Association Analysis of *Member RAS Oncogene Family* Gene Polymorphisms with Aspirin Intolerance in Asthmatic Patients

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Member RAS oncogene family (*RAB1A*), a member of the RAS oncogene family, cycles between inactive GDP-bound and active GTP-bound forms regulating vesicle transport in exocytosis. Thus, functional alterations of the *RAB1A* gene may contribute to aspirin intolerance in asthmatic sufferers. To investigate the relationship between single-nucleotide polymorphisms (SNPs) in the *RAB1A* gene and aspirin-exacerbated respiratory disease (AERD), asthmatics ($n = 1197$) were categorized into AERD and aspirin-tolerant asthma (ATA). All subjects were diagnosed as asthma on the basis of the Global Initiative for Asthma (GINA) guidelines. AERD was defined as asthmatics showing 15% or greater decreases in forced expiratory volume in one second (FEV₁) or naso-ocular reactions by the oral acetyl salicylic acid (ASA) challenge (OAC) test. In total, eight SNPs were genotyped. Logistic regression analysis identified that the minor allele frequency of +14444 $T > G$ and +41170 $C > G$ was significantly higher in the AERD group ($n = 181$) than in the ATA group ($n = 1016$) ($p = 0.0003$ – 0.03). Linear regression analysis revealed a strong association between the SNPs and the aspirin-induced decrease in FEV₁ ($p = 0.0004$ – 0.004). The *RAB1A* gene may play a role in the development of AERD in asthmatics and the genetic polymorphisms of the gene have the potential to be used as an indicator of this disease.

Introduction

ASPIRIN-EXACERBATED RESPIRATORY DISEASE (AERD) refers to the development of bronchoconstriction in asthmatics following the ingestion of aspirin (acetyl salicylic acid [ASA]) or other nonsteroidal anti-inflammatory drugs (NSAIDs) (Samter and Beers, 1967). This syndrome is characterized by ASA hypersensitivity, bronchial asthma, nasal polyposis, and chronic hyperplastic eosinophilic sinusitis (Lee and Stevenson, 2011). For other asthmatic individuals, the airways of patients with AERD show signs of persistent inflammation, with marked eosinophilia, epithelial disruption, and cellular proliferation as seen in nasal polyps (NPs) and hyperplastic sinusitis. Multiple points of overproduction or underproduction of critical mediators by a variety of inflammatory cells, including platelets, eosinophils, and neutrophils, probably account for the susceptibility to ASA. These inflammatory cells secrete proteins from intracellular vesicles or granules through exocytosis or

degranulation, which is common to all cell types (Siess and Grunberg, 1993; Zerial and McBride, 2001; Lova *et al.*, 2002; Logan *et al.*, 2003). The Ras-like Rab GTPases regulate vesicle transport in endocytosis and exocytosis. There is little definitive evidence supporting the role of Rab proteins in vesicle formation, but their role in subsequent transport and, in particular, targeting is well established. The Ras-related monomeric G proteins make up a diverse family thought to be involved in intracellular trafficking and distal stages of exocytosis in several kinds of inflammatory and immune cells, including platelets, eosinophils, and neutrophils, which probably account for the susceptibility to ASA. Considering the central role of the Rab GTPases in cellular biogenesis, it is not surprising that the loss or impairment of their activity is involved in a number of human diseases (Lacy *et al.*, 1995). These functions are critically important for all types of cellular growth. Indeed, defective protein transport is associated with the pathogenesis of a variety of human diseases (Guo *et al.*, 2000). A high prevalence of

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member RAS oncogene family (*RABIA*) overexpression (93%) has been observed not only in cancer, but also in premalignant lesions (Olkkonen and Ikonen, 2000). Cellular infiltration and proliferation are the characteristic findings of asthma. Asthmatics with ASA hypersensitivity are more prone to NPs, hyperplastic eosinophilic sinusitis, and increased cell proliferation in the airways, resulting in severe airway obstruction. These data indicate that the genetic alteration of the *RABIA* gene may be related to the development of asthma and ASA hypersensitivity through the modulation of intracellular protein trafficking. To reveal the genetic role of the *RABIA* gene in the development of ASA hypersensitivity in asthma, we evaluated the genetic association between single-nucleotide polymorphisms (SNPs) of the *RABIA* gene and the risk of AERD.

Materials and Methods

Ethical conduct of research

Consent to participate in the study was obtained in writing from all subjects. DNA from 181 patients with AERD and 1016 ATA subjects was donated by a biobank at Soonchunhyang University Hospital, Bucheon, Korea (Table 1). The protocol was approved by the ethics committee of Soonchunhyang University Hospital (approval No. SCHBC-IRB-2010-005).

Subjects

The subjects were recruited from the Asthma Genome Research Center, which comprises nine university hospitals in Korea. All subjects were Korean. All patients were diagnosed by physicians and met the definition of asthma set forth in the Global Initiative for Asthma (GINA) guidelines (Bateman *et al.*, 2008). All patients had a history of dyspnea and wheezing during the previous 12 months, plus one of the following: (1) >15% increase in forced expiratory volume in one second (FEV₁) or >12% increase plus 200 mL following inhalation of a short-acting bronchodilator, (2) <10 mg/mL PC₂₀ methacholine, and (3) >20% increase in FEV₁ following 2 weeks of treatment with inhaled steroids and long-acting bronchodilators. Twenty-four common inhalant allergens were used for a skin prick test. Total IgE was measured by the CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Atopy was defined as having a wheal reaction equal to or greater than histamine or 3 mm in diameter. All study subjects underwent the oral ASA challenge (OAC), and they had experienced no exacerbation of asthma and respiratory tract infection in the 6 weeks preceding OAC. The OAC was performed with increasing doses of ASA following previously described methods with slight modifications (Nizankowska-Mogilnicka *et al.*, 2007; Kim *et al.*, 2010). Changes in FEV₁ were followed for 5 h after the last ASA challenge dose. ASA-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 as follows: 15% or greater decreases in FEV₁ or naso-ocular reactions (AERD) and less than 15% decreases in FEV₁ without naso-ocular or cutaneous reactions (ATA). The protocols were approved by the local ethics committees of each hospital.

TABLE 1. CLINICAL CHARACTERISTICS OF THE STUDY SUBJECTS

<i>Clinical profile</i>	<i>AERD</i>	<i>ATA</i>
Number of subjects (<i>n</i>)	181	1016
Onset of age [year, median (range)]	40 (16–72)	45 (11–77)
Sex (<i>n</i> , male/female)	75/106	381/635
Current smoker/ex-smoker (%)	12.71/7.18*	13.39/16.44
Height (cm, mean ± SD)	162.69 ± 9.72	160.85 ± 8.97
Weight (kg, mean ± SD)	62.38 ± 11.03	63.18 ± 11.23
Body mass index (kg/m ²)	23.54 ± 3.49*	24.38 ± 3.58
% Decline of FEV ₁ by aspirin provocation	28.02 ± 15.31**	3.78 ± 4.82
Blood eosinophil (%)	5.33 ± 5.46	5.34 ± 5.02
FEV ₁ (% predicted)	86.39 ± 15.81	86.60 ± 18.13
PC ₂₀ methacholine (mg/mL)	4.80 ± 7.83*	6.71 ± 8.75
Total IgE (IU/mL)	369.30 ± 597.92	390.89 ± 629.42
Positive rate of skin test (%)	52.60	51.61
Positive rate of nasal polyp (%)	52.03**	22.83

Asterisks represent *p*-values for the comparison of the AERD group to ATA controls using the *t*-test or χ^2 -test. **p* < 0.01; ***p* < 0.0001.

AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; FEV₁, forced expiratory volume in one second.

Genotyping

A total of eight polymorphisms of the *RABIA* gene were selected from the National Center for Biotechnology Information (build 36) by the minor allele frequency (MAF) criteria (over 5% of MAF). Amplification and extension primers were designed for genotyping of the polymorphic sites by single-base extension. All primer extension reactions were performed using the SNaP shot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, CA). For subsequent cleanup, the reaction mixture was incubated at 37°C for 1 h with 1 U shrimp alkaline phosphatase, followed by 15 min at 72°C for enzyme inactivation. Then, the extension products and the GeneScan 120 Liz size standard solution were mixed with Hi-Di formamide (Applied Biosystems) according to the manufacturer's recommendations and incubated at 95°C for 5 min, followed by 5 min on ice. The mixture was electrophoresed on an ABI Prism 3100 Genetic Analyzer and the results were analyzed using GeneScan and GenoTyper (Applied Biosystems). Information on the primers is shown in Table 2.

Statistics

We applied widely used measures of linkage disequilibrium (LD) to all pairs of the biallelic loci: Lewontin's D' (|D'|) and *r*² (Barrett *et al.*, 2005). Haplotypes of each individual were inferred using the PHASE algorithm (ver. 2.0) developed by Stephens *et al.* (2001). The genotype distribution was analyzed using logistic regression models with age (continuous value), gender (male = 0, female = 1), smoking status (nonsmoker = 0, ex-smoker = 1, smoker = 2), atopy (absence = 0, presence = 1), and BMI as covariates.

TABLE 2. SEQUENCES OF AMPLIFYING AND EXTENSION PRIMERS FOR GENOTYPING OF *RAB1A* POLYMORPHISMS BY SINGLE-BASE EXTENSION METHOD

Locus	Primer sequence		
-2232G>T	Forward	5'-ACTTCGTCAGTAACGGACGGGGAGTATACTTCCCAGGGGAGGA-3'	
	Reverse	5'-GAGTCGAGGTCATATCGTGGGGAGTATACTTCCCAGGGGAGGC-3'	
	Extension	5'-AGATTGTATTTTGGCTGAGGTCCAAAGTCGGAATCGTAGTCGGGTCTGCC TATAGTGAGTC-3'	
-1279T>G	Forward	5'-ACTTCGTCAGTAACGGACTTGTAGGCACTATATAGTGATGAATTGCA-3'	
	Reverse	5'-GAGTCGAGGTCATATCGTTTGTAGGCACTATATAGTGATGAATTGCC-3'	
	Extension	5'-ATTTTATTGCTTATTATTTTCCAGATGCAAGGGCTGCGTTGTCTAGGTCTGCC TATAGTGAGTC-3'	
+1444T>C	Forward	5'-ACTTCGTCAGTAACGGACCACAATTCAAACCAACCTTCAGGT-3'	
	Reverse	5'-GAGTCGAGGTCATATCGTCACAATTCAAACCAACCTTCAGGC-3'	
	Extension	5'-GAAACACTTACTCCTTGCTGGCCTTGCGTCCGAGAGTAAGGGCGTCTGCCT ATAGTGAGTC-3'	
+15676T>C	Forward	5'-ACTTCGTCAGTAACGGACGGGAATGTTGAAAGTTGGGTAAATGTCA-3'	
	Reverse	5'-GAGTCGAGGTCATATCGTGGGAATGTTGAAAGTTGGGTAAATGTGC-3'	
	Extension	5'-TCAGCTTCTTTTGTAAAGTTCCTTATGAATGCGGAGGGTCAAAGGTCTGCC TATAGTGAGTC-3'	
+25391G>T	Forward	5'-ACTTCGTCAGTAACGGACGTGTTGCTCTTAAAAGAAATTCAGTGAAGTA-3'	
	Reverse	5'-GAGTCGAGGTCATATCGTGTGTGCTCTTAAAAGAAATTCAGTGAAGTC-3'	
	Extension	5'-GGTTATAAAGTCAGAATGTCTAATACTATTAGCAAGCGCCTCATGTATCGGGTC TGCCTATAGTGAGTC-3'	
+28356A>G	Forward	5'-ACTTCGTCAGTAACGGACAGGGTTTTCTTGGAGGCACAAACA-3'	
	Reverse	5'-GAGTCGAGGTCATATCGTAGGGTTTTCTTGGAGGCACAAACG-3'	
	Extension	5'-CTGAGGCTACAGCTAGATTCTTCACACGCGGGTAAACCATTGATCGTCTGCC TATAGTGAGTC-3'	
+29857A>C	Forward	5'-ACTTCGTCAGTAACGGACAGCAAGAAAACAACCAAATGGAAAA-3'	
	Reverse	5'-GAGTCGAGGTCATATCGTAGCAAGAAAACAACCAAATGGAAAC-3'	
	Extension	5'-GTATTTTGCAGGTTGATAATGGCGCAGTCAACGACGTATTCCGAGTCTGCCTA TAGTGAGTC-3'	
+41170C>G	Forward	5'-ACTTCGTCAGTAACGGACGTCCATTATGGATGGTAGAAATGCAG-3'	
	Reverse	5'-GAGTCGAGGTCATATCGTGTCCATTATGGATGGTAGAAATGCAC-3'	
	Extension	5'-AAGAATTAGTGAAAAAGATTTTCTGCACCGTAGACTGCTACAAGGGTC TGCCTATAGTGAGTC-3'	

RAB1A, member RAS oncogene family.

Haplotype associations were estimated using the HaploScore (www.biostat.wustl.edu/genetics/geneticssoft/). The differences in the rates of decline in FEV₁ following the ASA challenge among the genotypes and haplotypes were examined using a linear regression model. The data were managed and analyzed using SAS version 9.1 (SAS, Inc., Cary, NC) and SPSS version 11.0 (SPSS, Inc., Chicago, IL). For the correction of the *p*-values, the effective number of independent markers in *RAB1A* was calculated using the software SNPSpD (<http://genepi.qimr.edu.au/general/daledN/SNPSpD>), which is based on the spectral decomposition (SpD) of the matrices of pairwise LD between SNPs ($p\text{-value} \times 59.3221$) (Nyholt, 2004). The data are expressed as the mean $\pm$ standard error of the mean. Intergroup comparisons were assessed by the Mann–Whitney *U*-test. A *p*-value of $<5\%$ was considered statistically significant.

Results

Characteristics of the study subjects

A total of 1197 subjects were recruited from the asthma cohort. The clinical characteristics of the study subjects are summarized in Table 1. Significant differences in onset of age, prevalence of smoking, FVC, BMI, and rate (%) of FEV₁ decline after ASA challenge were found between the

AERD and the ATA groups ($p < 0.05$, Table 1). An ASA-induced decline in FEV₁ of -15% to 68% was observed.

Frequency, heterozygosity, and the Hardy–Weinberg equilibrium of SNPs in the *RAB1A* gene in the study population

A total of eight polymorphisms of the *RAB1A* gene, which were validated in the National Center for Biotechnology Information (build 36) database, were selected and genotyped in the study subjects. The MAFs of these eight SNPs are summarized in Table 3. The distributions of all loci followed the Hardy–Weinberg equilibrium ($p > 0.01$). The LD coefficients ($|D'|$) and r^2 among the SNPs of the *RAB1A* gene were calculated for all study subjects (Fig. 1A, C). Absolute LD ($|D'| = 1$ and $r^2 = 1$) was present between +1444T>C and +15676T>C, between +25391G>T and +41170C>G, and between +28356A>G and +29857A>C on the *RAB1A* gene. *RAB1A* was parsed into two LD blocks (BLs) and there was two major haplotypes for BL1 and three for BL2 (Fig. 1B, C). *RAB1A* BL1-ht1, BL1-ht2, BL2-ht1, BL2-ht2, and BL2-ht3 were equivalent to -2232G>T, -1279T>G, +41170C>G, +29857A>C, and +1444T>C, respectively. Therefore, the major haplotypes were omitted for further statistical analysis.

TABLE 3. THE FREQUENCIES, HETEROZYGOSITY AND HWE OF SNPs ON *RAB1A* GENE IN THE STUDY POPULATION

Loci	rs no.	Position	Genotype	Frequency	Heterozygosity	HWE ^a	
						AERD	ATA
-2232 G>T	rs 6748462	Promoter	G GT T N 523 558 114 1195	0.329	0.441	0.647	0.014
-1279 T>G	rs 2422437	Promoter	T GT G N 566 529 100 1195	0.305	0.424	0.715	0.060
+14444 T>C	rs 13409078	Intron1	T CT C N 995 192 7 1194	0.086	0.158	0.387	0.081
+15676 T>C	rs 17029863	Intron1	T CT C N 995 192 7 1194	0.086	0.158	0.387	0.081
+25391 G>T	rs 2302631	Intron2	G GT T N 509 553 129 1191	0.340	0.449	0.243	0.056
+28356 A>G	rs 10195328	Intron2	A AG G N 642 448 60 1190	0.255	0.308	0.148	0.021
+29857 A>C	rs 6725335	Intron2	A AC C N 642 448 60 1190	0.255	0.380	0.148	0.021
+41170 C>G	rs 1420184	Intron5	C CG G N 509 553 129 1191	0.340	0.449	0.243	0.056

^a*p*-Values of deviation from HWE in a Korean population.

MAF, minor allele frequency; SNP, single-nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium.

Associations of polymorphisms in the *RAB1A* gene with the risk of ASA intolerance in asthmatics

All of the polymorphisms of the *RAB1A* gene and haplotypes were analyzed for associations with the risk of ASA intolerance in asthmatic patients using multiple logistic re-

gression models (Table 4). The logistic analysis of the eight SNPs in the *RAB1A* gene between AERD (*n*=181) and ATA (*n*=1016) revealed that two SNPs on the intron (+14444 G>T, +41170 C>G) were associated with ASA hypersensitivity (*p*=0.0003–0.03, in the codominant and recessive model). The MAFs of +14444 G>T and +41170 G>T

FIG. 1. (A) Schematic gene map and single-nucleotide polymorphisms (SNPs) in the member RAS oncogene family (*RAB1A*) gene on chromosome 2p-14 (43 kb). Black blocks represent coding exons and white blocks represent 5' and 3'UTR. The first base of translation site was denoted as nucleotide +1. (B) Haplotype of *RAB1A*. (C) Linkage disequilibrium (LD) coefficient ($|D'|$) among *RAB1A* SNPs.

TABLE 4. LOGISTIC ANALYSIS FOR THE RISK OF ASPIRIN INTOLERANCE, CONTROLLING AGE, SEX, SMOKE STATUS, ATOPY, AND BMI, BETWEEN AERD AND ATA SUBJECTS

Loci	rs no.	Position	Geno-type	Distribution		Codominant			Dominant			Recessive		
				AERD	ATA	OR (95% CI)	p ^a	p ^{corr,b}	OR (95% CI)	p ^a	p ^{corr,b}	OR (95% CI)	p ^a	p ^{corr,b}
-2232 G>T	rs 6748462	Promoter	G	73 (40.6%)	450 (44.3%)									
			GT	81 (45.4%)	477 (47.0%)									
			T	26 (14.4%)	88 (8.7%)									
-1279 T>G	rs 2422437	Promoter	T	80 (44.2%)	486 (47.9%)									
			GT	79 (43.7%)	450 (44.4%)									
			G	22 (12.2%)	78 (7.7%)									
+14444 T>C	rs 13409078	Intron1	T	135 (74.6%)	860 (84.9%)									
			CT	41 (22.7%)	151 (14.9%)									
			C	5 (2.8%)	2 (0.2%)									
+29857 A>C	rs 6725335	Intron2	A	97 (53.6%)	545 (54.0%)									
			AC	76 (42.0%)	412 (40.8%)									
			C	8 (4.4%)	52 (5.2%)									
+41170 C>G	rs 1420184	Intron5	C	70 (38.7%)	439 (43.5%)									
			CG	79 (43.7%)	474 (46.9%)									
			G	32 (17.7%)	97 (9.6%)									

^ap-Values for logistic analyses controlling age, smoking status, atopy, and BMI as covariates with AERD patients assuming codominant, dominant, and recessive models.

^bp-Values after multiple testing corrections.

OR, odds ratio; CI, confidence interval; BMI, body mass index.

were significantly higher in the AERD group than in the ATA group, and the difference remained after correction for multiple comparisons ($p^{corr}=0.001-0.02$) (Table 2). The frequencies of the haplotypes in RAB1A were not different between the groups.

Because the ASA-induced decline in FEV₁ is the most important parameter for the diagnosis of ASA intolerance in asthmatics, we determined the association of the two SNPs with the rate of decline in FEV₁ following ASA challenge. Asthmatic patients carrying the minor allele of +14444 G>T showed a greater decline in FEV₁ in response to ASA provocation compared with those carrying the common allele (25.36±18.43% vs 6.94±10.73%, $p=0.0004$; Table 5). Heterozygotes showed the intermediate level of decline in FEV₁ between the homozygotes (9.09±13.47%). In addition, asthmatics carrying the minor allele of +41170 C>G showed a greater decline in FEV₁ compared with those carrying the common allele (10.03±14.13 vs 6.52±10.27%, $p=0.004$, Table 5). Heterozygotes showed the intermediate level of decline in FEV₁ between the homozygotes (7.62%±11.55%).

Discussion

The human RAB1A gene located on chromosome 2p14 contains six exons. The Ras-like Rab GTPases regulate vesicle transport in exocytosis, and cardiac rabs1, 4, and 6 are upregulated in a dilated cardiomyopathy model over-expressing β(2)-adrenergic receptors (Wu *et al.*, 2001). The results of the present study indicate that GTPase products are altered before and after exposure to ASA/NSAIDs in AERD compared with ATA. In the basal condition before the ASA challenge, metabolites of LTE₄ and RAB1A were increased in the urine and airways of AERD compared with ATA. ASA challenge induces an increase in the concentrations of cysteinyl leukotrienes and decreased concentrations of RAB1A in asthmatics (Mastalerz *et al.*, 2012). Based on these data, it is believed that changes to the synthesis of RAB1A are related to ASA hypersensitivity in asthma.

Rab GTPases, although they are the largest subfamily of Ras-like small G proteins, have been overlooked in this regard because they are not signaling molecules. Unlike other Ras-like G proteins, which are integral members of cell-signaling cascades, Rab GDP/GTP exchange regulates protein internalization and secretion by controlling vesicle docking and fusion throughout the cell (Somsel Rodman and Wandinger-Ness, 2000). These functions are critically important for all types of cellular growth, presumably including that which leads to NPs and hyperplastic eosinophilic sinusitis. The Ras superfamily of GTPases are members of the RAS oncogene family and are heavily expressed in various organs, including the bronchus of the rat lung and the peripheral blood cells, including eosinophils and platelets (Lacy *et al.*, 1995; Burgoyne and Morgan, 2003). Multiple points of overproduction or underproduction of critical inflammatory mediators may be determined by metabolism through the Ras family GTPase pathway (Ferro *et al.*, 2012). The release of specific granules from platelets, eosinophils, and neutrophils depends on the phosphorylation of the Rab proteins, including RAB1A, RAP2, RAB3B, RAB6 and RAB8 (Takai *et al.*, 2001).

The BL2 has six intronic SNPs, among which two (+14444 G>T and +41170 C>G) were associated with

TABLE 5. REGRESSION ANALYSIS FOR THE DECREASE OF FEV₁ % AFTER ASA INJECTION, CONTROLLING AGE, SEX, SMOKE STATUS, ATOPY, AND BMI, AMONG AERD AND ATA SUBJECTS

Loci	rs no.	C/C	C/R	R/R	pa*	pa ^{COT} **	pb*	pb ^{COT} **	pc*	pc ^{COT} **
-2232 G>T	rs 6748462	519 (6.73 ± 10.62)	557 (7.54 ± 11.61)	112 (9.49 ± 12.97)	0.03	0.11	0.10	0.35	0.05	0.18
-1279 T>G	rs 2422437	562 (6.73 ± 10.50)	528 (7.78 ± 11.98)	98 (9.13 ± 12.52)	0.04	0.13	0.06	0.21	0.16	0.57
+14444 T>C	rs 13409078	990 (6.94 ± 10.73)	190 (9.09 ± 13.47)	7 (25.36 ± 18.43)	0.0004	0.001	0.003	0.01	<0.0001	0.0003
+29857 A>C	rs 6725335	638 (7.05 ± 10.92)	486 (7.97 ± 12.17)	59 (6.79 ± 9.19)	0.40	1	0.24	0.83	0.68	1
+41170 C>G	rs 1420184	505 (6.52 ± 10.27)	553 (7.62 ± 11.55)	126 (10.03 ± 14.13)	0.004	0.01	0.02	0.08	0.01	0.04

*pa, p-values of the codominant model; *pb, dominant model; *pc, recessive model.

**p-Values after multiple testing corrections.

C/C, C/R, and R/R indicate the homozygote of the common allele, and the heterozygote and homozygote of the rare allele, respectively.
ASA, acetyl salicylic acid.

AERD in the present study. It is well known that intronic SNPs can affect transcript processing through alternative splicing or by producing different RNA secondary structures. Thus, we estimated the secondary structures of *RAB1A* mRNA possessing each alleles using Mfold Web Server (<http://mfold.rna.albany.edu/?q=mfold>). The free energy change (ΔG) of *RAB1A* mRNA containing the minor alleles of +14444 G>T and +41170 C>G was -78.72 kcal/mol, while that having the common allele was -76.10 kcal/mol. These suggest a possibility that these SNPs may affect the secondary structure of the *RAB1A* mRNA. In addition, we searched the splicing segments around +14444 G>T and +41170 C>G using a splicing prediction tool, Human Splicing Finder Version 2.4.1 (www.umd.be/HSF). +14444 G>T could not be analyzed using this tool because its location was far from the exon-intron boundary, but +41170 C>G in intron5 was predicted to be the third base upstream from the splicing branch point. There have been four transcription variants in the NCBI database. Among them, variant 2 is an alternative splicing form lacking exon 4 and 5. This suggests that the production of spliced variants of *RAB1A* might be affected by +41170C>G. However, the functional effects of these SNPs should be evaluated in the future.

It has been well known that the proportion of patients suffering from NPs is higher in AERD than in ATA (Fahrenholz, 2003). In the present study (Table 1), and our previous publication (Chang *et al.*, 2011), the proportion of patients with NP was significantly higher in Korean patients with AERD than in ATA subjects. Thus, the association of +14444 G>T and +41170 C>G with AERD in the present study could be a result of the association with NP. However, neither +14444 G>T nor +41170 C>G were associated with the presence of NP in the present study ($p>0.05$, Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/dna).

Conclusion

The present study investigated the associations of SNPs on the *RAB1A* gene with the risk of AERD. We genotyped eight human *RAB1A* SNPs and examined their links to AERD and the responsiveness of airways to ASA in asthmatic patients. Two SNPs (+14444 T>C and +41170 C>G) were associated with ASA hypersensitivity in asthma. The *RAB1A* gene may play a role in the development of AERD in asthmatics and the genetic polymorphisms of the gene have the potential to be used as an indicator of this disease.

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Disclosure Statement

No competing financial interests exist.

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