

ORIGINAL ARTICLE

Development of a genetic marker set to diagnose aspirin-exacerbated respiratory disease in a genome-wide association study

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We developed a genetic marker set of single nucleotide polymorphisms (SNPs) by summing risk scores of 14 SNPs showing a significant association with aspirin-exacerbated respiratory disease (AERD) from our previous 660 W genome-wide association data. The summed scores were higher in the AERD than in the aspirin-tolerant asthma (ATA) group ($P = 8.58 \times 10^{-37}$), and were correlated with the percent decrease in forced expiratory volume in 1 s after aspirin challenge ($r^2 = 0.150$, $P = 5.84 \times 10^{-30}$). The area under the curve of the scores for AERD in the receiver operating characteristic curve was 0.821. The best cutoff value of the summed risk scores was 1.01328 ($P = 1.38 \times 10^{-32}$). The sensitivity and specificity of the best scores were 64.7% and 85.0%, respectively, with 42.1% positive and 93.4% negative predictive values. The summed risk score may be used as a genetic marker with good discriminative power for distinguishing AERD from ATA.

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INTRODUCTION

Aspirin-exacerbated respiratory disease (AERD) refers to the development of bronchoconstriction in asthmatic individuals after the ingestion of aspirin or other nonsteroidal anti-inflammatory drugs,¹ and is characterized by aspirin hypersensitivity, asthma and chronic rhinosinusitis with nasal polypsis.² Aspirin hypersensitivity in asthmatics is associated with increased asthma severity, life-threatening asthma attacks and possible remodeling of both the upper and lower airways.³ The prevalence of aspirin hypersensitivity in asthmatics varies widely.⁴ On the basis of patient histories alone, the incidence of AERD in adults is 3–8%, but this percentage has been shown to increase by two- or threefold after challenge with ASA via the oral or bronchial route.^{5,6} Approximately 20% of patients are entirely unaware of suffering from aspirin intolerance,⁴ and we previously observed that one-third of AERD subjects do not have a history of aspirin hypersensitivity.⁵ In addition, 25% of those requiring emergency mechanical ventilation are aspirin intolerant.^{7,8} Nevertheless, AERD remains widely under-diagnosed because of insufficient awareness of the relationship between aspirin ingestion and asthma exacerbation^{4,5} and inadequate diagnostic tools.⁹ Thus, the identification of aspirin hypersensitivity is essential to avoid serious complications.^{3,4,7,10}

Several noninvasive methods for diagnosing AERD have been proposed in peripheral blood, including flow-cytometric determination of basophil activation in peripheral blood,¹¹ functional eicosanoid test in cultured peripheral blood leukocytes,¹² levels of

galectin-10 mRNA,¹³ plasma eotaxin²¹⁴ and plasma apolipoprotein H concentrations.¹⁵ Urine leukotriene E4, 9alpha and 11beta in exhaled breath condensate also show high sensitivity and specificity for discriminating between AERD and aspirin-tolerant asthma (ATA).¹⁶ Recently, using a microarray analysis of ~34 560 genes, we reported that the mRNA expression level of two genes, CNKSR3 and SPTBN2, could be markers for AERD.¹⁷ However, they have not been applied as diagnostic biomarkers in practice because of their weak biological effects on the risk for AERD and problems with assessments.

Several genomic and genetic studies have been performed to develop readily assessable biomarkers for AERD. However, less than 100 association studies have been performed to identify genetic variants associated with the development of AERD over the past three decades. Several early studies reported a positive association between AERD and biologically plausible genes responsible for over- or underproduction of critical modulators in the arachidonate pathways, including cysteinyl leukotriene receptor 2,¹⁸ prostaglandin E2 receptor subtype 2,¹⁹ leukotriene C4 synthase (LTC4S)²⁰ and arachidonate 5-lipoxygenase (ALOX5).²¹ More recently, AERD has been shown to be associated with genetic variants in the immune response and inflammation pathways such as *FcεR1β*,²² *HLA*,²³ *TBX21*,²⁴ *IL-4*²⁵ and *PPAR-γ*²⁶ genes. In a study regarding single nucleotide polymorphisms (SNPs), the statistical significance was marginal with odds ratios (ORs) ranging from 0.5 to 2.0.²⁷ Although recent genome-wide association studies (GWAS) for the risk of AERD

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have shown better results, with an OR of 2.63 for a non-synonymous rs7275857 on exon 2 of *CEP68*²⁸ and OR of 2.40 for rs1042151 (Met105Val) of *HLA-DPB1*,²⁹ the discriminative power of each SNP was low in these studies and clinical application was not practical. Thus, we developed a set of eight SNP markers for predicting AERD, which were identified as genetic variants associated with AERD in our first GWAS study consisting of 100 K SNPs.²⁸ This combination of SNP markers has high discriminative power to distinguish between AERD and ATA groups, with 78% sensitivity and 88% specificity.³⁰ These data suggest that an SNP combination may be used as a genetic marker to predict AERD. However, more powerful sets of SNPs are required for the clinical application of genetic markers for diagnosing AERD.

Therefore, in the present study, we extended our second GWAS study²⁹ using a denser BeadChip of 657 366 gene variants (660 W) to develop an SNP marker set for the discrimination of AERD in asthmatics.

MATERIALS AND METHODS

Study subjects

The subjects and GWAS data are provided in our previous report.²⁹ Briefly, a total of 685 asthmatic patients without AERD and 117 subjects with AERD were recruited from the Asthma Genome Research Center, Bucheon, Republic of Korea. All subjects met the definition of asthma as defined by the Global Initiative for Asthma guidelines.³¹ All patients were ethnic Koreans and provided written informed consent to participate in the study. Criteria for the diagnosis of AERD, clinical characteristics of study subjects, genome-wide SNP genotyping and GWAS analysis are provided in the previous study.²⁹ Clinical profiles of the study subjects were summarized as follows; Female, 62.3%; Age, 45.5 ± 14.9 years (mean ± s.d.); Onset age, 38.9 ± 16.3 years; Duration, 6.6 ± 9.2 years; Forced vital capacity predicted, 29.2 ± 17.5%; forced expiratory volume in one second (FEV1) predicted, 78.5 ± 18.1%; PC20 methacholine, 6.2 ± 8.5 mg ml⁻¹; Total IgE in serum, 386.0 ± 567.2 IU ml⁻¹; Skin test positive, 50.2%; % decline of FEV1 after oral aspirin challenge (OAC), 7.8 ± 12.2%.

Calculation of summed genetic risk scores and statistical methods

For association analysis, allele frequencies between asthmatics with and without AERD were compared using logistic regression analyses adjusted for age, sex, atopic status, smoking status and body mass index as covariates using the HelixTree® (Golden Helix, Bozeman, MT, USA) and SPSS software version 12.0 (SPSS, Chicago, IL, USA), as described in our previous report. Multiple logistic regression models were used to perform the association analysis of AERD. To exclude the effect of linkage

disequilibrium, we chose one SNP on each of 14 genes showing a significant association with AERD in our previous study regarding OR and *P* values. To calculate the genetic risk score of individuals, we assigned ORs to corresponding causative alleles, in contrast to 1 given to opposite allele. It was followed by summing ORs from multiple variants in different genetic loci into a single relative risk value using the following formula:

$$\text{Summed risk score} = \text{EXP} \left(\frac{\sum \ln(\text{Odds ratio} \times \text{risk allele})}{n_{\text{allele}}} \right)$$

Receiver-operating characteristic curves were obtained for all models, and the area under the curve was calculated to select marker SNPs and predict AERD.³² Youden's index was used to define cutoff values for the scores. Comparisons of the score between AERD and ATA and the discriminating power were performed using independent *t* test and χ^2 analysis, respectively. Correlations between the score and percent decrease in FEV1 after OAC were analyzed using the Pearson's correlation test.

RESULTS

Calculation of summed risk score for individuals with AERD

On the basis of association analyses using 142 AERD and 996 ATA subjects in our previous study, 14 genes (*HLA-DPB1*, *MEIS1*, *NAB1*, *NRP2*, *CLDN1*, *DCDC2*, *TMEM196*, *AK8* [C9orf98], *TSC1*, *ZFYVE1-RBM25*, *PCSK6*, *ILVBL*, *GP6* and *RDH13*) were selected on the basis of OR and *P* values (Table 1). Major and minor alleles were coded as 1 and the corresponding OR, respectively. The summed risk scores in the study subjects ranged from 0.774 to 1.142, with an average of 0.973 (0.058 s.d.).

Comparison of the summed risk scores between AERD and ATA and its correlation with percent decrease in FEV1 by OAC

The summed risk scores were significantly higher in the AERD than ATA group (1.033 ± 0.005 vs 0.963 ± 0.002, *P* = 8.58 × 10⁻³⁷, Figure 1a). In each group, the summed risk scores were normally distributed (Figure 1b). The scores were significantly correlated with the percent decrease in FEV1 after the OAC test across all study subjects (*r*² = 0.150, *P* = 1.76 × 10⁻²⁹, Figure 2). The area under the curve of the score for AERD was 0.821 ± 0.021 (*P* = 1.67 × 10⁻²⁸, Figure 3a). The best cutoff value with a score of 1.01328 was determined using the receiver-operating characteristic curve and Youden Index (Figure 3b).

Table 1. Odd ratio and *P*-values of 14 single nucleotide polymorphisms associated with the risk of AERD

SNPID	Chr.	Gene (nearest gene)	Location	Results of GWAS	
				Odd ratio (95%CI)	P value ^a
rs12619205	2	MEIS1	Intron	1.72 (1.30–2.28)	1.14 × 10 ⁻⁴
rs10931468	2	NAB1	Intron	1.45 (1.09–1.93)	9.94 × 10 ⁻³
rs849530	2	NRP2	Intron	1.80 (1.35–2.41)	6.91 × 10 ⁻⁵
rs10513846	3	CLDN1	Intron	0.68 (0.51–0.91)	8.15 × 10 ⁻³
rs2281389	6	HLA-DPB1	3'	2.40 (1.61–3.58)	5.60 × 10 ⁻⁶
rs3789224	6	DCDC2	Intron	1.98 (1.41–2.77)	8.55 × 10 ⁻⁵
rs9886152	7	(TMEM196)	Intron	0.54 (0.39–0.76)	3.09 × 10 ⁻⁴
rs2771994	9	AK8 (C9orf98)	Intron	1.87 (1.39–2.52)	2.48 × 10 ⁻⁵
rs1050700	9	TSC1	3'UTR	1.82 (1.35–2.45)	5.82 × 10 ⁻⁵
rs12432987	14	ZFYVE1-RBM25	Intron	1.88 (1.38–2.57)	4.36 × 10 ⁻⁵
rs3825915	15	PCSK6	Intron	0.51 (0.37–0.70)	2.50 × 10 ⁻⁵
rs2240299	19	ILVBL	Intron	0.51 (0.37–0.72)	7.61 × 10 ⁻⁵
rs11669150	19	GP6	Intron	0.68 (0.47–0.98)	3.74 × 10 ⁻²
rs1671215	19	RDH13	3'	0.49 (0.32–0.74)	6.34 × 10 ⁻⁴

Abbreviations: AERD, aspirin-exacerbated respiratory disease; CI, confidence interval; GWAS, genome-wide association study; SNP, single nucleotide polymorphism; UTR, untranslated region. ^a*P*-values were obtained using logistic regression analyses.

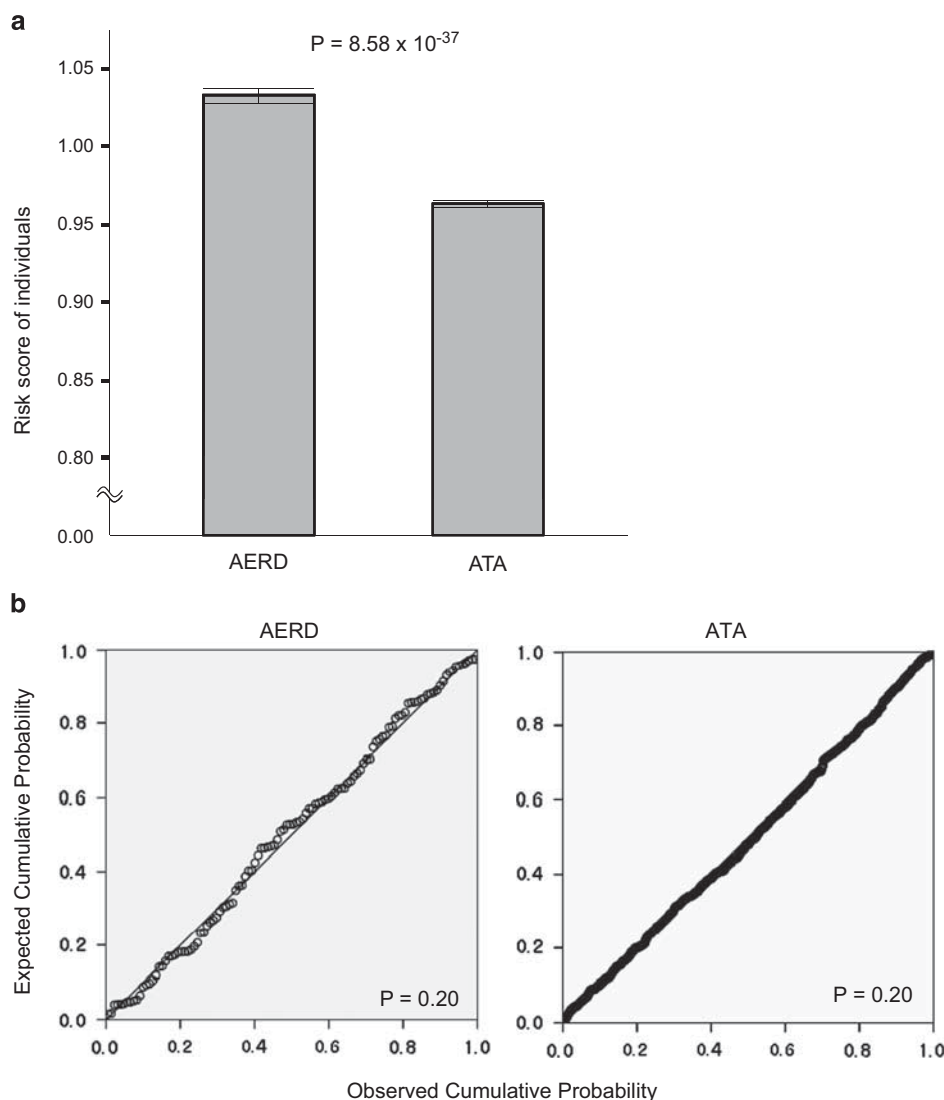


Figure 1. (a) Comparison of the relative risk score between AERD and ATA subjects using the independent *t*-test. The relative risk scores of individuals were calculated from a combination of 14 SNPs on the basis of our previous 660 W genome-wide association study (GWAS) data. (b) Q-Q plot for the relative risk score in each group.

Diagnostic value of the summed risk score for the prediction of AERD

After dividing the study subjects into two groups according to the cutoff value, diagnostic values were tested for predicting AERD. We found that a higher proportion of AERD subjects than ATA subjects showed summed risk scores above the cutoff value (64.66 vs 15.04%, $P = 1.38 \times 10^{-32}$, Table 2). The sensitivity and specificity of the scores were 64.7% and 85.0% with 42.1% positive and 93.4% negative predictive values, respectively. The overall accuracy of the test was 82.0%. The positive and negative likelihood ratios were 4.300 and 0.416, respectively.

DISCUSSION

We tested a summation method of OR values for 14 SNPs obtained from our previous GWAS data. The summed risk scores were significantly higher in AERD than in ATA subjects and showed a good correlation with percent decrease in FEV1 after the OAC test. We observed 64.7% sensitivity, 85.0% specificity and 82.0% overall accuracy. Although the positive predictive value was

relatively low (42.1%), the negative predictive value was 93.4% and the negative likelihood ratio was 0.416. These results suggest that the summed risk score may have sufficient power to discriminate between AERD and ATA subjects.

Integration of multi-locus association data has been performed by counting risk alleles or using a multifactor dimensionality reduction method.³³ However, these methods do not reflect the quantitative impact of genetic effects of each allele on phenotypes. Accordingly, we developed summed risk scores using ORs of candidate SNPs obtained from an association study. To date, several studies have explored whether OR summations of genetic and clinical risk factors are useful for diagnosing diseases and predicting drug responses. Shigemizu *et al.*³⁴ investigated risk prediction models using the ORs of clinical factors and nine SNPs from GWAS data in 7360 Japanese patients with type 2 diabetes. The best model with an area under the curve of 0.8085 was obtained, and application of the model to a prospective cohort accurately predicted disease-free survival. Similar approaches of OR summation have been applied to determine the optimal dose of anticoagulants such as warfarin for individuals. A pharmacogenetic algorithm using *CYP2C9* variants and *VKORC1-1639G>A* has

been successful for determining the initial and effective dose of warfarin in personalized therapy.³⁵ In addition, the pharmacogenetic algorithm explains approximately 70% of the variability

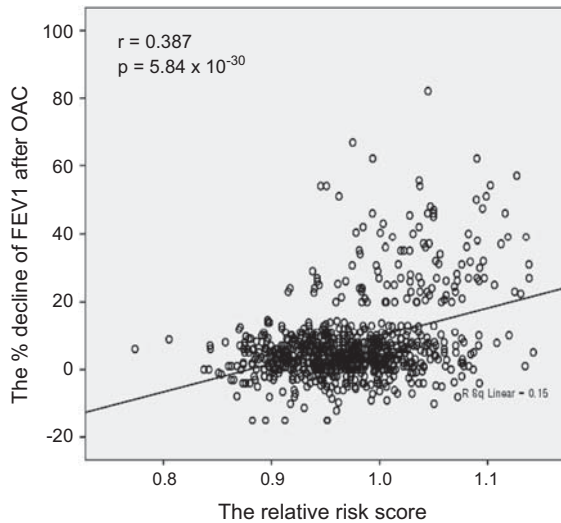


Figure 2. Correlation between the relative risk score and the percent decrease in FEV1 after the OAC test in asthmatics. The correlation was tested using the Pearson correlation test.

observed in therapeutic warfarin dose in large retrospective and prospective international study cohorts.³⁶ However, this algorithm was not useful for determining the loading and maintenance doses of phenprocoumon and acenocoumarol in a prospective study.³⁷ In this study, a summation method of ORs of polymorphisms on 14 genes obtained from a high-density GWAS was applied to discriminate AERD from ATA. To the best of our knowledge, this is the first report to apply the summation of ORs to risk evaluation of AERD.

Because the prevalence of AERD among asthmatics is approximately 15% in the Korean population,^{5,29} the estimated positive and negative post-test probability of the risk scores would be 0.431 and 0.068, respectively. This suggests that 93.2% of asthmatics showing risk scores under the cutoff value of 1.01328 are ATA, while 6.8% are AERD. This result suggests that the summed risk scores under 1.01328 may be very useful for excluding AERD in asthmatics. In our previous study, we integrated clinical parameters associated with AERD, including nasal polyposis, history of aspirin hypersensitivity, chronic sinusitis and PC20 methacholine values, to predict OAC results.⁵ The calculated risk scores were significantly higher in AERD patients than in ATA patients, and the sensitivity, specificity and accuracy were 78.7%, 81.8% and 81.4%, respectively. These values are comparable with those in the present study. However, the false-positive ratio was 0.630, which was higher than that of the present study (0.579).

In an additional study, we reported another method for combining SNPs based on GWAS data using a low-density 100 K

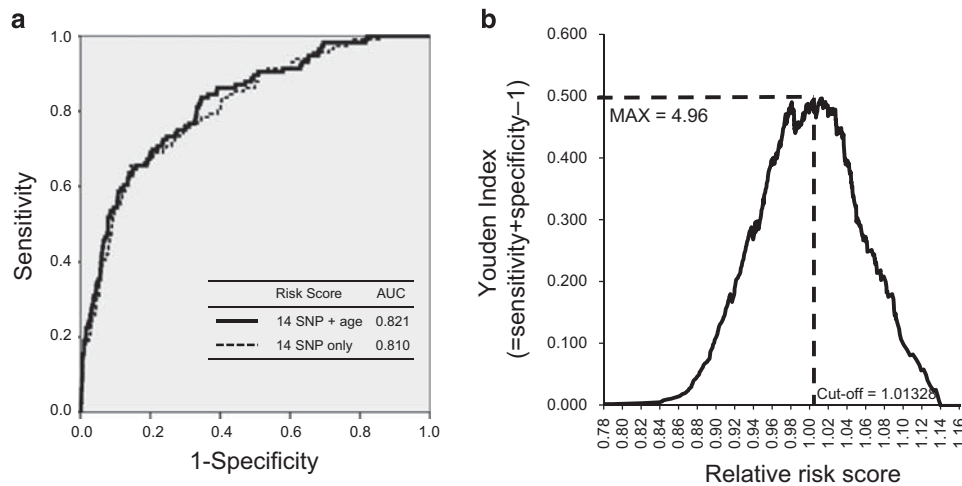


Figure 3. (a) Receiver-operating characteristic curves and the area under curve of the relative risk score. (b) Determination of the cutoff value for discriminating between AERD and ATA using the Youden index.

Table 2. Diagnostic values of the relative risk score to predict AERD

Relative risk score	Diagnosis			Statistics	Diagnostic value
	ATA	AERD	Total		
< 1.01328	582 (72.7%)	41 (5.1%)	623 (77.8%)	$\chi^2 = 141.31$ $P = 1.38 \times 10^{-32}$	Sensitivity 64.7% Specificity 85.0% +PV 42.1% -PV 93.4% Accuracy 82.0% LR+ 4.300 LR- 0.416
> 1.01328	103 (12.9%)	75 (9.4%)	178 (22.2%)		
Total	685 (85.5%)	116 (14.5%)	801 (100%)		

Abbreviations: AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; +PV, positive predictive value; -PV, negative predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio.

BeadChip.³⁰ In that study, eight SNPs were selected and combined to estimate the overall genetic risk for each subject by generating the power set of candidates (2^8-1), which was followed by multiple logistic regression analysis with elements in the power set. The sensitivity and specificity of the eight-SNP model were 78% and 88%, respectively, and the overall accuracy of the model was 82.01% (Supplementary Table S1). Although the combined overall genetic risk differed significantly between AERD and ATA, 100 AERD and 100 ATA subjects were included. Importantly, the candidate SNPs used for calculating the risk scores differed from those used in the present study. SNPs on *PARK2*, *WDR21*, *VRK2*, *CHST11*, *LINC00314*, *PDZK3*, *SGSM1* and *CENPF* were used in the previous study, which were not included in the present study because of the lack of relication.^{29,30} Additionally, although the nonsynonymous rs7572857 G>A variant of the CEP68 gene showed the most significant association with AERD compared with ATA controls ($P=6.0\times 10^{-5}$) in our previous low-density 100 K GWAS, CEP68 gene polymorphisms were not replicated in the next GWAS being the basis of the present study. Thus, we excluded these polymorphisms in this study.²⁸⁻³⁰ This difference may be due to different SNP contents between the 100 K BeadChip and the 660 W Chip. In contrast to the 100 K BeadChip that covered only 109 365 SNPs over the whole genome, the 660 W BeadChip contains 657 366 variants including 6000 copy number variants, which enables higher-powered GWAS. For example, the Illumina Human 660 W contains rs2302647 and rs10496123 on the CEP68 gene instead of rs7572857 of the former 100 K chip, and the two SNPs rs2302647 and rs10496123 showed a statistically insignificant association with AERD both in the former and in the latter GWAS.²⁹ However, the 660 W Chip includes only one-thirtieth of the SNP contents of the 100 K chip (3178 SNPs), and a combination of the two results would provide a more accurate tool for diagnosing AERD.

To define the summed risk scores, we selected 14 SNPs, each of which is located on a different gene to exclude the influence of LD. Among them, the genetic association between HLA-DPB1 and AERD has been reported.²³ Although no other genes selected in this study are known to be directly associated with AERD or the arachidonate pathway, some are associated with the mechanism of aspirin and airway functions. In addition, 15-epi-lipoxin A4 upregulates *NAB1*, a glucocorticoid-responsive gene encoding a transcriptional corepressor, in human neutrophils and in smooth muscle cells of mice lung.³⁸ Because 15-epi-lipoxin A4 is generated by aspirin,³⁹ *NAB1* may play a role in the pathophysiology of AERD. In addition, *CLDN1* encodes a member of the claudin family protein, a component of tight junction strands.⁴⁰ Aspirin treatment of epithelial monolayers downregulates claudin-7 protein, which decreases permeability.⁴¹ *TSC1* encodes a growth inhibitory protein also known as hamartin that may mediate the PI3K-mTOR/S6K1 pathway involved in smooth muscle remodeling in the airways.⁴² Finally, *GP6* encodes a membrane glycoprotein that acts as a receptor for collagen in platelets.⁴³ To obtain a rationale for the use of these genes as markers for AERD, the functional relationship between the genes and pathogenesis of AERD should be evaluated.

This study has several limitations. First, the study was retrospective and performed in Korean asthmatics. Thus, subsequent prospective and internationally corroborative studies with a large number of patients should be performed. Second, the 14 SNPs used in the present study have minor allele frequencies >5%. Thus, rare allele variants at less than 1% would be identified in association with AERD and included in the development of diagnostic markers. Additionally, because the primary purpose of this study was to develop a genetic marker set for discriminating AERD from ATA in asthmatics, we did not consider a comparison between AERD and normal subjects. Further development of a marker set supporting diagnosis of AERD in general population

would be important. Thus, it should be considered in a further study.

In conclusion, 14 SNPs were selected depending on *P*-values and ORs obtained from our previous GWAS data to develop an SNP marker to discriminate AERD in asthmatics. The risk scores were calculated by summing the ORs of the SNPs. The summated risk scores were significantly higher in AERD than in ATA patients. The cutoff value of 1.01328 for asthmatics showed 64.7% sensitivity and 85.0% specificity with 82.0% overall accuracy. Thus, the summed risk scores may be promising methods for integrating genetic markers for supporting the diagnosis of AERD in asthmatic patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (<http://www.nature.com/tpj>)

Supplement Table 1. Comparison of discriminating power of our previous GWAS and the present study.

	Previous Study ⁽³⁰⁾	Present Study
N	189	802
Genotyping Methods	100K BeadChip	660K BeadChip
Sensitivity, %	78.0	64.7
Specificity, %	87.7	85.0
Accuracy, %	82.0	82.0