR and asthma, and that eosinophilic inflammation may play a mediating role in this phenomenon.

A major strength of the current study is that the case-control study data were replicated in an independent general population study. This is significant because GWAS-detected asthma-susceptibility SNPs are irrelevant if they do not play the same role in other study subjects. The significant association of GWAS-detected polymorphisms in *GSDMB/ORMDL3* with asthma has been found repeatedly throughout the world, including in Caucasian, European, Mexican, and Asian populations [1,4–10,14,16]. Moreover, the present study shows for the first time that the *GSDMB/ORMDL3* polymorphisms may affect eosinophil-mediated BHR. This is important because, despite the many studies that show a relationship between 17q12–21 polymorphisms and asthma, far fewer have investigated the function and regulation of the genes that are located in that locus. The present study does not investigate these issues directly, and this constitutes a weakness. However, with regard to the mechanisms by which 17q12–21 polymorphisms may promote the development of asthma, several publications now suggest that *GSDMB* and *ORMDL3* polymorphisms play important roles in immune responses and inflammatory diseases. Most studies have focused on the rs7216389 polymorphism. Lluís et al. showed that when cord blood mononuclear cells with the T allele of rs7216389 are treated with *Dermatophagoides pteronissinus* (*Der P*), *ORMDL3* expression increases; moreover, phytohemagglutinin (PHA) treatment increases IL-17 secretion by these cells [20]. In addition, Qiu et al. demonstrated that the peripheral blood leukocytes of asthma patients with the TT genotype of rs7216389 express elevated levels of both *ORMDL3* and *GSDMB* [21]. These results suggest that SNPs on 17q12–21 affect *ORMDL3* and *GSDMB* expression at an early age, thereby influencing the regulation of allergen sensitization and immune responses.

The second weak point of the present study is that the *GSDMB/ORMDL3* polymorphisms found to play significant roles in asthma pathogenesis in the case-control study were not identical to the SNPs with significant associations in the general population. This may be because the definitions of asthma patients vs. normal controls differed in the two study populations. However, our BHR re-

sult is meaningful because the ISAAC questionnaire does not represent all current asthma cases. In any case, even though some differences were observed between the two independent populations, there are clearly some connections between the data.

The *Gasdermin* (*GSDM*) gene was originally identified as a candidate gene responsible for the phenotype of a mouse skin mutant, namely recombination-induced mutation 3 (Rim3) [22]. *GSDMB* is expressed as an oncogene product in stem cell-resident regions of normal epithelium [23]. *ORMDL3* is the third member of a gene family that encodes transmembrane proteins that are anchored in the ER [24]. Despite this heterogeneity in biological function, further studies examining the functions of *GSDMB* and *ORMDL3* should focus on their role in asthma pathogenesis because of the strong correlations observed between SNPs in these genes and asthma in many different ethnic groups.

In summary, the present study of two independent population groups of Korean children replicated previous findings that identified a relationship between the *GSDMB/ORMDL3*-bearing locus, 17q12–21, and asthma. The present observations also suggested that these polymorphisms may shape the development of eosinophil-mediated BHR, which plays an important role in the pathophysiology of asthma.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), which is funded by the Ministry of Education, Science, and Technology of Korea (2010-0004890).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.humimm.2012.06.009>.

References

- [1] Moffatt MF, Kabisch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants regulating *ORMDL3* expression contribute to the risk of childhood asthma. *Nature* 2007;448(7152):470–3.
- [2] Cantero-Recasens G, Fandos C, Rubio-Moscardo F, Valverde MA, Vicente R. The asthma-associated *ORMDL3* gene product regulates endoplasmic reticulum-mediated calcium signaling and cellular stress. *Hum Mol Genet* 2010;19:111–21.
- [3] Todd DJ, Lee AH, Glimcher LH. The endoplasmic reticulum stress response in immunity and autoimmunity. *Nat Rev Immunol* 2008;8:663–74.

- [4] Tavendale R, Macgregor DF, Mukhopadhyay S, Palmer CN. A polymorphism controlling ORMDL3 expression is associated with asthma that is poorly controlled by current medications. *J Allergy Clin Immunol* 2008;121(4):860–3.
- [5] Bouzigon E, Corda E, Aschard H, Dizier MH, Boland A, Bousquet J, et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. *N Engl J Med* 2008;359(19):1985–94.
- [6] Galanter J, Choudhry S, Eng C, Nazario S, Rodríguez-Santana JR, Casal J, et al. ORMDL3 gene is associated with asthma in three ethnically diverse populations. *Am J Respir Crit Care Med* 2008;177(11):1194–200.
- [7] Bisgaard H, Bønnelykke K, Sleiman PM, Brasholt M, Chawes B, Kreiner-Møller E, et al. Chromosome 17q21 gene variants are associated with asthma and exacerbations but not atopy in early childhood. *Am J Respir Crit Care Med* 2009;179(3):179–85.
- [8] Hirota T, Harada M, Sakashita M, Doi S, Miyatake A, Fujita K, et al. Genetic polymorphism regulating ORM1-like 3 (*Saccharomyces cerevisiae*) expression is associated with childhood atopic asthma in a Japanese population. *J Allergy Clin Immunol* 2008;121(3):769–70.
- [9] Leung TF, Sy HY, Ng MC, Chan IH, Wong GW, Tang NL, et al. Asthma and atopy are associated with chromosome 17q21 markers in Chinese children. *Allergy* 2009;64(4):621–8.
- [10] Yu J, Kang MJ, Kim BJ, Kwon JW, Song YH, Choi WA, et al. Polymorphisms in GSDMA and GSDMB are associated with asthma susceptibility, atopy and BHR. *Pediatr Pulmonol* 2011;46(7):701–8.
- [11] Kwon JW, Kim BJ, Song Y, Seo JH, Kim TH, Yu J, et al. Changes in the prevalence of childhood asthma in Seoul from 1995 to 2008 and its risk factors. *Allergy Asthma Immunol Res* 2011;3(1):27–33.
- [12] Jang AS, Son MH. The association of airway hyperresponsiveness and tuberculin responses. *Allergy* 2002;57(4):341–5.
- [13] Lee SY, Kim HB, Kim JH, Kim BS, Kang MJ, Jang SO, et al. Responsiveness to montelukast is associated with bronchial hyperresponsiveness and total immunoglobulin E but not polymorphisms in the leukotriene C4 synthase and cysteinyl leukotriene receptor 1 genes in Korean children with exercise-induced asthma (EIA). *Clin Exp Allergy* 2007;37(10):1487–93.
- [14] Sleiman PM, Annaiah K, Imielinski M, Bradfield JP, Kim CE, Frackelton EC, et al. ORMDL3 variants associated with asthma susceptibility in North Americans of European ancestry. *J Allergy Clin Immunol* 2008;122(6):1225–7.
- [15] Verlaan DJ, Berlivet S, Hunninghake GM, Madore AM, Larivière M, Moussette S, et al. Allele-specific chromatin remodeling in the ZBP2/GSDMB/ORMDL3 locus associated with the risk of asthma and autoimmune disease. *Am J Hum Genet* 2009;85(3):377–93.
- [16] Hrdlickova B, Holla LI. Relationship between the 17q21 locus and adult asthma in a Czech population. *Hum Immunol* 2011;72(10):921–5.
- [17] Craig TJ, King TS, Lemanske Jr RF, Wechsler ME, Icitovic N, Zimmerman Jr RR, et al. Aeroallergen sensitization correlates with PC(20) and exhaled nitric oxide in subjects with mild-to-moderate asthma. *J Allergy Clin Immunol* 2008;121(3):671–7.
- [18] Medoff BD, Thomas SY, Luster AD. T cell trafficking in allergic asthma: the ins and outs. *Annu Rev Immunol* 2008;26:205–32.
- [19] Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, et al. A critical role for eosinophils in allergic airways remodeling. *Science* 2004;305(5691):1776–9.
- [20] Lluís A, Schedel M, Liu J, Illi S, Depner M, von Mutius E, et al. Asthma-associated polymorphisms in 17q21 influence cord blood ORMDL3 and GSDMA gene expression and IL-17 secretion. *J Allergy Clin Immunol* 2011;127(6):1587–94.
- [21] Qiu R, Zhao H, Wang A, Gong Y, Liu Q. Association of genetic variants in chromosome 17q21 and adult-onset asthma in a Chinese Han population. *BMC Med Genet* 2011;12(1):133.
- [22] Saeki N, Kuwahara Y, Sasaki H, Satoh H, Shiroishi T. Gasdermin (Gsdm) localizing to mouse Chromosome 11 is predominantly expressed in upper gastrointestinal tract but significantly suppressed in human gastric cancer cells. *Mamm Genome* 2000 Sep;11(9):718–24.
- [23] Saeki N, Usui T, Aoyagi K, Kim DH, Sato M, Mabuchi T, et al. Distinctive expression and function of four GSDM family genes (GSDMA-D) in normal and malignant upper gastrointestinal epithelium. *Genes Chromosomes Cancer* 2009;48(3):261–71.
- [24] Hjelmqvist L, Tuson M, Marfany G, Herrero E, Balcells S, González-Duarte R. ORMDL proteins are a conserved new family of endoplasmic reticulum membrane proteins. *Genome Biol* 2002;3(6):RESEARCH0027.