

## MECHANISMS

# Putative Association of *SMAPIL* Polymorphisms with Risk of Aspirin Intolerance in Asthmatics

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**Background.** Aspirin-intolerant asthma (AIA), as a clinical syndrome caused by aspirin, is characterized by lung inflammation and reversible bronchoconstriction. Recently, the altered trafficking and diminished airway reactivity have been implicated in allergic airway remodeling. The stromal membrane-associated protein 1-like (*SMAPIL*) exerts common and distinct functions in vesicle trafficking including endocytosis. The disturbance of pulmonary surfactant synthesis has been elucidated to be associated with asthma experimentally. Moreover, in alveolar type II (ATII) cells that synthesize pulmonary surfactant, alterations of clathrin-dependent endocytosis cause disturbance at the surfactant function, suggesting that *SMAPIL*, which directly interacts with clathrin, could be associated with asthma and related phenotypes. **Objective.** To verify our hypothesis that *SMAPIL* could play a role in the development of AIA, this study investigated associations between single-nucleotide polymorphisms (SNPs) of the *SMAPIL* gene and AIA. **Methods.** We conducted an association study between 19 SNPs of the *SMAPIL* gene and AIA in a total of 592 Korean subjects including 163 AIA and 429 aspirin-tolerant asthma (ATA) patients. Associations between polymorphisms of *SMAPIL* and AIA were analyzed with sex, smoking status, atopy, and body mass index as covariates. **Results.** Logistic analyses revealed that three common polymorphisms, *rs2982510*, *rs2294752*, and *rs446738*, were putatively associated with the increased susceptibility to AIA ( $p = .003$ ,  $p^{\text{corr}} = .004$ , OR = 0.24, 95% CI = 0.09–0.62 for *rs2982510* and *rs2294752*;  $p = .008$ ,  $p^{\text{corr}} = .03$ , OR = 0.44, 95% CI = 0.24–0.80 for *rs446738*, in the recessive model). In addition, *rs2982510* and *rs2294752* were significantly associated with the fall of forced expiratory volume in 1 s (FEV<sub>1</sub>) by aspirin provocation ( $p = .001$ ,  $p^{\text{corr}} = .04$  in the recessive model for both SNPs). **Conclusions.** Our findings suggest that *SMAPIL* might be a susceptible gene to AIA, providing a new strategy for the control of aspirin intolerance.

**Keywords** aspirin-intolerant asthma (AIA), polymorphism, stromal membrane-associated protein 1-like (*SMAPIL*)

## INTRODUCTION

Aspirin-intolerant asthma (AIA) is a clinical syndrome with acute bronchoconstriction in asthmatic individuals following the ingestion of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) (1). This distinct clinical syndrome is characterized by symptoms of eosinophilic rhinosinusitis, nasal polyposis, aspirin sensitivity, and asthma (2). AIA consists of 5–15% of asthmatics, and occurs more frequently in women (3).

Recently, asthma is considered to be caused not only by a disorder of the smooth muscle function, but also

by a new airway remodeling for the pathogenesis of asthma (4). In addition, particular attention has been given to the airway's inflammatory response. Moreover, altered trafficking and diminished airway reactivity have been implicated in allergic airway remodeling (5). A number of intracellular signaling proteins are implicated in various aspects of the inflammatory processes that regulate asthma pathogenesis, and these proteins are thus targets for therapeutic intervention. Many factors associated with the inflammation reaction involve a protein invasion of the airway, which disturbs the surfactant function. The pulmonary surfactant synthesis activity is decreased in experimentally induced asthma when the 55 intracellular storage capacity and its physical activity are hindered in the induced asthma (6–8). Alveolar type II (ATII) cells synthesize pulmonary surfactant which is a surface-active lipoprotein complex. Several papers reported that a lot of hormones, pharmacological agents, and physicochemical factors increase or decrease

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pulmonary surfactant synthesis in ATII cells (9–11). This endocytosis in ATII cells was shown to be involved in the recycling of lamellar bodies (LB) associated proteins or in surfactant uptake from the extracellular space (12, 13). These results indicate that the disturbed endocytosis can have an influence on the interruption of the surfactant function, suggesting that endocytosis might be associated with asthma development.

The stromal membrane-associated protein 1-like (SMAP1L), also known as SMAP2, exhibits GTPase-activating protein (GAP) activity and interacts with clathrin heavy chain and clathrin assembly lymphoid-myeloid (CALM). SMAP1L co-localizes with the adaptor proteins for clathrin AP-1 and EpsinR on the early endosomes/trans-Golgi network (TGN). Moreover, the over expression of SMAP1L delays the accumulation of TGN38/46 molecule on the TGN. These facts suggest that SMAP1L functions in the retrograde, early endosome-to-TGN pathway in a clathrin- and AP-1-dependent manner. In addition, it is known that SMAP1L exerts both common and distinct functions in vesicle trafficking (14). Therefore, we hypothesized that polymorphisms of *SMAP1L* could affect aspirin hypersensitivity in asthmatics, and then performed a genetic association analysis of *SMAP1L* polymorphisms with AIA compared with aspirin-tolerant asthma (ATA) as a control group.

## SUBJECTS AND METHODS

### Study Subjects

This study was conducted in compliance with the Global Initiative for Asthma (GINA) Global Strategy for Asthma Management and Prevention study. Subjects were recruited from the Asthma Genome Research Center comprising hospitals of Soonchunhyang, Chunnam, Chungbuk, and Seoul National Universities in Korea. All subjects provided an informed consent, and the protocols were approved by the Institutional Review Board of each hospital. Each patient showed airway reversibility defined as a 15% improvement in forced expiratory volume in 1 sec (FEV<sub>1</sub>) or airway hyperresponsiveness defined as a 20% drop in FEV<sub>1</sub> in response to a methacholine dose <10 mg/ml (PC20). Oral aspirin challenge was performed in all asthmatic subjects using methods that were slightly modified from those described previously (15, 16). Subjects showing a decline rate of FEV<sub>1</sub> less than 15% and without extra-bronchial nasal or skin symptoms were included in ATA group. Subjects with hypertension/diabetes or those taking angiotensin-converting enzyme (ACE) inhibitors were excluded from this study.

### SNP Selection and Genotyping

We selected candidate single-nucleotide polymorphisms (SNPs) showing polymorphism in the National Center for Biotechnology Information database (build 36) and then genotyped SNPs of *SMAP1L* in 163 AIA and 429 ATA subjects. Genomic DNA was extracted from peripheral blood lymphocytes using Gentra Puregene kit

(Minneapolis, MN, USA), according to the manufacturer's protocol. Genotyping was performed with 20 ng of genomic DNA by TaqMan assay in the ABI PRISM 7900HT sequence-detection system (SDS; Applied Biosystems, Carlsbad, CA, USA). Genotyping data were obtained using the ABI PRISM SDS software version 2.3. The genotype quality score for keeping data was set at 0.25. A total of 19 SNPs on *SMAP1L* were successfully genotyped.

### Statistics

We examined Lewontin's  $D'$  ( $|D'|$ ) and the linkage disequilibrium (LD) coefficient  $r^2$  between all pairs of biallelic loci (17). LD was obtained using Haploview version 4.1 software downloaded from the Broad Institute (<http://www.broadinstitute.org/mpg/haploview>) (18). Haplotypes were first estimated using PHASE software (19), and then computed by logistic analyses using SAS version 9.1 (SAS Inc., Cary, NC, USA). Subjects with missing genotypes were omitted in the analysis of individual SNPs and haplotypes. All 19 SNPs were perfectly genotyped. The genotype and haplotype distributions were analyzed using logistic regression models with age (continuous value), gender (male = 0, female = 1), smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2), atopy (absence = 0, presence = 1), and BMI (continuous value) as covariates. The differences in the rates of decline in FEV<sub>1</sub> following aspirin challenge among the genotypes and haplotypes were examined using a linear regression model. The data were also managed and analyzed by using SAS version 9.1. To correct for multiple testing, permutation test with HelixTree software (Golden Helix, Inc., Bozeman, MT, USA) was performed. A total of 10,000 permutations were run on each SNP.

## RESULTS

### Subject Characteristics

There were a total of 592 asthma patients recruited for the study; among them, 163 of the subjects were AIA patients and 429 were ATA patients (Table 1). From the table, the fall rate by aspirin provocation in AIA patients (24.63%) showed a higher fall rate than that of ATA controls (3.54%) ( $p < .0001$ , Table 1). Besides fall rate of FEV<sub>1</sub> by aspirin provocation, there were also other characteristics of AIA patients that showed significant deviations from those of ATA patients, namely, age of onset, body mass index, predicted FEV<sub>1</sub>%, PC20 methacholine, and population percentage of smokers. The difference in percentage of smokers was especially notable as there were significantly less AIA patients who smoke (21.47%) than ATA patients (30.07%).

### Genotyping and Frequency of SNPs in the *SMAP1L* Gene

A total of 19 non-monomorphic SNPs of the *SMAP1L* gene were genotyped in 592 Korean subjects including 163 AIA patients and 429 ATA controls. One SNP

TABLE 1.—Characteristics of study subjects.

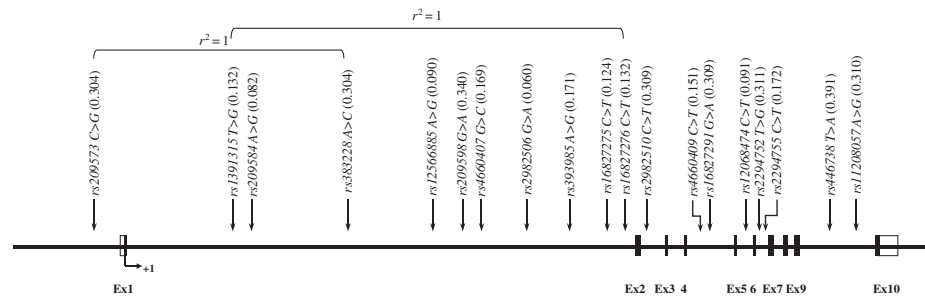
| Clinical profile                                                      | Asthmatics (all subjects) | AIA (n = 163)        | ATA (n = 429)       |
|-----------------------------------------------------------------------|---------------------------|----------------------|---------------------|
| Age of first medical examination (range)                              | 46.15 (15.40–77.88)       | 43.13 (17.22–72.73)* | 47.30 (15.40–77.88) |
| Body mass index (kg/m <sup>2</sup> )                                  | 24.24 ± 3.39              | 23.39 ± 3.25*        | 24.58 ± 3.39        |
| Fall rate of FEV <sub>1</sub> by aspirin provocation (%) <sup>a</sup> | 9.27 ± 13.24              | 24.63 ± 16.11**      | 3.54 ± 4.85         |
| Blood eosinophil (%) <sup>a</sup>                                     | 6.01 ± 5.73               | 5.96 ± 5.21          | 6.03 ± 5.92         |
| FVC%, predicted <sup>a</sup>                                          | 88.54 ± 14.08             | 90.35 ± 14.04        | 87.85 ± 14.05       |
| FEV <sub>1</sub> %, predicted                                         | 90.54 ± 16.97             | 87.58 ± 16.94*       | 91.66 ± 16.87       |
| PC20, methacholine (mg/mL) <sup>a</sup>                               | 6.43 ± 8.67               | 5.02 ± 7.83*         | 6.91 ± 8.90         |
| Total IgE (IU/mL) <sup>a</sup>                                        | 357.65 ± 604.09           | 348.60 ± 596.44      | 361.00 ± 607.56     |
| Sex (male/female)                                                     | 206/386                   | 59/104               | 147/282             |
| Smoker (current smoker/ex-smoker) (%)                                 | 27.70 (12.5/15.2)         | 21.47 (12.88/8.59)*  | 30.07 (12.35/17.72) |
| Positive rate of skin test (%)                                        | 56.42                     | 52.76                | 57.81               |

Note. Each clinical profile of AIA was compared to the respective ATA controls.

AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 s.

\* $p < .05$ ; \*\* $p < .0001$  compared to ATA control.

<sup>a</sup>This value was presented as mean ± SD.

(A) Map of *SMAPIL* (stromal membrane-associated protein 1-like) on chromosome 1p35.3-p34.1 (49kb)(B) Haplotypes in *SMAPIL*

| Hap    | rs209573 C>G | rs1391313 T>G | rs209584 A>G | rs382228 A>C | rs2566885 A>G | rs209596 G>A | rs4660407 G>C | rs2982510 G>A | rs393985 A>G | rs1682725 C>T | rs1682726 C>T | rs2982510 C>T | rs4660409 C>T | rs1682729 T>G | rs1206874 C>T | rs294752 T>G | rs294753 C>T | rs446738 T>A | rs1208057 A>G | Freq. |
|--------|--------------|---------------|--------------|--------------|---------------|--------------|---------------|---------------|--------------|---------------|---------------|---------------|---------------|---------------|---------------|--------------|--------------|--------------|---------------|-------|
| ht1    | C            | T             | A            | A            | G             | G            | G             | A             | C            | C             | C             | C             | C             | C             | C             | T            | T            | G            | G             | 0.288 |
| ht2    | G            | T             | A            | C            | A             | G            | G             | G             | C            | C             | C             | T             | T             | T             | T             | T            | T            | A            | A             | 0.167 |
| ht3    | C            | T             | A            | A            | A             | C            | G             | A             | C            | C             | T             | T             | G             | C             | G             | C            | A            | A            | A             | 0.155 |
| ht4    | G            | G             | A            | C            | A             | G            | G             | A             | T            | T             | C             | C             | C             | C             | C             | T            | T            | A            | A             | 0.123 |
| ht5    | C            | T             | A            | A            | G             | G            | G             | A             | C            | C             | C             | C             | C             | G             | T             | G            | C            | A            | A             | 0.088 |
| ht6    | C            | T             | G            | A            | A             | G            | G             | A             | C            | C             | C             | C             | C             | C             | C             | T            | C            | A            | A             | 0.080 |
| ht7    | C            | T             | A            | A            | A             | G            | G             | A             | C            | C             | C             | C             | C             | G             | C             | G            | C            | A            | A             | 0.049 |
| ht8    | C            | T             | A            | A            | A             | C            | G             | A             | C            | C             | C             | C             | C             | C             | C             | G            | C            | A            | A             | 0.016 |
| ht9    | C            | T             | A            | A            | A             | G            | G             | A             | C            | C             | C             | C             | C             | C             | C             | A            | C            | T            | G             | 0.009 |
| ht10   | G            | G             | A            | C            | A             | G            | G             | A             | C            | C             | T             | C             | C             | C             | C             | T            | C            | T            | A             | 0.005 |
| Others | -            | -             | -            | -            | -             | -            | -             | -             | -            | -             | -             | -             | -             | -             | -             | -            | -            | -            | -             | 0.020 |

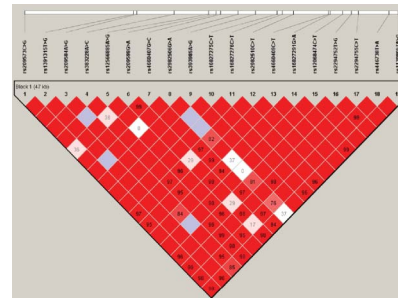
(C) LDs among *SMAPIL* polymorphisms

FIGURE 1.—Physical map, haplotypes, and LD of the *SMAPIL* gene. (A) Schematic gene map and SNPs in the *SMAPIL* gene on chromosome 1p35.3–p34.1. Black blocks represent coding exons and white blocks represent 5' and 3'-untranslated region. The first base of translation site was denoted as nucleotide +1. (B) Haplotypes of *SMAPIL*. (C) LD coefficient ( $|D'|$ ) among *SMAPIL* SNPs in a Korean population.

(*rs209573*) is positioned in the promoter region and others in intron regions (Figure 1A, Table 2). All 19 SNPs were perfectly genotyped, so they were analyzed with no bias for the association study. The frequencies of these 19 SNPs in the Korean asthmatics are shown in Table 2, and the genotype distributions of all loci were in Hardy–Weinberg equilibrium ( $p > .05$ ). After haplotypes of each individual were inferred using the algorithm of PHASE (version 2.0), six haplotypes with frequencies higher than 0.05 were selected for analysis (Figure 1B). The LD coefficients ( $|D'|$ ) and  $r^2$  among *SMAPIL* polymorphisms were calculated for all of the study subjects (Figure 1C). Two sets of SNPs in *SMAPIL* were in absolute LD ( $|D'| = 1$  and  $r^2 = 1$ ) (Figure 1A).

### Association of *SMAPIL* Polymorphisms with the Risk of AIA

Nineteen SNPs and six haplotypes were analyzed for the association with the risk of AIA using multiple logistic analyses adjusted for initial diagnosed age, sex, smoking status, and atopy (Table 3). In the association analysis, several SNPs showed significant associations with aspirin intolerance. However, most associations disappeared after correcting for multiple testing. Three SNPs (*rs2982510*, *rs2294752*, and *rs446738*) retained significances in the recessive model after correction for multiple testing ( $p = .003$ ,  $p_{\text{corr}} = .004$ , OR = 0.24, 95% CI = 0.09–0.62 for *rs2982510* and *rs2294752*;  $p = .008$ ,  $p_{\text{corr}} = .03$ , OR = 0.44, 95% CI = 0.24–0.80 for *rs446738*, in the

TABLE 2.—SNPs of human *SMAPIL* gene discovered in Korean subjects.

| SNP ID            | Allele | Position | Frequency | Heterozygosity | HWE   |
|-------------------|--------|----------|-----------|----------------|-------|
| <i>rs209573</i>   | C>G    | Promoter | 0.304     | 0.423          | 0.313 |
| <i>rs1391315</i>  | T>G    | Intron1  | 0.132     | 0.229          | 0.513 |
| <i>rs209584</i>   | A>G    | Intron1  | 0.082     | 0.151          | 0.900 |
| <i>rs383228</i>   | A>C    | Intron1  | 0.304     | 0.423          | 0.313 |
| <i>rs12566885</i> | A>G    | Intron1  | 0.090     | 0.164          | 0.131 |
| <i>rs209598</i>   | G>A    | Intron1  | 0.340     | 0.449          | 0.180 |
| <i>rs4660407</i>  | G>C    | Intron1  | 0.169     | 0.281          | 0.250 |
| <i>rs2982506</i>  | G>A    | Intron1  | 0.060     | 0.113          | 0.593 |
| <i>rs393985</i>   | A>G    | Intron1  | 0.171     | 0.283          | 0.935 |
| <i>rs16827275</i> | C>T    | Intron1  | 0.124     | 0.217          | 0.827 |
| <i>rs16827276</i> | C>T    | Intron1  | 0.132     | 0.229          | 0.513 |
| <i>rs2982510</i>  | C>T    | Intron2  | 0.309     | 0.427          | 0.682 |
| <i>rs4660409</i>  | C>T    | Intron4  | 0.151     | 0.256          | 0.394 |
| <i>rs16827291</i> | G>A    | Intron4  | 0.309     | 0.427          | 0.332 |
| <i>rs12068474</i> | C>T    | Intron5  | 0.091     | 0.166          | 0.123 |
| <i>rs2294752</i>  | T>G    | Intron6  | 0.311     | 0.428          | 0.770 |
| <i>rs2294755</i>  | C>T    | Intron6  | 0.172     | 0.284          | 0.897 |
| <i>rs446738</i>   | T>A    | Intron9  | 0.391     | 0.476          | 0.802 |
| <i>rs11208057</i> | A>G    | Intron9  | 0.310     | 0.428          | 0.350 |

Note. HWE, Hardy-Weinberg equilibrium.

TABLE 3.—Analyses of association of *SMAPIL* polymorphisms with risk of aspirin intolerance in asthma.

| SNP or haplotype      | Frequency <sup>a</sup> |       | Codominant       |             |                            | Dominant         |            |                            | Recessive        |             |                            |
|-----------------------|------------------------|-------|------------------|-------------|----------------------------|------------------|------------|----------------------------|------------------|-------------|----------------------------|
|                       | AIA                    | ATA   | OR (95% CI)      | <i>p</i> *  | <i>p</i> <sup>corr**</sup> | OR (95% CI)      | <i>p</i> * | <i>p</i> <sup>corr**</sup> | OR (95% CI)      | <i>p</i> *  | <i>p</i> <sup>corr**</sup> |
| <i>rs209573</i> C>G   | 0.319                  | 0.296 | 1.09 (0.8–1.44)  | .52         | 1.00                       | 1.08 (0.75–1.56) | .66        | 1.00                       | 1.24 (0.68–2.24) | .49         | 1.00                       |
| <i>rs1391315</i> T>G  | 0.120                  | 0.132 | 0.90 (0.60–1.35) | .62         | 1.00                       | 0.87 (0.57–1.34) | .53        | 1.00                       | 1.47 (0.27–7.98) | .66         | 1.00                       |
| <i>rs209584</i> A>G   | 0.096                  | 0.078 | 1.19 (0.76–1.87) | .45         | 1.00                       | 1.22 (0.75–1.98) | .42        | 1.00                       | 0.91 (0.09–9.20) | .94         | 1.00                       |
| <i>rs383228</i> A>C   | 0.319                  | 0.296 | 1.09 (0.8–1.44)  | .52         | 1.00                       | 1.08 (0.75–1.56) | .66        | 1.00                       | 1.24 (0.68–2.24) | .49         | 1.00                       |
| <i>rs12566885</i> A>G | 0.069                  | 0.099 | 0.66 (0.40–1.09) | .10         | .58                        | 0.67 (0.40–1.11) | .12        | .67                        |                  | .98         | 1.00                       |
| <i>rs209598</i> G>A   | 0.352                  | 0.339 | 1.05 (0.81–1.37) | .71         | 1.00                       | 1.03 (0.71–1.48) | .89        | 1.00                       | 1.17 (0.68–1.99) | .57         | 1.00                       |
| <i>rs4660407</i> G>C  | 0.154                  | 0.176 | 0.87 (0.62–1.24) | .45         | .99                        | 0.92 (0.61–1.37) | .68        | 1.00                       | 0.47 (0.1–1.63)  | .23         | .69                        |
| <i>rs2982506</i> G>A  | 0.033                  | 0.069 | 0.44 (0.2–0.87)  | <b>.02</b>  | .10                        | 0.44 (0.22–0.88) | <b>.02</b> | .13                        |                  | .98         | 1.00                       |
| <i>rs393985</i> A>G   | 0.199                  | 0.163 | 1.24 (0.89–1.74) | .20         | .89                        | 1.28 (0.87–1.88) | .21        | .91                        | 1.35 (0.47–3.93) | .58         | 1.00                       |
| <i>rs16827275</i> C>T | 0.111                  | 0.125 | 0.89 (0.59–1.35) | .58         | 1.00                       | 0.86 (0.55–1.33) | .49        | 1.00                       | 1.47 (0.27–7.98) | .66         | 1.00                       |
| <i>rs16827276</i> C>T | 0.120                  | 0.132 | 0.90 (0.60–1.35) | .62         | 1.00                       | 0.87 (0.57–1.34) | .53        | 1.00                       | 1.47 (0.27–7.98) | .66         | 1.00                       |
| <i>rs2982510</i> C>T  | 0.253                  | 0.332 | 0.68 (0.51–0.92) | <b>.01</b>  | .08                        | 0.76 (0.5–1.10)  | .14        | .73                        | 0.24 (0.09–0.62) | <b>.003</b> | <b>.004</b>                |
| <i>rs4660409</i> C>T  | 0.133                  | 0.160 | 0.82 (0.56–1.18) | .28         | .89                        | 0.85 (0.56–1.28) | .43        | .99                        | 0.38 (0.08–1.72) | .21         | .63                        |
| <i>rs16827291</i> G>A | 0.337                  | 0.297 | 1.22 (0.9–1.60)  | .15         | .67                        | 1.27 (0.88–1.83) | .20        | .78                        | 1.36 (0.76–2.43) | .29         | .93                        |
| <i>rs12068474</i> C>T | 0.069                  | 0.100 | 0.65 (0.39–1.08) | .09         | .52                        | 0.66 (0.39–1.10) | .11        | .62                        |                  | .98         | 1.00                       |
| <i>rs2294752</i> T>G  | 0.253                  | 0.334 | 0.67 (0.50–0.91) | <b>.009</b> | .07                        | 0.75 (0.52–1.08) | .12        | .67                        | 0.24 (0.09–0.62) | <b>.003</b> | <b>.004</b>                |
| <i>rs2294755</i> C>T  | 0.196                  | 0.164 | 1.21 (0.86–1.69) | .27         | .95                        | 1.23 (0.84–1.81) | .29        | .97                        | 1.35 (0.47–3.93) | .58         | 1.00                       |
| <i>rs446738</i> T>A   | 0.349                  | 0.409 | 0.77 (0.58–1.01) | .06         | .31                        | 0.87 (0.60–1.27) | .47        | .99                        | 0.44 (0.24–0.80) | <b>.008</b> | <b>.03</b>                 |
| <i>rs11208057</i> A>G | 0.339                  | 0.296 | 1.23 (0.94–1.62) | .13         | .62                        | 1.29 (0.89–1.86) | .17        | .72                        | 1.37 (0.77–2.45) | .29         | .93                        |
| <i>SMAPIL_h1</i>      | 0.328                  | 0.276 | 1.31 (0.99–1.73) | .06         | .32                        | 1.37 (0.95–1.97) | .09        | .48                        | 1.55 (0.85–2.83) | .15         | .72                        |
| <i>SMAPIL_h2</i>      | 0.196                  | 0.161 | 1.24 (0.88–1.73) | .22         | .91                        | 1.27 (0.86–1.87) | .23        | .93                        | 1.35 (0.47–3.93) | .58         | 1.00                       |
| <i>SMAPIL_h3</i>      | 0.130                  | 0.160 | 0.79 (0.55–1.15) | .23         | .82                        | 0.82 (0.54–1.25) | .35        | .97                        | 0.38 (0.08–1.72) | .21         | .63                        |
| <i>SMAPIL_h4</i>      | 0.105                  | 0.125 | 0.84 (0.55–1.28) | .41         | .99                        | 0.80 (0.51–1.25) | .33        | .98                        | 1.47 (0.27–7.98) | .66         | 1.00                       |
| <i>SMAPIL_h5</i>      | 0.069                  | 0.097 | 0.68 (0.41–1.12) | .13         | .66                        | 0.69 (0.41–1.15) | .15        | .76                        |                  | .98         | 1.00                       |
| <i>SMAPIL_h6</i>      | 0.096                  | 0.075 | 1.25 (0.80–1.97) | .33         | .99                        | 1.30 (0.80–2.11) | .29        | .99                        | 0.91 (0.09–9.20) | .94         | 1.00                       |

AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; OR, odds ratio; CI, confidence interval; *p*<sup>corr</sup>, corrected *p*-value. *p*-values below 0.05 are in bold to highlight the significance.

\**P*-values of logistic analysis controlling sex, smoking status, and atopy as covariates.

\*\**P*-value corrected for the multiple testing using permutation test (*n* = 10,000).

<sup>a</sup>Frequency of SNP indicates the minor allele frequency.

recessive model). The minor allele frequencies of these SNPs were lower in aspirin-intolerant asthmatics than those of aspirin-tolerant asthmatics.

As the percentage FEV<sub>1</sub> fall after aspirin provocation is the most important parameter for the diagnosis of aspirin intolerance in asthmatics, we investigated the associations

of the percentages of FEV<sub>1</sub> fall following aspirin provocation with genotypes or haplotypes using multiple regression analysis (Table 4). Two SNPs (*rs2982510* and *rs2294752*) were also associated with the percentage FEV<sub>1</sub> fall induced by aspirin provocation in the asthmatics after correction for multiple testing (*p* = .001,

TABLE 4.—Regression analysis for the association of *SMAPIL* with the decline of FEV<sub>1</sub> by aspirin provocation.

| SNP or haplotype         | C/C                 | C/R                 | R/R                | <i>Pa</i> * | <i>Pa</i> <sup>corr**</sup> | <i>Pb</i> * | <i>Pb</i> <sup>corr**</sup> | <i>Pc</i> * | <i>Pc</i> <sup>corr**</sup> |
|--------------------------|---------------------|---------------------|--------------------|-------------|-----------------------------|-------------|-----------------------------|-------------|-----------------------------|
| <i>rs209573 C&gt;G</i>   | 293 (8.48 ± 12.21)  | 242 (9.95 ± 14.38)  | 57 (10.07 ± 13.07) | .23         | .85                         | .18         | .78                         | .67         | 1.00                        |
| <i>rs1391315 T&gt;G</i>  | 447 (8.75 ± 12.81)  | 138 (10.79 ± 14.39) | 7 (9.46 ± 15.02)   | .14         | .68                         | .14         | .69                         | .72         | 1.00                        |
| <i>rs209584 A&gt;G</i>   | 498 (8.95 ± 12.78)  | 90 (10.99 ± 15.54)  | 4 (4.93 ± 7.73)    | .44         | 1.00                        | .33         | .98                         | .54         | 1.00                        |
| <i>rs383228 A&gt;C</i>   | 293 (8.48 ± 12.21)  | 242 (9.95 ± 14.38)  | 57 (10.07 ± 13.07) | .23         | .85                         | .18         | .78                         | .67         | 1.00                        |
| <i>rs12566885 A&gt;G</i> | 486 (9.63 ± 13.47)  | 104 (7.50 ± 12.02)  | 2 (3.00 ± 1.41)    | .14         | .77                         | .15         | .83                         | .58         | 1.00                        |
| <i>rs209598 G&gt;A</i>   | 263 (9.72 ± 13.41)  | 254 (8.95 ± 13.32)  | 75 (8.47 ± 12.25)  | .38         | .99                         | .39         | .99                         | .61         | 1.00                        |
| <i>rs4660407 G&gt;C</i>  | 412 (9.56 ± 13.51)  | 159 (9.08 ± 13.03)  | 21 (4.01 ± 6.23)   | .19         | .83                         | .37         | .99                         | .10         | .55                         |
| <i>rs2982506 G&gt;A</i>  | 524 (9.43 ± 13.38)  | 66 (8.01 ± 11.93)   | 2 (−0.55 ± 9.26)   | .21         | .86                         | .27         | .94                         | .28         | .97                         |
| <i>rs393985 A&gt;G</i>   | 405 (9.19 ± 13.06)  | 171 (9.14 ± 13.51)  | 16 (11.29 ± 14.82) | .84         | 1.00                        | .94         | 1.00                        | .66         | 1.00                        |
| <i>rs16827275 C&gt;T</i> | 456 (9.00 ± 13.22)  | 129 (10.05 ± 13.19) | 7 (9.46 ± 15.02)   | .43         | .98                         | .44         | .99                         | .72         | 1.00                        |
| <i>rs16827276 C&gt;T</i> | 447 (8.75 ± 12.81)  | 138 (10.79 ± 14.39) | 7 (9.46 ± 15.02)   | .14         | .68                         | .14         | .69                         | .72         | 1.00                        |
| <i>rs2982510 C&gt;T</i>  | 279 (10.13 ± 13.81) | 259 (9.44 ± 13.35)  | 54 (3.64 ± 6.61)   | <b>.01</b>  | .12                         | .15         | .83                         | <b>.001</b> | <b>.04</b>                  |
| <i>rs4660409 C&gt;T</i>  | 428 (9.60 ± 13.43)  | 148 (8.78 ± 13.05)  | 16 (3.56 ± 6.65)   | .15         | .75                         | .28         | .95                         | .11         | .62                         |
| <i>rs16827291 G&gt;A</i> | 285 (8.49 ± 12.56)  | 247 (9.98 ± 14.29)  | 60 (9.69 ± 11.63)  | .24         | .94                         | .17         | .89                         | .75         | 1.00                        |
| <i>rs12068474 C&gt;T</i> | 485 (9.66 ± 13.45)  | 105 (7.36 ± 12.07)  | 2 (3.00 ± 1.41)    | .11         | .68                         | .11         | .74                         | .58         | 1.00                        |
| <i>rs2294752 T&gt;G</i>  | 277 (10.14 ± 13.86) | 261 (9.43 ± 13.30)  | 54 (3.64 ± 6.61)   | <b>.01</b>  | .12                         | .15         | .83                         | <b>.001</b> | <b>.04</b>                  |
| <i>rs2294755 C&gt;T</i>  | 404 (9.23 ± 13.05)  | 172 (9.06 ± 13.52)  | 16 (11.29 ± 14.82) | .91         | 1.00                        | .98         | 1.00                        | .66         | 1.00                        |
| <i>rs446738 T&gt;A</i>   | 214 (9.96 ± 13.16)  | 292 (9.61 ± 13.92)  | 86 (6.16 ± 10.31)  | <b>.05</b>  | .43                         | .36         | .99                         | <b>.01</b>  | .20                         |
| <i>rs11208057 A&gt;G</i> | 285 (8.36 ± 12.43)  | 246 (10.02 ± 14.30) | 60 (9.69 ± 11.63)  | .20         | .90                         | .13         | .83                         | .74         | 1.00                        |
|                          | +/+                 | +/-                 | -/-                | <i>Pa</i> * | <i>Pa</i> <sup>corr**</sup> | <i>Pb</i> * | <i>Pb</i> <sup>corr**</sup> | <i>Pc</i> * | <i>Pc</i> <sup>corr**</sup> |
| <i>SMAPIL_h1</i>         | 300 (8.63 ± 13.06)  | 238 (9.85 ± 13.70)  | 54 (9.87 ± 11.95)  | .25         | .95                         | .23         | .95                         | .61         | 1.00                        |
| <i>SMAPIL_h2</i>         | 407 (9.19 ± 13.02)  | 169 (9.15 ± 13.59)  | 16 (11.29 ± 14.82) | .84         | 1.00                        | .94         | 1.00                        | .66         | 1.00                        |
| <i>SMAPIL_h3</i>         | 428 (9.60 ± 13.43)  | 148 (8.78 ± 13.05)  | 16 (3.56 ± 6.65)   | .15         | .75                         | .28         | .95                         | .11         | .62                         |
| <i>SMAPIL_h4</i>         | 457 (9.07 ± 13.28)  | 128 (9.82 ± 12.98)  | 7 (9.46 ± 15.02)   | .55         | 1.00                        | .58         | 1.00                        | .72         | 1.00                        |
| <i>SMAPIL_h5</i>         | 488 (9.62 ± 13.44)  | 102 (7.51 ± 12.14)  | 2 (3.00 ± 1.41)    | .14         | .77                         | .15         | .84                         | .58         | 1.00                        |
| <i>SMAPIL_h6</i>         | 501 (8.91 ± 12.77)  | 87 (11.32 ± 15.66)  | 4 (4.93 ± 7.73)    | .32         | .97                         | .23         | .92                         | .54         | 1.00                        |

C/C, C/R, and R/R indicate the homozygote of common allele, and the heterozygote and homozygote of rare allele, respectively. Fall rate values are mean ± SD. *p*-values below 0.05 are in bold to highlight the significance.

\**P*-values of regression analysis represent the codominant (*Pa*), dominant (*Pb*), and recessive (*Pc*) models controlling sex, smoking status, and atopy as covariates.

\*\**P*-value corrected for the multiple testing using permutation test (*n* = 10,000).

$p^{\text{corr}} = .04$  in the recessive model for both the SNPs). The asthmatic patients who carried the minor allele homozygote for *rs2982510* and/or *rs2294752* showed a decreased fall in FEV<sub>1</sub> induced by aspirin provocation than those who carried heterozygote or major allele homozygote. In the haplotype association analysis with the FEV<sub>1</sub> decrease, no associations were found.

## DISCUSSION

The *SMAPIL* gene has been recently discovered, therefore its comprehensive functions and mechanisms are still unknown. The *SMAPIL* gene is a homolog of the *SMAP1*, which directly interacts with clathrin and regulates the clathrin-dependent endocytosis of transferrin receptors from the plasma membrane (14). The *SMAPIL* is associated with endocytosis, which is important to molecular secretion that is also associated with many diseases including asthma. Therefore, considering the direct interaction of *SMAPIL* with clathrin, polymorphisms of *SMAPIL* could be associated with the development of asthma and related phenotypes such as AIA.

In ATII cells, endocytosis was shown to be involved in surfactant uptake depending predominantly on clathrin (12, 13). In addition, there is evidence that Ca<sup>2+</sup> could be associated with endocytosis in epithelial cell types. In addition, Ca<sup>2+</sup> has been shown to mediate the formation of protein complexes, which initiate clathrin accumulation at the site of internalization in different cell types

(20, 21). Moreover, changes of the concentration of Ca<sup>2+</sup> in epithelioid cells demonstrate that they modulate the protein composition of plasma membrane by regulating endocytosis. Furthermore, the elevation of the concentration of Ca<sup>2+</sup> in ATII cells triggers not only exocytosis but also an opposing endocytotic mechanism (22). Finally, ATII cells sense mechanical stress and respond by Ca<sup>2+</sup>-dependent release of surfactant. These facts suggest that the interruption of endocytosis leads to disturbance of the surfactant function. Therefore, we hypothesized that *SMAPIL*, which directly interacts with clathrin, could be associated with aspirin hypersensitivity in asthmatics.

Interestingly, the 19 SNPs investigated in this study showed strong LDs among each other (Figure 1C). According to the logistic analyses for susceptibility of *SMAPIL* polymorphism to AIA, three polymorphisms that showed significant associations with aspirin hypersensitivity in asthmatics were initially found. However, after corrections for multiple testing, only three common polymorphisms (*rs2982510*, *rs2294752*, and *rs446738*) were associated with the increased susceptibility to the risk of aspirin intolerance in asthma. Consistent with the case-control association, minor allele homozygotes of *rs2982510* and *rs1194752* also affected the decrease in the maximum fall of FEV<sub>1</sub> induced by aspirin provocation significantly even after multiple testing corrections ( $p^{\text{corr}} = .04$  in both of them). From these results, it is suggested that *rs2982510* and *rs2294752* polymorphisms of

the *SMAPIL* gene may be associated with aspirin intolerance in asthmatics. Two SNPs that were discovered to be associated with AIA are located in the introns 2 and 6 of the gene. Although further functional studies are required to establish whether these SNPs have influence on the expression and/or function of *SMAPIL*, it is suggested that their location in the intronic region could affect overall transcription in different ways. Recently, studies showed that introns can affect transcription efficiency (23), and variations in intronic region can lead to abnormal splicing, which in turn may lead to human diseases (24). It has been also reported that a SNP located in the intronic region showed an association with asthma (25). Therefore, it is possible that *rs2982510* and *rs2294752* of the *SMAPIL* gene affected the onset of AIA by affecting the transcription procedure.

To observe the associations depending on the severity of aspirin hypersensitivity, intermediate AIA (AIA-I) patients who showed a lower rate (%) of FEV<sub>1</sub> decline (15–19%) and severe AIA (AIA-Y) patients with 20% or greater decreases in FEV<sub>1</sub> were additionally classified. The *rs2982506* showed evidence of a significant frequency difference between the three groups. ( $p = .02$  under codominant model, Supplementary Table 1, available online at [http://informahealthcare.com/doi/suppl/\[doinumber\]](http://informahealthcare.com/doi/suppl/[doinumber])). However, because of the infrequency of *rs2982506* and small sample size of AIA-I, further replications in a larger-scale group of subjects are needed. For other SNPs, although there were no significant differences in allele frequencies between AIA-I and AIA-Y, three SNPs (*rs2982510*, *rs2294752*, and *rs446738*) still showed significant associations (Supplementary Table 1). This observation suggests that combinations of AIA-I and AIA-Y may be important to investigate in future association studies.

The mechanisms and pathways of the aspirin hypersensitivity in asthmatics are not fully understood and seem to be intricate because genetic variations of several other genes have been reported to have a susceptibility to AIA. Cysteinyl-leukotriene receptor 2 gene (*CYSLTR2*), membrane-spanning 4-domains, subfamily A, member 2 gene (*MS4A2*), peroxisome proliferator-activated receptor gamma gene (*PPARG*), and adenosine A1 receptor gene (*ADORA1*) are just some of the examples that were recently found to be associated with AIA (26–29). Meanwhile, the overproduction of pro-inflammatory cysteinyl-leukotrienes, which is caused by diverting the arachidonic acid metabolism from the cyclooxygenase pathway to the 5-lipoxygenase pathway, has been considered as a major characteristic of AIA (30, 31). Trafficking of lung dendritic cells to draining lymph nodes has been shown to be decreased in the treatment of leukotriene A4 hydrolase inhibitor, which is used to examine the role of leukotriene B4 (32). In addition, leukotriene B4 has been implicated as a potential target for reducing immune cell trafficking in chronic inflammatory diseases (33). Therefore, when considering the functions of *SMAPIL* in vesicle trafficking and altered airway reactivity through

leukotriene-related trafficking (4, 5, 14), our findings suggest that *SMAPIL* might play a role in the susceptibility to AIA. Despite no direct functional evidence for the relationship between aspirin and *SMAPIL* protein or surfactant secretion, our findings suggest a new insight into the relation between *SMAPIL* gene and aspirin hypersensitivity.

## CONCLUSION

In summary, based on the genotyping of 19 frequent polymorphisms of the *SMAPIL* gene, that is known to be involved in trafficking and is possibly associated with asthma and related phenotypes, and statistical analyses of the polymorphisms, we found that *rs2982510* and *rs2294752* are putatively associated with an increased susceptibility risk for AIA. Our findings provide additional information for the development of new methods for diagnosis of aspirin intolerance and new strategies for its control.

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## DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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