

Association Analysis Between *FILIP1* Polymorphisms and Aspirin Hypersensitivity in Korean Asthmatics

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Purpose: Aspirin exacerbated respiratory disease (AERD) results in a severe asthma attack after aspirin ingestion in asthmatics. The *filamin A interacting protein 1 (FILIP1)* may play a crucial role in AERD pathogenesis by mediating T cell activation and membrane rearrangement. We investigated the association of *FILIP1* variations with AERD and the fall rate of forced expiratory volume in one second (FEV1). **Methods:** A total of 34 common *FILIP1* single nucleotide polymorphisms (SNPs) were genotyped in 592 Korean asthmatic subjects that included 163 AERD patients and 429 aspirin-tolerant asthma (ATA) controls. **Results:** This study found that 5 SNPs ($P=0.006-0.01$) and 2 haplotypes ($P=0.01-0.03$) of *FILIP1* showed nominal signals; however, corrections for the multiple testing revealed no significant associations with the development of AERD ($P_{corr}>0.05$). In addition, association analysis of the genetic variants with the fall rate of FEV1, an important diagnostic marker of AERD, revealed no significant evidence ($P_{corr}>0.05$). **Conclusions:** Although further replications and functional evaluations are needed, our preliminary findings suggest that genetic variants of *FILIP1* might be not associated with the onset of AERD.

Key Words: *Filamin A interacting protein 1*; single nucleotide polymorphism; haplotype; asthma; aspirin exacerbated respiratory disease

INTRODUCTION

Asthma is a common disease worldwide that affects a large number of people, with approximately 300 million patients all over the world.¹ Some of the symptoms of the disease include lung inflammation and difficulty of breathing when the patient is under the influence of various asthma-inducing factors. Among them, non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin are known to cause aspirin exacerbated respiratory disease (AERD), which was first described in 1922.^{2,3} Usual symptoms of AERD patients include aspirin sensitivity, bronchial asthma, and chronic rhinosinusitis with nasal polyposis,^{4,6} and the airway inflammation is often initiated by T lymphocytes. Aspirin (acetylsalicylic acid) is primarily used as a pain and fever reliever as well as an anti-inflammatory medication.

The protein product from *filamin A interacting protein 1 (FILIP1)* is closely associated with the protein filamin A. The main role of filamin A is commonly known as an actin-binding protein that links actin filaments in cortical cytoplasm and helps form an actin cytoskeleton. This remodeling of the cytoskeleton

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Received: March 28, 2012; Revised: May 4, 2012; Accepted: May 21, 2012

• There are no financial or other issues that might lead to conflict of interest.
• Jason Yongha Kim and Jeong-Hyun Kim contributed equally on this paper.

is essential for cell shape modification, spreading and cell migration (OMIM).⁷ Along with filamin A, *FILIP1* has also been associated with cell migration and motility on various accounts;⁸⁻¹⁰ many researchers have shown that these two proteins interact together in most cases. Recently, a group of researchers found that filamin A is also closely associated with T cell activation and membrane rearrangement, which are correlated with asthma and related diseases.^{11,12} Furthermore, a recent study has found that T cells are linked with AERD via the leukotriene B₄ (LTB₄) pathway.¹³

Single nucleotide polymorphisms (SNPs) in *CYSLTR2*, *ALOX5*, *LTC4S*, and *SLC6A12* are shown to be associated with AERD,¹⁴⁻¹⁷ and suggest that the small effects of various genetic factors may play a role in the development of the disease. Furthermore, our previous genome-wide and follow-up studies show associations between *FILIP1* polymorphisms and AERD.¹⁸ Considering that, *FILIP1* is related to T-cell modification, we further investigated associations of *FILIP1* genetic variations with the fall rates of forced expiratory volume by aspirin provocation as well as AERD development.

MATERIALS AND METHODS

Study subjects

This study was conducted in compliance with the Global Initiative for Asthma (GINA) report Global Strategy for Asthma Management and Prevention. A total of 592 subjects were recruited from the Asthma Genome Research Center comprised of Soonchunhyang University Hospital (both Bucheon and Cheonan), Chungbuk National University Hospital, Chonnam National University Hospital, Chung-Ang University Yongsan Hospital, and Seoul National University Hospital. All subjects provided informed consent, and the protocols were approved

by the Institutional Review Board of each hospital. Each patient showed airway reversibility such as inhalant bronchodilator-induced improvement with over 15% of forced expiratory volume in 1 second (FEV1) and/or airway hyperresponsiveness to the provocative concentration of P20 methacholine.¹⁹

All patients had a history of dyspnea and wheezing in the past 12 months, plus one of the following: 1) >15% increase in FEV1 or >12% increase plus 200 mL following the 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.²⁰ Total IgE was measured with the CAP system (Pharmacia Diagnostics, Uppsala, Sweden), and atopy was defined as having a wheal reaction equal to or greater than histamine or 3 mm in diameter. All asthmatic patients had experienced no exacerbation of asthma and respiratory tract infection 6 weeks prior to the oral aspirin challenge (OAC). OAC was performed with increasing doses of aspirin using methods slightly modified from those previously described.^{20,21} Changes in FEV1 were followed for 5 hours after the last aspirin challenge dose. Aspirin-induced bronchospasms, as reflected by rate (%) of FEV1 decline, were calculated as the pre-challenge FEV1 minus the post-challenge FEV1 divided by the pre-challenge FEV1. OAC reactions were categorized into 2 groups of a 15% or greater decrease in FEV1 with naso-ocular or cutaneous reactions (AERD) and less than a 15% decrease in FEV1 without naso-ocular or cutaneous reactions (aspirin tolerant asthma, ATA). The clinical profiles of the subjects are listed in Table 1.

SNP selection and genotyping

Thirty-four common (MAF>0.05) SNPs of *FILIP1*, with MAF>0.05, were selected based on linkage disequilibrium (LD) status

Table 1. Clinical profiles of AERD patients and ATA cases

Clinical profile	Asthmatics (all subjects)	AERD	ATA
Subject number (n)	592	163	429
Age of first medical examination (mean [range])	46.15 (15.40-77.88)	43.13 (17.22-72.73)	47.30 (15.40-77.88)
BMI	24.19±3.39	23.39±3.25	24.58±3.39
Fall rate (%)	9.27±13.24	24.63±16.11	3.54±4.85
Blood eosinophil (%)	6.01±5.73	5.96±5.21	6.03±5.92
FVC%, predicted	88.54±14.08	90.35±14.04	87.85±14.05
FEV1%, predicted	90.54±16.97	87.58±16.94	91.66±16.87
PC20, methacholine (mg/mL)	6.43±8.67	5.02±7.83	6.91±8.90
Total IgE (IU/mL)*	156 (62, 394)	164 (78, 357)	154 (53, 416)
Sex (male/female)	206/386	59/104	147/282
Current Smoker (%)	27.7	21.47	30.07
Positive rate of skin test (%)	56.42	52.76	57.81

Fall rate refers to the decline of FEV1% by aspirin provocation.

*Total IgE value is shown as median (25% interquartile interval, 75% interquartile interval).

AERD, aspirin exacerbated respiratory disease; ATA, aspirin-tolerant asthma; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second.

from an Asian (Chinese and Japanese) population database of the International HapMap Project (<http://hapmap.ncbi.nlm.nih.gov/>). Then, the selected SNPs were genotyped in 163 AERD and 429 ATA subjects. Genotyping was performed on a multiplex level using the Illumina Golden Gate genotyping system (Illumina, San Diego, CA, USA) and data quality was assessed by duplicate DNAs (n=10). The SNPs were scanned using BeadXpress® (Illumina). SNPs that could not meet the following criteria were excluded: 1) a minimum call rate of 95%, 2) no duplicate error, and 3) a Hardy-Weinberg equilibrium of $P > 0.05$. As a result, 34 SNPs on *FILIP1* were successfully genotyped.

Statistics

We examined Lewontin's D' ($|D'|$) and the linkage disequilibrium (LD) coefficient r^2 between all pairs of biallelic loci. LD was obtained using the algorithm developed by the Broad Institute (using the program Haploview).²² Haplotypes were first estimated using PHASE software,²³ and then computed by logistic analyses using SAS. Subjects harboring missing genotypes were omitted in the analysis of individual single-nucleotide polymorphisms and haplotypes. The genotype and haplotype association with AERD were analyzed using logistic 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 body mass index (BMI) as covariates compared to ATA patients as a control; significant associations are shown in bold character ($P < 0.05$). The association analysis of differences in the fall rates in FEV1, following the aspirin challenge with the genotypes and haplotypes, were exam-

ined using SAS. To correct for the multiple testing error, we calculated the correction number of 27.21 using SNPSpD software (<http://gump.qimr.edu.au/general/daleN/SNPSpD/>).

RESULTS

Characteristics of study subjects

For our study, 592 subjects were recruited, with 163 AERD patients as cases and 429 ATA patients as controls. The phenotype data of all subjects are summarized in Table 1. As expected, the fall rate of AERD subjects was significantly higher than that of the controls (24.63% for AERD subjects and 3.54% for ATA controls, $P < 0.0001$). Other notable differences between cases and controls include age of first medical examination, BMI, FEV1, PC20 methacholine, and current smoking status ($P < 0.05$).

Genotyping, LD and haplotype of *FILIP1* polymorphisms

From the samples, we successfully genotyped a total of 34 common SNPs located in the intronic regions of *FILIP1*, spanning over 186 kb region in chromosome 6q14.1 (Fig. 1A). Results from the Hardy-Weinberg Equilibrium (HWE) test showed no significant difference between the distribution of the observed genotypes and the expected distributions ($P > 0.05$; Table 2). Several SNPs were found to have absolute LD ($|D'| = 1$ and $r^2 = 1$, Fig. 1A) and indicated that these SNPs are always interlinked. Haplotypes were estimated (Fig. 1B), and only those with a frequency higher than 0.05 were analyzed for associations. The SNPs comprised two tight haplotype blocks, with 19 SNPs in haplotype block 1 and 15 SNPs in haplotype block 2 (Figs. 1, 2).

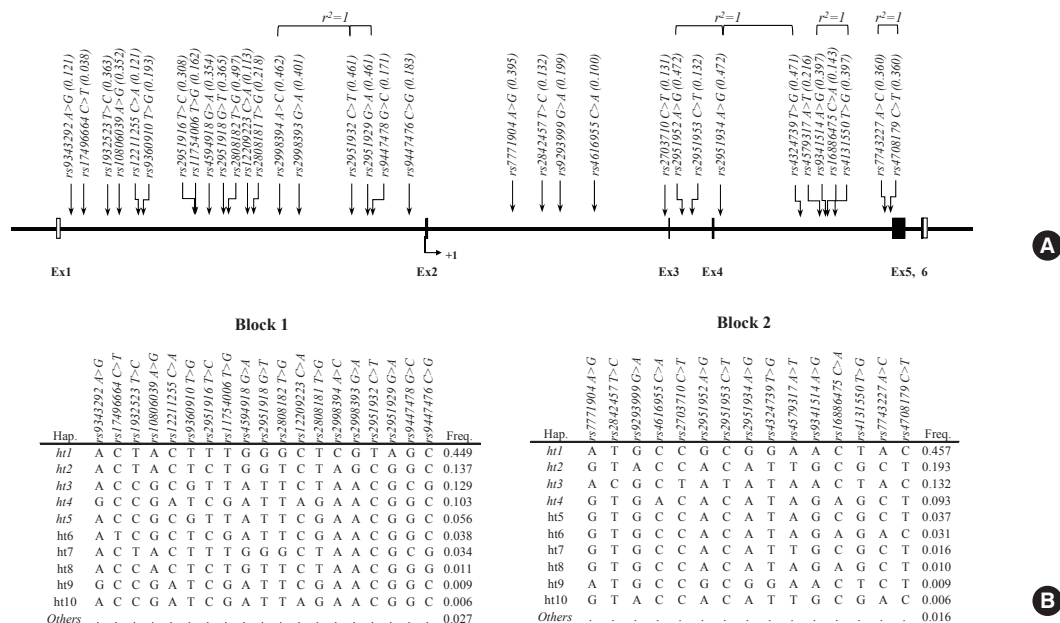


Fig. 1. Gene maps and haplotypes of the *FILIP1*. (A) A map of *FILIP1* on chromosome 6q14.1, in which coding exons, UTR, and SNPs are shown. Coding exon (Ex) is marked by shaded blocks while UTRs are marked by white blocks. (B) Haplotypes of *FILIP1* in the Korean population. SNPs are divided into 2 haplotype blocks. Only common haplotypes (frequency > 0.05) were used for an association analysis.

Table 2. Associations of *FILIP1* SNPs and haplotypes with AERD

SNP/Haplotype	Position	MAF			Heterozygosity	HWE*	OR (95% CI)	P	P _{corr} [†]
		AERD (n=163)	ATA (n=429)	Total (n=592)					
<i>rs9343292A>G</i>	Intron	0.126	0.118	0.121	0.213	0.932	1.04 (0.70-1.55)	0.84	-
<i>rs17496664C>T</i>	Intron	0.028	0.040	0.038	0.072	0.896	0.60 (0.28-1.31)	0.20	-
<i>rs1932523T>C</i>	Intron	0.402	0.347	0.363	0.463	0.525	1.30 (0.98-1.72)	0.07	-
<i>rs10806039A>G</i>	Intron	0.393	0.334	0.352	0.456	0.521	1.33 (1.00-1.76)	0.05	-
<i>rs12211255C>A</i>	Intron	0.126	0.117	0.121	0.213	0.404	1.06 (0.71-1.58)	0.79	-
<i>rs9360910T>G</i>	Intron	0.239	0.178	0.193	0.312	0.722	1.59 (1.14-2.21)	0.006	0.16
<i>rs2951916T>C</i>	Intron	0.322	0.294	0.308	0.426	0.549	1.10 (0.82-1.46)	0.53	-
<i>rs11754006T>G</i>	Intron	0.153	0.160	0.162	0.271	0.696	0.90 (0.62-1.30)	0.57	-
<i>rs4594918G>A</i>	Intron	0.393	0.337	0.354	0.457	0.444	1.31 (0.99-1.74)	0.06	-
<i>rs2951918G>T</i>	Intron	0.402	0.350	0.365	0.464	0.447	1.28 (0.97-1.69)	0.08	-
<i>rs2808182T>G</i>	Intron	0.439	0.524	0.497	0.500	0.471	0.69 (0.52-0.91)	0.008	0.22
<i>rs12209223C>A</i>	Intron	0.120	0.107	0.113	0.201	0.676	1.05 (0.70-1.59)	0.82	-
<i>rs2808181T>G</i>	Intron	0.242	0.203	0.218	0.341	0.321	1.19 (0.87-1.61)	0.28	-
<i>rs2998394A>C</i>	Intron	0.405	0.490	0.462	0.497	0.477	0.70 (0.53-0.92)	0.01	0.27
<i>rs2998393G>A</i>	Intron	0.436	0.386	0.401	0.480	0.348	1.25 (0.95-1.64)	0.12	-
<i>rs2951932C>T</i>	Intron	0.405	0.488	0.461	0.497	0.448	0.70 (0.53-0.92)	0.01	0.27
<i>rs2951929G>A</i>	Intron	0.404	0.488	0.461	0.497	0.472	0.70 (0.53-0.92)	0.01	0.27
<i>rs9447478G>C</i>	Intron	0.184	0.169	0.171	0.283	0.716	1.20 (0.84-1.70)	0.31	-
<i>rs9447476C>G</i>	Intron	0.193	0.183	0.183	0.299	0.801	1.15 (0.82-1.61)	0.43	-
<i>FILIP1_BL1_ht1</i>		0.402	0.474	0.450	0.495	0.742	0.74 (0.57-0.98)	0.03	0.82
<i>FILIP1_BL1_ht2</i>		0.156	0.122	0.135	0.234	0.244	1.32 (0.91-1.93)	0.15	-
<i>FILIP1_BL1_ht3</i>		0.153	0.125	0.131	0.228	0.928	1.40 (0.96-2.05)	0.08	-
<i>FILIP1_BL1_ht4</i>		0.117	0.094	0.102	0.184	0.268	1.22 (0.79-1.87)	0.37	-
<i>FILIP1_BL1_ht5</i>		0.083	0.044	0.055	0.104	0.399	1.95 (1.15-3.30)	0.01	0.27
<i>rs7771904A>G</i>	Intron	0.414	0.392	0.395	0.478	0.633	1.08 (0.83-1.42)	0.56	-
<i>rs2842457T>C</i>	Intron	0.153	0.122	0.132	0.229	0.316	1.32 (0.91-1.93)	0.15	-
<i>rs9293999G>A</i>	Intron	0.190	0.203	0.199	0.319	0.574	0.87 (0.63-1.21)	0.42	-
<i>rs4616955C>A</i>	Intron	0.110	0.098	0.101	0.181	0.771	1.28 (0.83-1.98)	0.27	-
<i>rs2703710C>T</i>	Intron	0.150	0.122	0.131	0.228	0.336	1.29 (0.88-1.89)	0.19	-
<i>rs2951952A>G</i>	Intron	0.433	0.485	0.472	0.498	0.811	0.82 (0.63-1.07)	0.14	-
<i>rs2951953C>T</i>	Intron	0.150	0.124	0.132	0.229	0.316	1.28 (0.88-1.88)	0.20	-
<i>rs2951934A>G</i>	Intron	0.433	0.485	0.472	0.498	0.811	0.82 (0.63-1.07)	0.14	-
<i>rs4324739T>G</i>	Intron	0.433	0.484	0.471	0.498	0.746	0.82 (0.63-1.07)	0.14	-
<i>rs4579317A>T</i>	Intron	0.227	0.216	0.216	0.339	0.694	1.00 (0.73-1.36)	0.99	-
<i>rs9341514A>G</i>	Intron	0.417	0.393	0.397	0.479	0.659	1.09 (0.83-1.42)	0.54	-
<i>rs16886475C>A</i>	Intron	0.147	0.142	0.143	0.245	0.795	1.13 (0.78-1.65)	0.52	-
<i>rs4131550T>G</i>	Intron	0.417	0.393	0.397	0.479	0.659	1.09 (0.83-1.42)	0.54	-
<i>rs7743227A>C</i>	Intron	0.387	0.353	0.360	0.461	0.575	1.15 (0.88-1.52)	0.31	-
<i>rs4708179C>T</i>	Intron	0.387	0.353	0.360	0.461	0.575	1.15 (0.88-1.52)	0.31	-
<i>FILIP1_BL2_ht1</i>		0.420	0.474	0.461	0.497	0.634	0.80 (0.62-1.05)	0.11	-
<i>FILIP1_BL2_ht2</i>		0.187	0.193	0.192	0.310	0.607	0.91 (0.65-1.27)	0.57	-
<i>FILIP1_BL2_ht3</i>		0.147	0.121	0.129	0.225	0.217	1.28 (0.87-1.89)	0.21	-
<i>FILIP1_BL2_ht4</i>		0.104	0.090	0.093	0.169	0.469	1.31 (0.84-2.04)	0.24	-

Age, sex, smoking status, atopy, and body mass index are adjusted as co-variables for logistic analysis. OR (95% CI) and *P* values are analyzed from the co-dominant model as shown in the supporting information of our previous report.¹⁸ Bold values indicate the statistical significance of *P* < 0.05.

* *P* value of the Hardy-Weinberg equilibrium (HWE). † Correction for multiple testing is calculated by SNPSpD program.

MAF, minor allele frequency; AERD, aspirin exacerbated respiratory disease; ATA, aspirin-tolerant asthma; OR, odds ratio; CI, confidence interval.

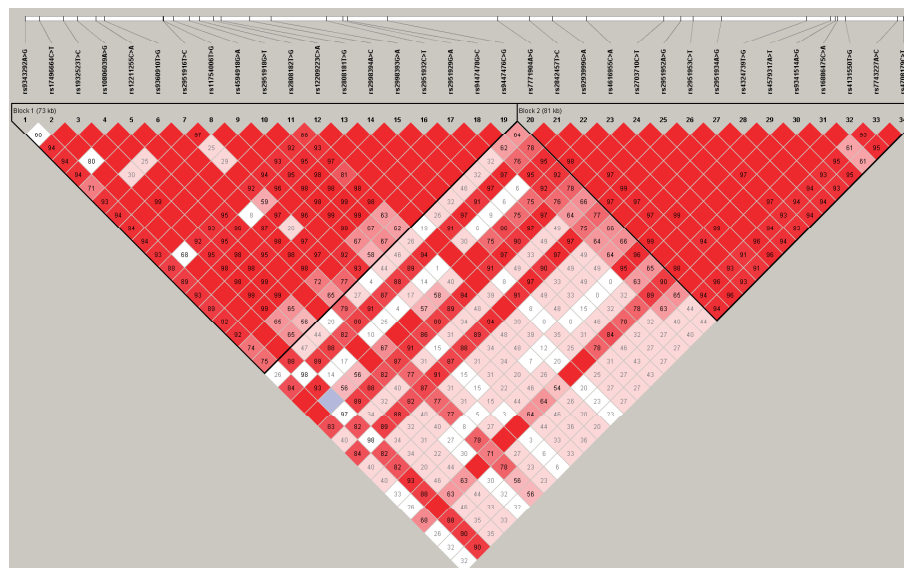


Fig. 2. LD coefficients graphical plot ($|D'|$ and r^2) among SNPs based on the genotypes of Korean study subjects ($n=592$).

Logistic analysis of *FILIP1* SNPs

Logistic analyses on each SNP and haplotype showed that 5 SNPs (*rs93690910*, *rs2808182*, *rs2998394*, *rs2951932*, and *rs2951929*) in the haplotype block 1 were nominally associated with AERD ($P=0.006-0.01$, Table 2). Two haplotypes in the haplotype block 1, *FILIP1_BLI_ht1* and *FILIP1_BLI_ht5*, also showed associations with AERD. However, all significant signals disappeared after corrections for multiple testing ($P_{corr}>0.05$, correction number=27.21, Table 2). On the other hand, no genetic polymorphisms and haplotypes in the haplotype block 2 showed significant associations with AERD ($P>0.05$, Table 2).

Association analysis of *FILIP1* polymorphisms with FEV1 decline by aspirin provocation

We additionally conducted association analysis between the genetic polymorphisms in *FILIP1* and FEV1 by aspirin provocation (Table 3). Most of SNPs that showed nominal associations with AERD revealed no significant relation with the fall rate of FEV1, except *rs93690910* and *FILIP1_BLI_ht5* in the haplotype block 1, which showed significant signals ($P_a=0.04$ and $P_b=0.03$ for *rs93690910*; $P_a=0.003$ and $P_b=0.004$ for *FILIP1_BLI_ht5*, Table 3). However, similar to the AERD case/control association, the significant signals disappeared after corrections for multiple testing ($P_{corr}>0.05$, Table 3). In addition, genetic variants in the haplotype block 2 again showed no association with the fall rate of FEV1 ($P>0.05$, Table 3).

DISCUSSION

During the inflammation process, the rolling and adhesion of activated leukocytes are mediated by endothelium, which is an important part of airway inflammation. In addition, adhesion

molecules are associated with airway remodeling, as evidenced in a recent study by a group in Canada.²⁴ In their study, vascular cell adhesion molecule (VCAM-1) played a significant role in the airway remodeling via interaction with T cells. In addition, airway remodeling is closely related with aspirin hypersensitivity.²⁵ Intracellular signaling in endothelial cells is induced by integrin-mediated adhesion to endothelial ligands of the immunoglobulin family; in addition, intracellular adhesion molecule-1 (ICAM-1) has recently been revealed to interact with filamin B.²⁶ Since filamin A and B share similar roles and interact with each other,²⁷ *FILIP1* has been suggested as associated with the airway inflammation process via the interaction between filamins and adhesion molecules.

The possible relation between *FILIP1* and AERD is also implicated in the role of LTB_4 . LTB_4 is a family of proinflammatory lipid mediators and plays a critical role in allergic inflammation.²⁸ Furthermore, LTB_4 is expressed in various inflammatory cells (including T cells), which are known to be modified by filamin A.^{11,12} A recent study has observed an increased LTB_4 release after an OAC,¹³ and suggests that LTB_4 may play an important role for AERD in the involvement of T cells. Our present study revealed that genetic variations of *FILIP1* might have no direct effect on the subsequent association with AERD development; however, other evidence also suggests a possible association between the potential transcript derived from the alternative splicing and LTB_4 receptor. The potential splice variant of *FILIP1* may also have a role in various allergic inflammations (such as aspirin hypersensitivity-related diseases) and requires further investigation since a recent study suggests that discrepant LTB_4 receptor antagonists derived from alternative splicing of the receptor in airway smooth muscle may affect bronchoconstriction.²⁹

Table 3. Association of *FILIP1* SNPs and haplotypes with the fall of FEV1 by aspirin provocation

SNP/Haplotype	Position	C/C		P*	P _{corr} [†]	C/R		P*	P _{corr} [†]	R/R		P*	P _{corr} [†]
		n	Fall rate			n	Fall rate			n	Fall rate		
<i>rs9343292A>G</i>	Intron	456	9.44 ± 13.60	0.31	-	127	8.74 ± 11.99	0.37	-	9	5.53 ± 10.13	0.46	-
<i>rs17496664C>T</i>	Intron	549	9.28 ± 13.26	0.61	-	43	8.59 ± 12.87	0.61	-
<i>rs1932523T>C</i>	Intron	231	8.40 ± 12.48	0.47	-	290	10.02 ± 14.00	0.26	-	71	8.75 ± 12.23	0.83	-
<i>rs10806039A>G</i>	Intron	240	8.27 ± 12.37	0.47	-	286	10.29 ± 14.08	0.18	-	66	8.16 ± 12.14	0.56	-
<i>rs12211255C>A</i>	Intron	455	9.48 ± 13.58	0.27	-	130	8.55 ± 12.08	0.30	-	7	5.61 ± 9.08	0.55	-
<i>rs9360910T>G</i>	Intron	380	8.45 ± 12.24	0.04	1.00	193	10.63 ± 14.98	0.03	0.82	19	10.75 ± 12.43	0.62	-
<i>rs2951916T>C</i>	Intron	285	9.24 ± 13.78	0.52	-	254	9.58 ± 12.85	0.82	-	53	7.52 ± 11.92	0.29	-
<i>rs11754006T>G</i>	Intron	414	9.59 ± 13.68	0.17	-	164	8.57 ± 12.25	0.20	-	13	5.96 ± 10.20	0.41	-
<i>rs4594918G>A</i>	Intron	238	8.31 ± 12.41	0.51	-	288	10.24 ± 14.05	0.21	-	66	8.16 ± 12.14	0.56	-
<i>rs2951918G>T</i>	Intron	229	8.44 ± 12.53	0.51	-	292	9.97 ± 13.96	0.30	-	71	8.75 ± 12.23	0.83	-
<i>rs2808182T>G</i>	Intron	143	9.38 ± 12.91	0.34	-	308	9.81 ± 13.82	0.77	-	141	7.82 ± 12.13	0.21	-
<i>rs12209223C>A</i>	Intron	465	9.47 ± 13.49	0.26	-	120	8.55 ± 12.36	0.27	-	7	5.51 ± 9.13	0.60	-
<i>rs2808181T>G</i>	Intron	366	8.82 ± 12.66	0.53	-	196	9.95 ± 14.21	0.52	-	30	9.60 ± 13.40	0.79	-
<i>rs2998394A>C</i>	Intron	164	10.45 ± 13.94	0.21	-	307	8.88 ± 13.03	0.13	-	121	8.48 ± 12.68	0.64	-
<i>rs2998393G>A</i>	Intron	203	8.82 ± 12.79	0.35	-	302	9.09 ± 13.24	0.64	-	87	10.68 ± 14.15	0.24	-
<i>rs2951932C>T</i>	Intron	164	10.45 ± 13.94	0.21	-	307	8.88 ± 13.03	0.13	-	120	8.49 ± 12.73	0.65	-
<i>rs2951929G>A</i>	Intron	164	10.45 ± 13.94	0.21	-	306	8.83 ± 13.03	0.13	-	120	8.49 ± 12.73	0.66	-
<i>rs9447478G>C</i>	Intron	402	9.18 ± 13.07	0.61	-	175	9.18 ± 13.86	0.73	-	15	11.36 ± 9.32	0.48	-
<i>rs9447476C>G</i>	Intron	390	9.23 ± 13.17	0.63	-	184	8.99 ± 13.63	0.84	-	18	11.73 ± 10.06	0.33	-
<i>FILIP1_BL1_ht1</i>		174	10.16 ± 13.68	0.33	-	301	8.94 ± 13.10	0.23	-	117	8.60 ± 12.87	0.74	-
<i>FILIP1_BL1_ht2</i>		444	9.09 ± 13.27	0.65	-	140	9.62 ± 12.70	0.65	-	8	10.43 ± 19.88	0.87	-
<i>FILIP1_BL1_ht3</i>		444	9.14 ± 13.19	0.55	-	139	9.43 ± 13.60	0.62	-	9	11.06 ± 9.24	0.58	-
<i>FILIP1_BL1_ht4</i>		474	9.33 ± 13.42	0.58	-	114	8.90 ± 12.48	0.58	-	4	7.45 ± 12.26	0.87	-
<i>FILIP1_BL1_ht5</i>		530	8.70 ± 12.55	0.003	0.08	59	13.42 ± 17.22	0.004	0.11	3	21.02 ± 24.05	0.13	-
<i>rs7771904A>G</i>	Intron	211	8.45 ± 12.22	0.51	-	291	9.90 ± 14.56	0.27	-	90	8.90 ± 10.70	0.84	-
<i>rs2842457T>C</i>	Intron	446	9.17 ± 13.44	0.94	-	138	9.77 ± 12.51	0.82	-	8	3.68 ± 13.16	0.24	-
<i>rs9293999G>A</i>	Intron	383	8.87 ± 12.81	0.52	-	184	10.00 ± 14.09	0.41	-	25	9.06 ± 13.09	0.88	-
<i>rs4616955C>A</i>	Intron	478	9.17 ± 13.18	0.53	-	109	9.25 ± 13.62	0.69	-	5	15.20 ± 8.13	0.22	-
<i>rs2703710C>T</i>	Intron	447	9.19 ± 13.43	0.89	-	137	9.70 ± 12.53	0.88	-	8	3.68 ± 13.16	0.24	-
<i>rs2951952A>G</i>	Intron	163	9.73 ± 12.29	0.52	-	300	9.15 ± 13.95	0.52	-	129	8.81 ± 12.66	0.69	-
<i>rs2951953C>T</i>	Intron	446	9.20 ± 13.44	0.87	-	138	9.67 ± 12.49	0.90	-	8	3.68 ± 13.16	0.24	-
<i>rs2951934A>G</i>	Intron	163	9.73 ± 12.29	0.52	-	300	9.15 ± 13.95	0.52	-	129	8.81 ± 12.66	0.69	-
<i>rs4324739T>G</i>	Intron	163	9.73 ± 12.29	0.52	-	301	9.13 ± 13.93	0.52	-	128	8.85 ± 12.70	0.70	-
<i>rs4579317A>T</i>	Intron	365	8.74 ± 12.85	0.36	-	197	10.05 ± 13.87	0.30	-	30	9.86 ± 13.38	0.86	-
<i>rs9341514A>G</i>	Intron	210	8.48 ± 12.25	0.44	-	291	9.78 ± 14.45	0.29	-	91	9.24 ± 11.14	0.97	-
<i>rs16886475C>A</i>	Intron	432	9.41 ± 13.43	0.94	-	150	8.55 ± 12.93	0.77	-	10	11.86 ± 7.12	0.47	-
<i>rs4131550T>G</i>	Intron	210	8.48 ± 12.25	0.44	-	291	9.78 ± 14.45	0.29	-	91	9.24 ± 11.14	0.97	-
<i>rs7743227A>C</i>	Intron	237	8.76 ± 12.70	0.44	-	282	9.48 ± 13.94	0.47	-	73	9.82 ± 12.12	0.63	-
<i>rs4708179C>T</i>	Intron	237	8.76 ± 12.70	0.44	-	282	9.48 ± 13.94	0.47	-	73	9.82 ± 12.12	0.63	-
<i>FILIP1_BL2_ht1</i>		168	9.84 ± 12.98	0.46	-	303	9.05 ± 13.56	0.43	-	121	8.84 ± 12.77	0.70	-
<i>FILIP1_BL2_ht2</i>		390	8.90 ± 12.76	0.51	-	179	9.92 ± 14.17	0.44	-	23	9.52 ± 13.57	1.00	-
<i>FILIP1_BL2_ht3</i>		448	9.22 ± 13.43	0.87	-	137	9.52 ± 12.51	0.95	-	7	4.49 ± 14.00	0.37	-
<i>FILIP1_BL2_ht4</i>		487	9.24 ± 13.34	0.70	-	100	8.93 ± 12.83	0.91	-	5	15.20 ± 8.13	0.22	-

C/C, C/R and R/R indicate the homozygote of common allele, heterozygote and homozygote of rare allele, respectively. Fall rate values are mean ± standard deviation. Bold values indicate the statistical significance of $P < 0.05$.

* P values of regression analysis are adjusted for sex, smoking status, atopy, and body mass index as covariates. [†]Correction for multiple testing is calculated by SNPSpD program (<http://gump.qimr.edu.au/general/daleN/SNPSpD/>).

This study conducted an association analysis of the genetic variants of *FILIP1* with aspirin hypersensitivity (case-control study) and the fall rate of FEV1 (phenotypic regression analysis) in asthmatics. In order to achieve an optimal correction for the multiple testing of markers representing SNPs in LD and to verify if the association signals on *FILIP1* polymorphisms are false positive, this study applied multiple testing corrections using the effective number of independent marker loci that explains the eigenvalue spectral decomposition of all genotypes represented in the correlation matrix.³⁰ As a result, multiple testing corrections failed to show consistently significant associations with the FEV1 decline by aspirin provocation as well as AERD development. The results indicate that the previously observed association signals might be false positive and/or *FILIP1* polymorphisms might not affect AERD development and FEV1 decline by aspirin provocation; however, further replications in other larger cohorts and functional evaluations are required.

Although significant associations disappeared after correction for multiple testing, we investigated whether a *FILIP1* variant with nominal associations could have potential functions using *in silico* prediction. In addition, genetic variations in intronic regions are shown to affect the splicing event that subsequently leads to different disease onset between populations. As a result, EMBL-EBI splice site prediction (<http://www.ebi.ac.uk/asss/srv/wb.cgi?method=2>) found that the TTTAT sequence containing 'A' variant of *rs2951929G>A* could act as a potential branch point (BP) site for an alternative splicing, with BP score = 3.09 (Supplementary Fig. 1). Despite the need for further studies, higher 'A' minor allele frequency of the SNP in ATA controls than in AERD patients suggest a possibility that this SNP could affect other aspirin hypersensitivity-related diseases.

Another interesting fact is that *rs9360910* might have a susceptible effect (odds ratio [OR] = 1.59), while the latter 4 associated SNPs could have protective effects (ORs of 0.69-0.70) in the *FILIP1*-related pathophysiology and functions in other diseases. Variant *rs9360910* had the most significant *P* value and was the only SNP associated with the fall rate of FEV1; however, *rs2808182* had the lowest *P* value among the 4 SNPs with protective effects. We suspect that these 2 SNPs might be related with other aspirin hypersensitivity-related diseases; however, further functional studies are required. In addition, the findings suggest that genetic and/or phenotypic balances between SNPs with susceptible effects and those with protective effects may regulate the susceptibility of diseases.

This study has a few limitations such as a small sample size and ethnicity. Although replications are needed in large AERD cohorts, this study was conducted with relatively small numbers of AERD patients due to the rareness of the disease. In addition, the study subjects consisted only of Korean population. Considering the ethnic diversity in antibiotic hypersensitivity including aspirin³¹ and the important role of filamin A on the immune cell activation and membrane rearrangement,^{11,12} fur-

ther studies in a broader population sample will be needed to confirm the genetic effect of *FILIP1* on AERD. However, although we were not able to compare with the frequencies of Korean normal controls, minor allele frequencies of 34 *FILIP1* SNPs among Korean asthmatics (AERD plus ATA in this study) and other Asian control populations (Chinese and Japanese) from the International HapMap Project database are shown in Supplementary Table 1.

This study first conducted an association analysis between genetic polymorphisms on the candidate gene of *FILIP1* and AERD, and found that no SNPs or haplotypes were associated with AERD even though there were nominal evidences that showed possible links between *FILIP1* and AERD. In addition, no significant relations between the SNPs and FEV1 decline by aspirin provocation were found. Although further replications are needed, our findings suggest that the genetic variants of *FILIP1* have no affect on aspirin-exacerbated respiratory diseases in the Korean population.

ACKNOWLEDGMENTS

This work was supported by a grant from the Korea Health 21 R&D Project (A010249); a grant from Korea Science and Engineering Foundation (KOSEF) funded by the Korea government (MEST) (No. 2009-0080157); and a Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-0006690).

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