

Genetic variants of the gasdermin B gene associated with the development of aspirin-exacerbated respiratory diseases

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ABSTRACT

Background: Aspirin-exacerbated respiratory disease (AERD) is characterized by a severe and sudden asthma attack after aspirin ingestion in patients with asthma. We studied associations with six common single nucleotide polymorphisms (SNP) of the gasdermin B gene (GSDMB).

Objective: DNA obtained from 572 patients with asthma (with AERD, $n = 165$; and with aspirin-tolerant asthma, $n = 407$) and 391 normal controls was subjected to genotyping of six SNPs of GSDMB.

Methods: An association analysis between GSDMB variants and AERD, with a fall rate of the forced expiratory volume in the first second of expiration (FEV₁), was performed by using logistic and regression models.

Results: Two SNPs in the intron (rs870830, rs7216389) showed significant associations with AERD (minimum $p = 7.00 \times 10^{-4}$ in the dominant model), even after Bonferroni correction ($p_{\text{corr}} = 0.01$ for the rs870830). Regression analysis of the genetic variants with FEV₁ revealed significant associations with rs870830 and the haplotype 2 ($p_{\text{corr}} = 4.71 \times 10^{-4}$ for rs870830 and $p_{\text{corr}} = 1.14 \times 10^{-3}$ for haplotype 2, respectively).

Conclusion: We found strong associations among GSDMB polymorphisms and the presence of AERD and FEV₁ in Korean patients with asthma. Our findings indicated that genetic variations of GSDMB may be associated with the development of AERD and aspirin-induced bronchospasm.

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Asthma is a common chronic inflammatory disease that involves the airways of the lungs and affects 235 million patients worldwide.¹ Asthma causes recurring periods of wheezing, chest tightness, difficulty breathing, and coughing. Acetylsalicylic acid (aspirin) or pain-killer groups called nonsteroidal anti-inflam-

matory drugs are used as analgesic and antipyretic agents as well as anti-inflammatory medication. However, a sensitivity or hypersensitivity to these drugs occurs in 10% of all adults with asthma and in 40% of patients with asthma and nasal polyps.^{2,3} Aspirin-exacerbated respiratory disease (AERD) is a clinical tetrad of chronic hypertrophic eosinophilic sinusitis, nasal polyps, asthma, and sensitivity to nonsteroidal anti-inflammatory drugs.^{4,5} The diagnosis of AERD is based on a reported history of adverse reactions precipitated by taking nonsteroidal anti-inflammatory drugs. Individuals with aspirin sensitivity and with asthma can develop bronchospasm, rhinorrhea, respiratory arrest, urticarial, and angioedema after oral aspirin challenge (OAC).⁴ Furthermore, OAC is a time-consuming procedure and requires special equipment and expertise.⁶ Thus, noninvasive diagnostic methods are needed for patients with suspected AERD. Until now, many association studies were performed to discover the genetic variants associated with the development of AERD.^{7–13} Among these studies, some results have not been replicated due to ethnic differences and/or small sample sizes.^{7–13} Replicated genetic variants associated with AERD may be used as AERD biomarkers.

Gasdermin B (GSDMB) encodes the gasdermin domain that contains the protein family. GSDMB is linked

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Table 1 Clinical profiles of the study subjects

	AERD (n = 165)	ATA (n = 407)	NC (n = 391)
Age, median (range), y	41 (17–72)*,**	46.6 (15–77)**	53.7 (11–86)
Sex, total no. (no. male /female)	165 (58/107)	407 (120/287)**	391 (169/222)
Smoker/ex-smoker, %	11.51/6.06**	8.10/14.00**	18.41/15.35
Decline of FEV ₁ by aspirin provocation, median (range), %	28.10 (17.6–100)*	3.05 (-15.00 to 14.7)	N/A
P20 methacholine, median (range), mg/mL	1.00 (0.07–25.00)*,**	1.85 (0.01–25.00)*,**	25.00 (13.6–25.00)
Positive skin-prick test results, %	53.33**	55.03**	23.94
Body mass index, mean $\pm$ SD, kg/m ²	23.77 $\pm$ 3.73*,**	25.08 $\pm$ 3.79	24.13 $\pm$ 3.31

AERD = Aspirin-exacerbated respiratory disease; ATA = aspirin-tolerant asthma; NC = normal control; FEV₁ = forced expiratory volume in the first second of expiration; N/A = not available; P20 = methacholine provocation test; SD = standard deviation.

* $p < 0.01$ vs ATA, ** $p < 0.01$ vs NC.

to oral squamous cell carcinoma and bronchial disease, and is associated with apoptosis in epithelial cells. This gene plays an important role as a metabolic component of the secretory pathway and maintains the final differentiation state of epithelial cells.^{14,15} The first genome-wide association study (GWAS) for asthma showed that chromosome 17q21, which encompasses the genes *ORMDL3* sphingolipid biosynthesis regulator 3 (*ORMDL3*), *GSDMB*, and zona pellucida binding protein 2 (*ZPBP2*), is associated with the development of asthma, and these three genes showed allele-specific differences in expression levels in lymphoblastoid cell lines and CD4⁺ T cells.^{16,17} Other researchers showed that genetic variants of *GSDMB* and *ORMDL3* are associated with childhood-onset asthma but not with adult-onset asthma.^{18–20} Chromosome 17q12-q21 is a well-replicated predisposition region for childhood asthma. In our recent exome GWAS, when using a large-scale platform with 240,000 polymorphic markers, five *GSDMB* single nucleotide polymorphisms (SNP) (rs2290400, rs7216389, exm1317549, exm1317550, and exm1317567) showed a significant association with AERD ($p = 0.002$).⁷ These SNPs were in absolute linkage disequilibrium ($D' = 1$ and $r^2 = 1$). To find the causal loci in *GSDMB*, we selected tagging SNPs of *GSDMB* and performed a further genetic association study.

METHODS

Study Subjects and OAC

Written informed consent was obtained from all the subjects to participate in the study. The study protocol was approved by the Soonchunhyang Bucheon Hospital's ethics committee (Institutional Review Board no. 05-02 and schbc-biobank-2012–004). Genomic DNA from ethnic Koreans study subjects was obtained from a biobank at Soonchunhyang Bu-

cheon Hospital. All the patients had a history of dyspnea and wheezing in the past 12 months, plus one of the following: (1) >15% increase in forced expiratory volume in the first second of expiration (FEV₁) or a >12% increase plus 200 mL after inhalation from a short-acting bronchodilator, (2) <10 mg/mL methacholine provocation test (PC20), or (3) >20% increase in FEV₁ after 2 weeks of treatment with inhaled steroids and long-acting bronchodilators, and they met the criteria for asthma according to the Global Initiative for Asthma guidelines.²¹ Twenty-four common inhalant allergens were used for a skin-prick test.

Total immunoglobulin E was measured by using the CAP system (Pharmacia Diagnostics, Uppsala, Sweden), and atopy was defined as a positive reaction to one or more of the allergens with weal size of at least 3 mm or a histamine control in diameter. None of the patients with asthma had experienced an exacerbation of asthma and respiratory tract infection 6 weeks before OAC. OAC was performed with increasing doses of aspirin by using methods slightly modified from those previously described.^{22,23} The patients were followed-up for 5 hours for changes in FEV₁ after the last aspirin challenge dose. Aspirin-induced bronchospasms, as reflected by the rate (%) of FEV₁ decline, were calculated as the prechallenge FEV₁ minus the postchallenge FEV₁ divided by the prechallenge FEV₁. OAC reactions were categorized into two groups: a >15% decrease in FEV₁ with nasoocular or cutaneous reactions (AERD), and <15% decrease in FEV₁ without nasoocular or cutaneous reactions (aspirin-tolerant asthma [ATA]). The clinical profiles of the subjects are summarized in Table 1.

SNP Genotyping

Thirty-eight SNPs were detected in *GSDMB* according to the information on Asian populations (Chinese

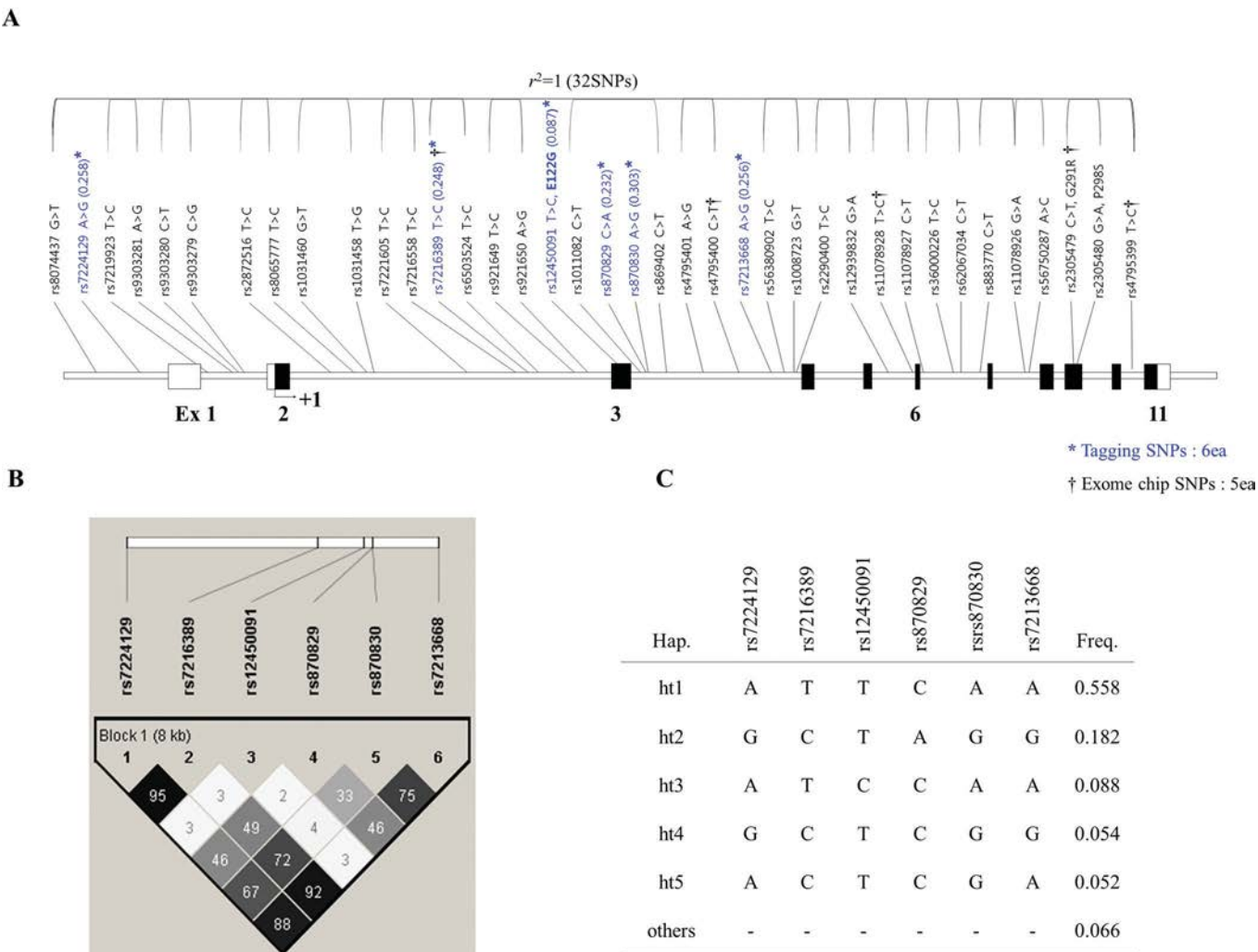


Figure 1. Gene map of gasdermin-B (GSDMB). (A) Map of GSDMB on chromosome 17q12.1. Coding exons are marked by black blocks, and 5' and 3' untranslated regions (UTRs) by white blocks (Ref. NM no. NM 001165958). The frequencies in parentheses are based on the Korean patients with asthma and normal controls of a fine mapping study ($n = 963$). (B) Linkage disequilibrium (LD) among GSDMB polymorphisms. LD values are given as r^2 . (C) Haplotypes in GSDMB. Six GSDMB polymorphisms were used for haplotype construction. Five haplotypes with $>5\%$ frequency were used in statistical analyses.

and Japanese) from the 1000 genomes data base, and 32 SNPs were in absolute LD ($D' = 1$, and $r^2 = 1$) (Fig. 1 A). Among the 32 absolute LD SNPs, only one SNP (rs7216389) in a regulatory region was selected because, in a previous study, it had shown a significant association with early onset of asthma.²⁴ Thus, six tagging SNPs were selected for genotyping that contained rs7216389. Genotyping was performed by using a total of 963 samples, which consisted of 165 AERD, 407 ATA, and 391 controls. Commercially available predesigned TaqMan probes and primers (Applied Biosystems, Foster City, CA) were used in genotyping (Supplemental Table 1). To assess the genotype data quality, duplicate DNA checking was carried out ($n = 10$) (the rate of concordance in duplicates was $>99\%$).

Statistical Analysis

We examined a widely used measure of linkage disequilibrium between all pairs of biallelic loci, D' (the correlation coefficient Lewontin's D' (iD')), and r^2 by using Haploview.²⁵ Haplotypes were inferred by using the PHASE algorithm version 2.0²⁶ and then computed by logistic analysis with statistical analysis software (SAS Institute, Cary, NC). Subjects who harbored missing genotypes were omitted in the analysis of individual SNPs and haplotypes. Association with AERD was analyzed under a logistic model by adjusting for sex (male, 0; female, 1), age (continuous value), body mass index (continuous value), and smoking status (nonsmoker, 0; ex-smoker, 1; smoker, 2) as covariates by using the SAS software. The association analysis of differences in

the fall rates in FEV₁ after aspirin challenge with the genotypes and haplotypes was done by using a regression analysis model. To correct for multiple comparisons, Bonferroni correction was applied based on the number of independent SNPs ($n = 6$) and three different models ($n = 3$).

***In Silico* Analysis**

The Web-based splice-site analysis tool, Human Splicing Finder (v3.0),⁴⁰ was used to analyze the effect of the SNP rs870830 on putative splicing regulatory sequences. Human Splicing Finder arranged all available matrices to identify exon and intron motifs: exon splicing enhancer motifs from Human Splicing Finder

for serine/arginine-rich (SR) proteins (SRp40, pre-mRNA-splicing factor SF2/alternative splicing factor 1, serine/arginine-rich splicing factor 2, immunoglobulin M/breast cancer 1, and SRp55), putative 8-mer exon splicing enhancers, and putative 8-mer exonic splicing silences, exon-identity and intron-identity elements, and heterogeneous nuclear ribonucleoprotein binding motifs.

RESULTS

Study Subjects

A total of 963 samples were used for the association study: 572 patients with asthma (165 AERD, 407 ATA) and 391 normal controls. Clinical characteristics of the study subjects are depicted in Table 1. Patients with asthma were divided into two groups, according to degree of aspirin sensitivity: patients with AERD and patients with ATA. The percentage of decrease in FEV₁ by aspirin provocation in AERD (28.1%) was significantly higher compared with ATA (3.05%; $p < 0.01$). Smoker status, sex, and methacholine provocation test (PC20) were significantly different among the AERD, ATA, and control groups.

Associations of GSDMB SNPs and Haplotypes with AERD

A total of six common SNPs of *GSDMB* on chromosome 17q12 (14 kb) were selected (Fig. 1 A). Pairwise comparisons revealed one LD block (Fig. 1 B). Haplotypes were estimated, and only common haplotypes with a frequency of >0.05 were analyzed for further associations (Fig. 1 C). Logistic analysis showed a significant associations between five SNPs and AERD development (minimum $p = 1.17 \times 10^{-4}$ at rs870830 in the dominant model; odds ratio, 0.52) (Table 2), even after Bonferroni correction ($p_{\text{corr}} = 0.01$). Differences in the frequencies of the SNPs were found between AERDs and normal controls, but there was no difference between subjects with ATA and normal controls (Supplemental Table 2). More importantly, two polymorphisms (rs7216389 and rs870830) in the intron of

GSDMB were significantly associated with AERD. Further haplotype analysis showed that the second common haplotype (ht2, GCTAGG) was also associated with AERD ($p = 0.002$; $p_{\text{corr}} = 0.04$) (Table 2).

Associations of GSDMB SNPs with FEV₁ Declined by Aspirin Provocation

We conducted an association analysis between the genetic variants in *GSDMB* and a fall rate of FEV₁ by aspirin provocation (Table 3). Most of the SNPs that were associated with AERD also showed significant signals with the fall rate of FEV₁ (minimum $p = 2.61 \times 10^{-5}$ at rs870830 in a codominant model) (Table 3), even after Bonferroni correction ($p_{\text{corr}} = 1.17 \times 10^{-4}$).

In addition, *GSDMB*-ht2 showed a significant association with the fall rate of FEV₁ ($p = 6.32 \times 10^{-7}$, $p_{\text{corr}} = 1.14 \times 10^{-3}$) (Table 3).

***In Silico* Analysis of rs12738007 in the Intron**

To predict the potential role of rs870830, which showed a significant association with AERD in the intron 3 region of *GSDMB*, *in silico* analysis was carried out by using HSF, web-based analysis of potential splicing aberrations.⁴⁰ The analysis showed that the rs870830 variant induced the creation of enhancer and splice motif sites (Table 4). The exonic splicing regulatory sequence was abolished by minor allele C of rs870830.

DISCUSSION

In our recent exome GWAS, *GSDMB* was identified as a potential association locus for AERD, which showed significance at rs2290400 ($p = 2.0 \times 10^{-3}$) in the exon region. Furthermore, results of other research indicate the potential susceptibility of *GSDMB* in childhood asthma and severe asthma in several populations.^{24,27–29} *GSDMB* is involved in leukocyte- and eosinophil-mediated bronchial hyperresponsiveness.^{20,30} It is also possibly involved in airway remodeling and epithelial barrier function.³¹ In Table 1, the presence of positive skin-prick test results for aeroallergens (*i.e.*, the marker for atopy) was identical in the AERD and ATA groups (53.3 and 55.03%). Atopy has been thought to be a comorbid condition, which could make AERD more aggressive. Depending on the case series, between 30⁸ and 70%³² of patients with AERD are atopic.^{8,32} Thus, atopy should be evaluated in the suspicion of AERD if the other risk factors, such as severe asthma or nasal polyposis, exist. However, subject numbers of the studies were <100 with AERD, and it would not be large enough to test statistical assumption. Recently, in studies that recruited >100 with AERD and several hundreds of individuals with asthma and aspirin tolerant, the prevalence of atopy became similar between the two groups^{9,10} Therefore, this is an interesting result, which indicated that the

Table 2 Logistic analysis of association between *GSDMB* polymorphisms and AERD

SNP/ haplotype	Allele	Position (AA)	MAF		Codominant			Dominant			Recessive			Statistical Power
			AERD (<i>n</i> = 165)	ATA (<i>n</i> = 407)	OR (95% CI)	<i>p</i> *	<i>p</i> _{corr} **	OR (95% CI)	<i>p</i> *	<i>p</i> _{corr} **	OR (95% CI)	<i>p</i> *	<i>p</i> _{corr} **	
rs7224129	A>G	5'-flanking	0.186	0.271	0.65 (0.47–0.90)	0.008#	NS	0.65 (0.44–0.95)	0.03#	NS	0.47 (0.2–1.08)	0.07	—	0.46
rs7216389	T>C	Intron 2	0.167	0.265	0.59 (0.43–0.84)	0.003#	NS	0.58 (0.4–0.86)	0.006#	NS	0.38 (0.15–1.01)	0.05	—	0.61
rs12450091	T>C	Exon 3 (E122G)	0.104	0.084	1.34 (0.83–2.16)	0.23	—	1.42 (0.88–2.3)	0.15	—	1.84 (0.16–20.74)	0.62	—	0.31
rs870829	G>T	Intron 3	0.166	0.253	0.64 (0.45–0.90)	0.009#	NS	0.67 (0.45–0.99)	0.04#	NS	0.25 (0.08–0.85)	0.03#	NS	0.58
rs870830	A>G	Intron 3	0.219	0.332	0.60 (0.44–0.82)	0.001#	0.02	0.52 (0.35–0.76)	7.00 × 10 ⁻⁴ #	0.01#	0.56 (0.27–1.16)	0.12	—	0.72
rs7213668	A>G	Intron 3	0.180	0.280	0.61 (0.44–0.85)	0.003#	NS	0.6 (0.41–0.89)	0.01#	NS	0.36 (0.14–0.96)	0.04#	NS	0.61
<i>GSDMB</i> -ht1			0.379	0.468	0.74 (0.57–0.96)	0.02#	NS	0.63 (0.43–0.92)	0.02#	NS	0.74 (0.46–1.2)	0.22	—	0.47
<i>GSDMB</i> -ht2			0.118	0.209	0.56 (0.38–0.81)	0.002#	0.04#	0.55 (0.36–0.83)	0.005#	NS	0.23 (0.05–0.98)	0.05	—	0.61
<i>GSDMB</i> -ht3			0.100	0.084	1.36 (0.86–2.13)	0.19	—	1.37 (0.85–2.2)	0.20	—	1.74 (0.15–19.61)	0.66	—	0.26
<i>GSDMB</i> -ht4			0.056	0.063	0.91 (0.52–1.59)	0.73	—	0.93 (0.52–1.64)	0.80	—	—	0.99	—	0.07
<i>GSDMB</i> -ht5			0.041	0.057	0.68 (0.36–1.27)	0.22	—	0.62 (0.32–1.2)	0.15	—	—	0.98	—	0.17

GSDMB = Gasdermin B gene; AERD = aspirin-exacerbated respiratory disease; SNP = single nucleotide polymorphism; AA = amino acid; MAF = minor allele frequency; ATA = aspirin-tolerant asthma; OR = odds ratio; CI = confidence interval; *p*_{corr} = Bonferroni correction; ht = haplotype; NS = not significant.

*The *p* values of regression analysis are adjusted for age, sex, body mass index, and smoking status as covariates.

**The *p*_{corr} value after Bonferroni correction by multiplying the number of independent makers SNPs (*n* = 6) and three different models (*n* = 3).

#Indicate the statistical significance of *p* < 0.05.

Table 3 Association of GSDMB SNPs and haplotypes with the fall of FEV₁ by aspirin provocation

SNP/ht	Allele	Position	C/C			C/R			R/R		
			No. FEV ₁ (mean ± SD)	p*	p _{corr} **	No. FEV ₁ (mean ± SD)	p*	p _{corr} **	No. FEV ₁ (mean ± SD)	p*	p _{corr} **
rs7224129	A>G	Promoter	328(14.05±16.08)	9.59 × 10 ⁻⁴ #	0.02#	194(10.97±14.3)	6.34 × 10 ⁻³ #	NS	40(6.00±12.53)	5.29 × 10 ⁻³ #	NS
rs7216389	T>C	Intron 2	328(14.24±16.1)	3.46 × 10 ⁻⁴ #	5.40 × 10 ⁻³ #	198(10.48±14.03)	2.32 × 10 ⁻³ #	0.04#	36(5.47±12.86)	6.23 × 10 ⁻³ #	NS
rs12450091	T>C	Exon 3	465(11.62±14.73)	0.04#	NS	94(14.84±17.31)	0.04#	NS	3(10.70±12.85)	0.94	—
rs870829	C>A	Intron 3	334(13.72±15.74)	2.10 × 10 ⁻³ #	0.04#	192(10.57±14.2)	0.01#	NS	31(5.13±12.55)	9.30 × 10 ⁻³ #	NS
rs870830	A>G	Intron 3	276(14.81±16.65)	2.61 × 10 ⁻⁵ #	4.71 × 10 ⁻⁴ #	228(10.26±13.44)	1.17 × 10 ⁻⁴ #	1.87 × 10 ⁻³ #	53(6.47±11.5)	4.97 × 10 ⁻³ #	NS
rs7213668	A>G	Intron 3	316(14.18±16.09)	1.60 × 10 ⁻⁴ #	3.60 × 10 ⁻³ #	204(10.46±13.87)	1.50 × 10 ⁻³ #	0.02#	38(4.92±10.94)	2.48 × 10 ⁻³ #	NS
GSDMB-ht1			182(15.11±16.68)	0.01#	NS	269(11.44±14.36)	7.87 × 10 ⁻³ #	NS	111(10.22±15.16)	0.14	—
GSDMB-ht2			381(14.11±16.17)	6.32 × 10 ⁻⁵ #	1.14 × 10 ⁻³ #	160(9.47±13.14)	3.47 × 10 ⁻⁴ #	5.47 × 10 ⁻³ #	21(3.38±10.19)	4.97 × 10 ⁻³ #	NS
GSDMB-ht3			466(11.88±14.98)	0.07	—	93(14.84±17.31)	0.06	—	3(10.7±12.85)	0.97	—
GSDMB-ht4			498(12.38±15.44)	0.89	—	63(12.61±15.1)	0.98	—	1	0.18	—
GSDMB-ht5			507(12.73±15.8)	0.11	—	54(8.93±10.67)	0.08	—	1	0.47	—

GSDMB = Gasdermin B gene; SNP = single nucleotide polymorphism; FEV₁ = forced expiratory volume in the first second of expiration; C/C = homozygote of common allele; C/R = heterozygote of rare allele; R/R = homozygote of rare allele; SD = standard deviation; p_{corr} = Bonferroni correction; NS = not significant; ht = haplotype.

*The p values of regression analysis are adjusted for age, sex, body mass index, and smoking status as covariates.

**The p_{corr} values after Bonferroni correction by multiplying the number of independent makers SNPs (n = 6) and three different models (n = 3).

#Statistical significance, p < 0.05.

Table 4 *In silico* analysis of the SNP rs870830 located in intron 3 of *GSDMB**

Sites	Motif		Threshold	Result
	T Allele	C Allele\$		
Enhancer motif	—	Cgcaagc (74.38)#	72.98	New SF2/ASF (IgM-BRCA1) enhancer motif
Silencer motif	—	ttaCgcaa (60.7)#	60	New sironi's silencer motif
Potential splice site	ccttta T gcaagca (75.12)#	ccttta C gcaagca (75.77)#	—	Splice acceptor site value increased
Exonic splicing regulatory sequences	ta T gca#	—	—	Splicing regulatory sequences broken

SNP = Single nucleotide polymorphism; *GSDMB* = gasdermin B gene; SF = pre-mRNA-splicing factor SF2; ASF = alternative splicing factor 1; IgM = immunoglobulin M; BRCA = breast cancer 1.

**In silico* analysis was performed by using the Human Splicing Finder (v.3.0) (Ref. 40).

#Bold capital characters indicate the polymorphic site of rs870830.

\$Sequence of rs870830 was changed to positive strand.

The range of value in parentheses is 0–100. High score means an increased specificity for the prediction of SF2/ASF-specific and silencer motif.

presence of atopy might be not related with the development of AERD.

The purpose of our study was to scrutinize the genetic associations between six tagging SNPs of *GSDMB* and clinical subtypes of asthma in a Korean population. We observed several *GSDMB* SNPs and haplotypes to have significant associations with the development of AERD. The possible susceptibility of *GSDMB* SNPs to AERD was reinforced by the strong association of these variants with the increased fall rate of FEV₁ by aspirin provocation (minimum $p_{corr} = 4.71 \times 10^{-4}$ in a codominant model) (Table 3). Halapi *et al.*²⁴ and Kang *et al.*³⁰ found a significant association between rs7216389 and childhood asthma susceptibility. Yu *et al.*¹⁹ reported that rs7216389 was also associated with asthma susceptibility and intermediate phenotypes, such as elevated immunoglobulin E and bronchial hyper-responsiveness, in Korean children with asthma. In sum, rs7216389 in *GSDMB* is significantly associated with susceptibility of children to asthma and AERD.

The first GWAS of childhood asthma discovered the novel associated loci on 17q21, which included *ORMDL3*, *GSDMB*, and *ZPBP2* genes.¹⁶ Since then, this finding has been consistently replicated across different ethnic groups. Replication studies of *GSDMB* gene variants are summarized in Supplemental Table 3. The three SNPs in *GSDMB*, rs2305480 on exon 9, rs7216389 on intron 2, and rs7219923 on intron 1, showed significant or nominal associations with the risk of asthma or childhood asthma in European populations and in other Asian ethnic groups ($p = 2 \times 10^{-2}$ in childhood asthma of Chinese, $p = 6 \times 10^{-23}$ in childhood asthma of European) (Supplemental Table 3).^{27–29,33–37} Interestingly, 32 variants of *GSDMB* that contain these three

SNPs were in absolute linkage disequilibrium according to our results, as presented in Fig. 1 A. It would be useful to understand and interpret these data for association research of *GSDMB* gene variants because one variant could act as the surrogate for the 31 other variants.

In previous studies, rs7216389 in *GSDMB* showed a strong association with childhood asthma, based on meta-analysis of five published studies in nine populations and differences in DNA methylation, depending on the alleles.³⁸ Although C-phosphate-G sites in the neighborhood of rs7216389 in *GSDMB* were highly methylated, DNA methylation levels did not differ between children with asthma and the controls in a Swedish population. Halapi *et al.*²⁴ reported that susceptibility allele T of rs7216389 was correlated with the expression of *ORMDL3* or *GSDMB* in peripheral blood leukocytes. Other sequence variants at 17q21 also affected the expression level of *GSDMB*.²⁴ The susceptibility allele A of rs2872507 near the *GSDMB* gene associated with inflammatory bowel disease was correlated with a reduced expression level of *GSDMB* in intestinal biopsies from patients with inflammatory bowel disease.³⁹ Thus, the expression level of *GSDMB* was associated with other adjacent variants as well as the rs7216389 variant.

The *in silico* analysis was conducted to determine the effect of rs870830 on splicing because its role had not been investigated. The substitution of a C allele in the position of rs870830 led to high development of enhancer motif sites, which indicated that rs870830 may alter splicing efficacy by creating an enhancer motif that functions by binding the enhancer regulatory protein. The noncoding variants rs7216389 and rs870830

may contribute to genetically complex disease phenotypes through the alteration of splicing efficiency and/or the regulation of downstream gene expression. However, it is difficult to be sure that these are causal loci because there are many SNPs with absolute LD with rs7216389. The variant may be in LD with several nearby polymorphisms, any one of which could be the causal locus. Thus, further studies are needed to identify the causative SNP in *GSDMB* and to establish a mechanistic link between the *GSDMB* gene and asthma-related traits. One limitation of this study was the insufficient number of AERD cases due to the rareness of the condition.

CONCLUSION

To investigate the genetic association of *GSDMB* with the risk of AERD based on our previous exome GWAS, we performed an extended replication study, including haplotype analysis. We found a strong association among *GSDMB* polymorphisms (such as rs870830 and rs7216389 in the intron) and AERD and the fall rate of FEV₁ in a Korean population. Our result was different from previous studies^{27–29,33–37} that found an association of several *GSDMB* variants and childhood-onset asthma. To our knowledge, our study was the first to report the association of genetic variants in the *GSDMB* and clinical subtypes of asthma. Although further studies are needed to explain the biologic mechanism of these associations, the *GSDMB* variants might be useful in the development of noninvasive diagnostic methods for AERD.

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