

ORIGINAL ARTICLE

Association analysis of *FABP1* gene polymorphisms with aspirin-exacerbated respiratory disease in asthma

Hun Soo Chang,^{1,#} Jong Sook Park,^{2,#} Hye-Rim Shin,¹ Byung Lae Park,³ Hyoung Doo Shin,^{3,4} and Choon-Sik Park^{1,2,}

¹Department of Medical Bioscience, Graduate School, Soonchunhyang University, Chungcheongnam-do, Republic of Korea

²Division of Allergy and Respiratory Medicine, Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, Gyeonggi-Do, Korea

³Department of Genetic Epidemiology, SNP Genetics, Inc., Seoul, Republic of Korea

⁴Department of Life Sciences, Sogang University, Seoul, Republic of Korea

ABSTRACT

Previously, we used a proteomic approach to demonstrate that the protein level of fatty acid-binding protein 1 (FABP1) is increased in nasal polyps in patients with aspirin-exacerbated respiratory disease (AERD). To reveal the genetic effect of *FABP1* variants, we evaluated the association of *FABP1* polymorphisms with the risk of AERD in 207 asthmatics with AERD and 1019 aspirin-tolerant asthmatics (ATA). Seven polymorphisms of *FABP1* were selected from the National Center for Biotechnology Information (build 36) using minor allele frequency and linkage disequilibrium criteria. The genotype and haplotype distributions were not significantly different between the AERD and ATA groups in all of the genetic models. The percent decline of forced expiratory volume in 1 second (FEV1) after the oral aspirin challenge (OAC) test did not differ according to single-nucleotide polymorphism (SNP) genotypes. In haplotype analysis, asthmatic patients who were *BL2ht2* homozygotes showed a greater decline in FEV1 after the OAC test than subjects who possessed 1 or no copy of *BL2ht2* ($P = 0.035$). However, these observations were not significant after correction for multiple comparisons (corrected P value = 1.00). Neither genotype nor haplotype was associated with the presence of nasal polyposis in the study subjects. Although we did not find a significant association between the *FABP1* polymorphisms and AERD, our data suggest that the 7 SNPs are not associated with the increased expression of *FABP1* in asthmatic patients with AERD. Further studies of epigenetic factors that may contribute to the increased expression of *FABP1* in AERD should be performed.

KEYWORDS AERD, association, asthma, *FABP1*, SNP

INTRODUCTION

Aspirin (acetylsalicylic acid, ASA) hypersensitivity refers to the development of bronchoconstriction, nasal symptoms (aspirin-exacerbated respiratory dis-

ease, AERD), and ocular and skin manifestations following ingestion of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs) [1]. This syndrome is characterized by ASA hypersensitivity, bronchial asthma, nasal polyposis, and chronic hyperplastic eosinophilic sinusitis [2]. The airways of asthmatic individuals with AERD show signs of persistent inflammation, with marked eosinophilia, epithelial disruption, and cellular proliferation, as seen in nasal polyps and hyperplastic sinusitis. Multiple points of over- or under-production of critical mediators, including leukotrienes, lipoxins, thromboxane, and prostaglandins, by various inflammatory cells, including platelets, eosinophils, and neutrophils, likely account for the susceptibility to ASA [3].

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[#]Hun Soo Chang and Jong Suk Park contributed equally to this work as first authors.

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Address correspondence to Choon-Sik Park, M.D., Ph.D., Division of Allergy and Respiratory Medicine, Department of Internal Medicine Soonchunhyang University Bucheon Hospital, 1174, Jung Dong, Wonmi Ku, Bucheon, Gyeonggi Do, 420–021, Republic of Korea. E-mail: mdcspark@hanmail.net and Hyoung Doo Shin, E-mail: hdshin@sogang.ac.kr

Previously, we reported that 7 proteins were significantly upregulated in the nasal polyps of AERD patients ($n = 5$) compared with those in patients with aspirin-tolerant asthma (ATA; $n = 8$) using a proteomic approach [4]. Among the 7 proteins, expression of fatty acid-binding protein 1 (FABP1), a member of the superfamily of the fatty-acid-binding proteins (FABPs), was significantly increased in the nasal polyps of AERD patients compared with those in ATA patients. On immunohistochemical staining, FABP1 is strongly expressed in epithelial cells, macrophages, eosinophils, and smooth muscle cells of the nasal polyps of AERD subjects compared with those of ATA subjects. The latter finding suggests that an increase in FABP1 protein in the nasal polyps may be related to the development of AERD. FABP1 serves as a key regulator of lipid metabolism by influencing the uptake, trafficking, oxidation, and esterification of fatty acids [5]. In addition, FABPs play a role in the trafficking of intracellular arachidonic acid metabolism, conversion of fatty acids to eicosanoid intermediates, and stabilization of leukotrienes [6], which are main factors in the development of AERD [7]. Among the mediators of the eicosanoid pathway, LTA4 is an intermediate in the formation of biologically active leukotrienes. LTA4 in the binding site of E-FABP (*FABP5*), another member of the FABP family, is stabilized for subsequent biochemical processing to LTB4 and cysteinyl leukotrienes [6]. An additional function of FABP1 is the reduction of 15-lipoxygenase (LO) activity [8]. The end-products of 15-LO are lipoxin and aspirin-triggered lipoxin, which have anti-inflammatory effects. Taken together, the elevation of FABP levels may switch the tissue environment to LTB4 or cysteinyl leukotriene overproduction and downregulation of lipoxin family members, a finding that has been demonstrated in the nasal polyps or airways of patients with AERD [9]. Variation within the genes of the arachidonate pathway is responsible for changes in the production and metabolism of these mediators [10]. However, no study has evaluated the genetic association of *FABP1* variants with AERD. To evaluate a possible relationship between the genetic effects on increased expression of FABP1 in AERD, we investigated the association of *FABP1* polymorphisms with the risk of AERD in asthmatic patients.

MATERIALS AND METHODS

Subjects

Two hundred and seven patients with AERD and 1019 ATA subjects were recruited from the Asthma Genome Research Center, which comprises 9 uni-

versity hospitals in Korea. All subjects were Korean. The protocol was approved by the Ethics Committee of Soonchunhyang University Hospital (approval No. SCHBC-IRB-2010-005). All patients were diagnosed by physicians and met the definition of asthma set forth in the Global Initiative for Asthma (GINA) guidelines [11]. All patients had a history of dyspnea and wheezing during the previous 12 months plus 1 of the following features: (1) >15% increase in forced expiratory volume in 1 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. The total immunoglobulin E (IgE) level was measured using the CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Atopy was defined as having a wheal reaction equal to or greater than that of histamine or equal to or greater than 3 mm in diameter. All study subjects underwent the oral ASA challenge (OAC), and they experienced neither exacerbation of asthma nor had a respiratory tract infection in the 6 weeks preceding OAC. The OAC was performed with increasing doses of ASA following previously described methods but with slight modifications [4, 12]. Changes in FEV1 were followed for 5 hours after the last ASA challenge dose. ASA-induced bronchospasms, as reflected by the 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 as follows: 15% or greater decreases in FEV1 or appearance of naso-ocular reactions, including rhinorrhea, nasal congestion, ocular injection, periorbital swelling, and erythema of the skin of the upper thorax and face (AERD), and less than 15% decreases in FEV1 without naso-ocular or cutaneous reactions (ATA). All subjects gave informed written consent to participate in the present study. The protocols were approved by the local institutional ethics committees.

Genotyping

Seven *FABP1* polymorphisms were selected from the National Center for Biotechnology Information (build 36) using minor allele frequency (>5%) and linkage disequilibrium criteria. Amplification and extension primers were designed for the 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, USA). For subsequent

TABLE 1. Probe Information Used for Genotyping of *FABP1* Polymorphisms by Single Base Extension Method

Loci	ID of Assay-on-demand (Applied Biosystems) or primer sequence	
rs2197076	C___15941447_10	
rs1545224	C___7933023_1_	
rs2241883	C___25473098_10	
rs1530273	C___8695438_10	
rs2919872	C___15941441_10	
rs2970902	C___7933035_10	
rs1545223	Forward	TTCTTTGCCCCCAGAATATG
	Reverse	ACCTATCAAAGGGCGTTGG
	Extension primer	GGGAGTCACAGACAACAGAACACTGCC

clean-up, the reaction mixture was incubated at 37°C for 1 hour with 1 U of shrimp alkaline phosphatase, followed by 15 minutes at 72°C for enzyme inactivation. Next, the extension products and 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 minutes, followed by incubation for 5 minutes 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 regarding the primers is shown in Table 1.

Statistical Analysis

We used Lewontin's D' ($|D'|$) and r^2 to measure linkage disequilibrium between the biallelic loci [13]. Haplotypes of each individual were inferred using the PHASE algorithm (ver. 2.0) [14]. The genotype distribution was 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 as covariates. Haplotype associations were estimated using HaploScore (<http://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 type III generalized linear model. The normality of the distributions of variables were tested using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Prior to statistical tests, blood eosinophil%, PC20 methacholine concentration, and the level of total IgE values were log-transformed to achieve normal distribution due to their positive skewness. The data were managed and analyzed using SAS version 9.1 (SAS Inc., Cary, NC, USA) and SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). For the correction of P values, the effective number of independent markers in *FABP1* was calculated using the software SNPSpD (<http://genepi.qimr.edu.au/general/daleN/SNPSpD>) [15]. The data are expressed as the mean $\pm$ standard error of the mean (SEM). P values less than 5% were deemed to indicate statistical significance.

RESULTS

Characteristics of the Study Subjects

In total, 1226 subjects were recruited from the asthma cohort. The clinical characteristics of the study subjects are summarized in Table 2. Significant

TABLE 2. Clinical Characteristics of the Study Subjects

Clinical profile	AERD	ATA	P
Number of subjects (n)	207	1019	-
Age (year)	33.63 $\pm$ 15.65	38.79 $\pm$ 16.73	<.001
Sex (n, male/female)	85/122	389/630	.437
Current smoker/Ex-smoker (%)	12.6/6.8	13.6/16.6	.001
Body mass index (kg/m ²)	23.63 $\pm$ 3.54	24.38 $\pm$ 3.58	.007
Blood eosinophil (%)	6.17 $\pm$ 5.67	5.57 $\pm$ 5.18	.145
FVC (% predicted)	83.15 $\pm$ 19.38	83.75 $\pm$ 19.83	.691
FEV1 (% predicted)	85.69 $\pm$ 17.82	83.37 $\pm$ 17.90	.092
PC20 methacholine (mg/ml)	5.08 $\pm$ 7.88	6.51 $\pm$ 8.59	.105
Total IgE (IU/ml)	340.81 $\pm$ 543.26	390.05 $\pm$ 628.47	.262
Positive rate of skin test (%)	61.9	56.4	.151
% decline of FEV1 by aspirin provocation	24.73 $\pm$ 16.56	3.76 $\pm$ 4.81	<.001
Positive rate of nasal polyp (%)	50.6	23.1	<.001

differences in age, the prevalence of smoking, BMI, the percentile of FEV1 decline after ASA challenge, and the ratio of patients with nasal polyposis were found between the AERD and ATA groups ($P < .05$; Table 2). Thus, the parameters, except the percentage decline of FEV1 and presence of nasal polypsis, were considered covariates in further analyses for evaluating genetic association.

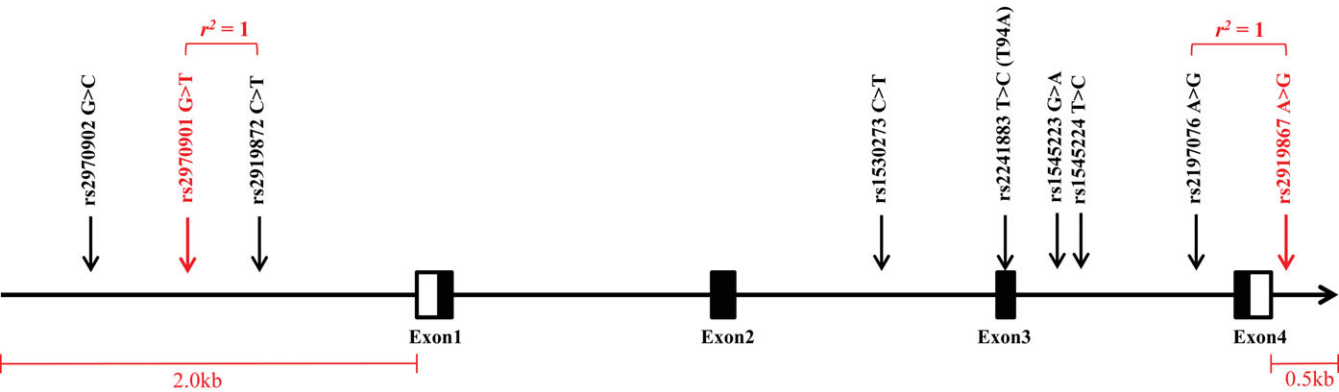
Frequency, Heterozygosity, and the Hardy–Weinberg Equilibrium of SNPs in *FABP1*

According to dbSNP (<http://www.ncbi.nlm.nih.gov/SNP>) and Hapmap DB (<http://hapmap.ncbi.nlm.nih.gov>), there are 9 SNPs, which have minor allele frequencies greater than 0.05, in the region from 2 kb upstream to 0.5 kb downstream of *FABP1*

in Asian the population (Han Chinese and Japanese); rs2970902, rs2970901, rs2919872, rs1530273, rs2241883, rs1545224, rs1545223, rs2197076, and rs2919867. Among them, rs2970901 and rs2919867 showed absolute linkage disequilibrium ($D = 1$, $r^2 = 1$) with rs2919872 and rs2197076, respectively. Thus, we excluded these 2 SNPs in further analysis. The remaining 7 SNPs of *FABP1* comprise 2 in the promoter (rs2970902 and rs2919872), 4 in the intronic sequences (rs1530273, rs1545224, rs1545223, and rs2197076), and 1 in the coding region (rs2241883, T94A). The gene map and location of the SNPs are presented in Figure 1A.

The MAFs and Hardy–Weinberg equilibrium of the 7 SNPs are summarized in Table 3. The distributions of all loci were in Hardy–Weinberg equilibrium in both AERD patients and ATA subjects

A. Physical map of fatty acid binding protein 1 (*FABP1*) on chromosome 2p11



B. Haplotype of *FABP1*

Block1				Block2					
rs2970902 G>C				rs1530273 C>T					
rs2919872 C>T				rs2241883 T>C					
Hap.	rs2970902	rs2919872	Freq.	Hap.	rs1530273	rs2241883	rs1545224	rs1545223	rs2197076
ht1	G	C	0.815	ht1	C	T	G	T	A
ht2	C	T	0.111	ht2	T	T	A	T	G
ht3	G	T	0.073	ht3	T	C	A	C	G
Others	C	C	0.0007	Others	C	C	A	C	G
					C	T	A	C	G
					C	T	A	T	G
					C	C	G	C	G
					T	T	G	T	A
					T	T	A	C	G
					T	C	A	C	A
					C	T	G	T	G
					T	C	A	T	G
					C	T	G	C	A
					C	T	G	C	G
					C	C	G	T	A

C. LDs among *FABP1* polymorphisms

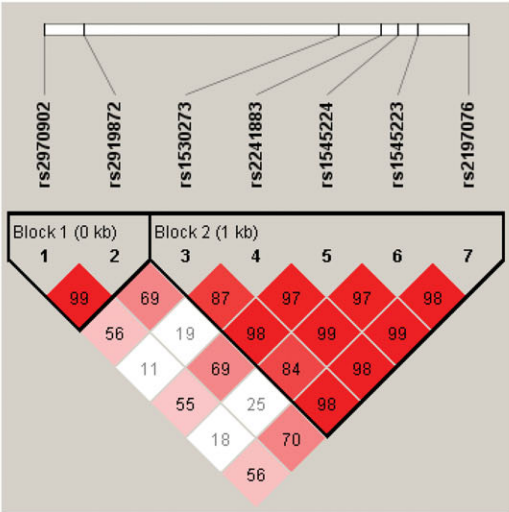


FIGURE 1. (A) Schematic gene map and SNPs in the *FABP1* gene on chromosome 2p11. Black blocks represent coding exons and white blocks represent 5' and 3'UTR. (B) Haplotype of *FABP1*. (C) Pairwise linkage disequilibrium among *FABP1* SNPs.

TABLE 3. Genotype and Minor Allele Frequencies of SNPs in FABP1 and Hardy–Weinberg Equilibrium

rs#	Diag	A1A1	A1A2	A2A2	MAF	χ^2	P
rs2970902	AERD	160	42	4	0.121	0.398	.528
	ATA	800	202	11	0.111	0.195	.659
rs2919872	AERD	139	58	10	0.188	1.453	.228
	ATA	672	310	32	0.184	0.269	.604
rs1530273	AERD	59	103	45	0.466	0.000	.997
	ATA	294	495	225	0.466	0.371	.542
rs2241883	AERD	122	78	7	0.222	1.679	.195
	ATA	595	373	41	0.225	3.444	.063
rs1545224	AERD	59	101	46	0.468	0.049	.824
	ATA	273	503	240	0.484	0.079	.779
rs1545223	AERD	121	79	7	0.225	1.891	.169
	ATA	591	381	46	0.232	2.470	.116
rs2197076	AERD	59	101	47	0.471	0.090	.764
	ATA	270	503	242	0.486	0.067	.796

Note. A1, common allele; A2, minor allele; MAF, minor allele frequency

($P > .01$). The calculated linkage disequilibrium coefficients $|D'|$ and r^2 among the SNPs revealed that *FABP1* was parsed into 2 LD Blocks (BLs) and that 3 major haplotypes each (frequency > 0.05) existed for BL1 and BL2 (Figure 1B and 1C). Among the 3 common haplotypes of BL1, only haplotype3 (BL1ht3) was used for further statistical analysis because ht1 and ht2 were almost equivalent to *rs2919872* and *rs2970902*, respectively (Figure 1B).

Associations of FABP1 Polymorphisms with the Risk and Phenotypes of AERD in Asthmatics

All of the *FABP1* polymorphisms and haplotypes were analyzed for associations with the risk of AERD using multiple logistic regression models. As shown in Table 4, the genotypes and allele distributions of all the SNPs and haplotype distributions were not significantly different between the AERD and ATA groups in all genetic models tested—namely, codominant, dominant, and recessive. Similar results were observed in analyses which were not adjusted with covariates (data not shown).

Because the ASA-induced decline in FEV₁ is the most important parameter for the diagnosis of ASA intolerance in asthmatics, we tested the association of the SNPs and haplotypes with the rate of decline in FEV₁ following ASA challenge. The percentage decline of FEV₁ after the OAC test did not differ according to SNP genotypes (Table 4). In haplotype analysis, haplotype2 in block 2 (BL2ht2) showed an association with the percentage reduction of FEV₁ in the recessive model with a marginal P value ($P = .035$; Table 5). Patients with asthma who were *BL2ht2* ho-

mozygotes showed a larger decline in FEV₁ after the OAC test than subjects who possess 1 or no copy of *BL2ht2* ($7.80 \pm 0.39\%$ vs. $6.55 \pm 0.45\%$, respectively). However, the difference was not significant after the correction for multiple comparisons (corrected P value = 1.00). In covariates-unadjusted models of the analyses using independent t test and one-way ANOVA, similar results were observed; only *BL2ht2* showed an association with the percentage reduction of FEV₁ in the recessive model with a marginal P value ($P = .032$; data not shown).

Because the occurrence of nasal polyps is a concomitant phenotype of AERD, we analyzed associations of SNPs and haplotypes with the risk of nasal polyposis in the study subjects. Neither genotypes nor haplotypes were associated with the presence of nasal polyposis in the study subjects (Table 6). Similar results were observed in analyses which were not adjusted with covariates (data not shown).

DISCUSSION

In the present study, we demonstrated no association of *FABP1* SNPs with the development of AERD or nasal polyp. Based on the elevated *FABP1* protein level in the nasal polyps of patients with AERD, genetic variants of *FABP1* may not affect the expression of *FABP1* protein in the nasal polyps. Several genetic studies of *FABP1* have been reported. A threonine (T) to alanine (A) mutation of *FABP1* at position 94 (T94A; *rs2241883*) is associated with elevated fasting serum cholesterol and triacylglycerol levels, which are associated with an increased risk of cardiovascular disease, type 2 diabetes, and the metabolic syndrome [16–18]. Recently, Peng et al. reported that 2 genetic variants in *FABP1*, *rs2241883* and *rs1545224*, are associated with the susceptibility to non-alcoholic fatty liver disease in a Chinese population [19]. These observations clearly suggest that *FABP1* polymorphisms are likely to be associated with diseases caused by impairment of lipid metabolism.

The pathogenesis of AERD is related to inflammatory or anti-inflammatory lipid mediators, such as cysteinyl leukotrienes and lipoxins, which are derived from arachidonic acid [20–24]. Arachidonic acid is oxidized by 5-LO to form the unstable intermediate LTA₄, which is then conjugated to reduced glutathione by the terminal enzyme LTC₄ synthase (LTC₄S) to form LTC₄ [24]. *FABP1* increases the half-life of LTA₄ competitively to arachidonic acid, and can bind to other arachidonic acid metabolites, Hydroperoxyeicosatetraenoic acid (HPETE) and hydroxyeicosatetraenoic acid (HETE), with high affinity [6, 25]. In addition, *FABP1* is known to reduce

TABLE 4. Association of Genotypes and Haplotypes of *FABP1* Gene with the Risk of Aspirin Intolerance

Locus	Diag	Genotype				Codominant		Dominant		Recessive	
		A1A1	A1A2	A2A2	Total	P*	OR [95% CI]	P*	OR [95% CI]	P*	OR [95% CI]
rs2970902	AERD	77.7% (160)	20.4% (42)	1.9% (4)	100% (206)	.816	0.96 [0.68–1.36]	.929	0.98 [0.67–1.44]	.531	0.66 [0.18–2.46]
	ATA	79% (800)	19.9% (202)	1.1% (11)	100% (1013)						
rs2919872	AERD	67.1% (139)	28% (58)	4.8% (10)	100% (207)	.699	1.06 [0.79–1.42]	.586	1.1 [0.78–1.54]	.803	0.9 [0.38–2.1]
	ATA	66.3% (672)	30.6% (310)	3.2% (32)	100% (1014)						
rs1530273	AERD	28.5% (59)	49.8% (103)	21.7% (45)	100% (207)	.901	0.99 [0.79–1.23]	.696	0.93 [0.66–1.33]	.831	1.04 [0.71–1.53]
	ATA	29% (294)	48.8% (495)	22.2% (225)	100% (1014)						
rs2241883	AERD	58.9% (122)	37.7% (78)	3.4% (7)	100% (207)	.669	0.94 [0.72–1.24]	.573	0.91 [0.66–1.26]	.864	1.08 [0.47–2.46]
	ATA	59% (595)	37% (373)	4.1% (41)	100% (1009)						
rs1545224	AERD	28.6% (59)	49% (101)	22.3% (46)	100% (206)	.634	1.06 [0.85–1.32]	.78	1.05 [0.74–1.5]	.612	1.1 [0.75–1.61]
	ATA	26.9% (273)	49.5% (503)	23.6% (240)	100% (1016)						
rs1545223	AERD	58.5% (121)	38.2% (79)	3.4% (7)	100% (207)	.854	0.98 [0.74–1.28]	.673	0.93 [0.68–1.28]	.62	1.23 [0.54–2.8]
	ATA	58.1% (591)	37.4% (381)	4.5% (46)	100% (1018)						
rs2197076	AERD	28.5% (59)	48.8% (101)	22.7% (47)	100% (207)	.599	1.06 [0.85–1.33]	.701	1.07 [0.75–1.52]	.631	1.1 [0.75–1.6]
	ATA	26.6% (270)	49.6% (503)	23.8% (242)	100% (1015)						
		+/+	–/+	–/–	Total	P*	OR [95% CI]	P*	OR [95% CI]	P*	OR [95% CI]
BL1ht3c	AERD	88.4% (183)	10.1% (21)	1.4% (3)	100% (207)	.304	1.27 [0.81–1.99]	.764	0.78 [0.16–3.91]	.23	1.35 [0.83–2.22]
	ATA	85.7% (873)	13.6% (139)	0.7% (7)	100% (1019)						
BL2ht1c	AERD	23.2% (48)	48.8% (101)	28% (58)	100% (207)	.629	0.95 [0.76–1.18]	.715	0.94 [0.66–1.33]	.669	0.92 [0.63–1.34]
	ATA	24.4% (249)	49.5% (504)	26.1% (266)	100% (1019)						
BL2ht2c	AERD	58.9% (122)	33.3% (69)	7.7% (16)	100% (207)	.396	1.12 [0.86–1.47]	.336	0.73 [0.39–1.39]	.143	1.27 [0.92–1.76]
	ATA	54.7% (557)	40% (408)	5.3% (54)	100% (1019)						
BL2ht3c	AERD	60.9% (126)	35.7% (74)	3.4% (7)	100% (207)	.47	0.9 [0.68–1.19]	.808	0.9 [0.39–2.09]	.46	0.89 [0.64–1.22]
	ATA	61.7% (629)	34.9% (356)	3.3% (34)	100% (1019)						

Note. A1, common allele; A2, minor allele; OR, odds ratio; 95% CI, 95% confidence interval

*P values were obtained using logistic regression analysis controlling for age, sex, smoke status, atopy, and BMI as covariates.

TABLE 5. Association of Genotypes and Haplotypes of *FABP1* Gene with the Percentage Decline of FEV1 After Oral ASA Challenge Test in the Study Subjects

Locus	Genotype			Pc	Pd	Pr
	A1A1	A1A2	A2A2			
rs2970902	7.21 ± 0.36 (950)	7.36 ± 0.76 (239)	7.80 ± 4.14 (15)	.717	.900	.415
rs2919872	7.36 ± 0.40 (803)	7.14 ± 0.58 (361)	6.40 ± 2.06 (42)	.404	.494	.194
rs1530273	7.24 ± 0.57 (352)	7.57 ± 0.48 (590)	6.57 ± 0.68 (264)	.530	.776	.353
rs2241883	7.04 ± 0.39 (710)	7.82 ± 0.60 (445)	5.82 ± 1.21 (47)	.162	.136	.432
rs1545224	7.31 ± 0.58 (331)	7.64 ± 0.48 (596)	6.35 ± 0.64 (280)	.309	.864	.169
rs1545223	7.03 ± 0.40 (705)	7.79 ± 0.58 (454)	5.54 ± 1.14 (51)	.125	.148	.307
rs2197076	7.34 ± 0.59 (328)	7.64 ± 0.48 (596)	6.34 ± 0.63 (283)	.298	.931	.152
	+/+	-/+	-/-	Pc	Pd	Pr
BL1ht3c	7.42 ± 0.36 (1043)	6.23 ± 0.74 (158)	4.92 ± 4.27 (10)	.351	.645	0.150
BL2ht1c	6.46 ± 0.63 (291)	7.61 ± 0.48 (597)	7.29 ± 0.59 (323)	.406	.837	0.239
BL2ht2c	7.80 ± 0.45 (673)	6.49 ± 0.48 (469)	6.98 ± 1.30 (69)	.108	.644	0.035
BL2ht3c	7.02 ± 0.39 (748)	7.73 ± 0.60 (424)	6.18 ± 1.46 (39)	.240	.610	0.149

Note. A1, common allele; A2, minor allele; Pc, *P* values in codominant model; Pd, *P* values in dominant model; Pr, *P* values in recessive model

**P* values were obtained using type III generalized linear model controlling for age, sex, smoke status, atopy, and BMI as covariates.

15-LO-induced oxygenation of linoleic acid and arachidonic acid [8]. In cooperation with 5-LO, 15-LO of epithelial cells is a key enzyme for the production of lipoxins, unstable anti-inflammatory derivatives of arachidonic acid [21]. FABPs themselves are controlled by transcription factors, including PPAR α / γ [26], which is upregulated in various cells via the overproduction of eicosanoid metabolites, including 15-hydroxyeicosatetraenoic acid (15-HETE), and 15-deoxy-12, 14-prostaglandin J2 (15d-PG J2). Interestingly, a polymorphism in the gene encoding PPAR γ is associated with aspirin hypersensitivity [27].

The lack of a genetic association in the present study indicates that the increased expression of FABP1 in AERD may be not determined by genetic factors but by other factors such as epigenetic controls, including DNA methylation, histone modification, or post-transcriptional regulation by microRNA. L-FABP is expressed in alveolar macrophages, although other myeloid cells, including immature and mature dendritic cells, peritoneal macrophages, and bone marrow macrophages, do not express FABP1 [28]. Although this report represents indirect evidence that epigenetic modulation may be involved in the regulation of FABP1 expression, no study has investigated the epigenetics of *FABP1*. We demonstrated previously that 337 genes, including *FABP1*, as well as genes involved in arachidonic acid metabolism, were differentially methylated compared with those in ATA subjects using the genome-wide methylation profile of nasal polyps in patients with AERD and ATA: AERD patients showed 3.3-fold more methylation at a

CpG site of *FABP1*, cg19910382, than did ATA subjects (*P* = .00008) [29]. Although the result was restricted to a single methylation site in *FABP1* due to the limitation of the array used in the study, the observation suggests that epigenetic regulation of FABP1 expression can affect the development of AERD. In addition, we searched possible miRNAs targeting the 3'UTR of FABP1 mRNA using 2 search tools: miRDB (<http://mirdb.org/miRDB>) and TargetScanHuman 6.2 (<http://www.targetscan.org>). Both tools consistently predicted hsa-miR-603 and has-miR-3941 with an 8-mer site, representing an exact match to positions 2–8 of the mature miRNA (the seed + position 8) followed by an 'A', although the relationship between the genes embedded by the miRNAs and AERD has not been reported. Thus, to evaluate the possible epigenetic factors involved in the increased expression of the gene in AERD, further studies should be performed.

A limitation of the present study was that we genotyped only 7 SNPs with a MAF > 0.05. To date, 124 SNPs are registered in dbSNP (<http://www.ncbi.nlm.nih.gov/snp>), including 12 synonymous and 8 missense SNPs in the coding region of the gene. Although the SNPs analyzed in the present study tagged haplotypes on each hapblock (Figure 1B), the other SNPs may be associated with AERD itself or related phenotypes. This possibility should be confirmed in future genetic studies that include high-density markers with low frequencies and use next-generation sequencing or exome variant analyses. In addition, we cannot exclude the possibility of a population stratification bias [30]. However, because the Korean population is characterized by a relatively

TABLE 6. Association of Genotypes and Haplotypes of *FABP1* Gene with the Presence of Nasal Polyposis in the Study Subjects

Locus	NP	Genotype			Codominant		Dominant		Recessive	
		A1A1	A1A2	A2A2	Total	P*	OR [95% CI]	P*	OR [95% CI]	P*
rs2970902	N	79.7% (556)	18.9% (132)	1.4% (10)	100% (698)	.268	0.84[0.61–1.15]	.161	0.78[0.56–1.1]	.463
	Y	75.9% (202)	23.3% (62)	0.8% (2)	100% (266)					
rs2919872	N	67.2% (471)	29% (203)	3.9% (27)	100% (701)	.644	1.07[0.81–1.39]	.894	0.98[0.72–1.33]	.086
	Y	67.2% (178)	30.6% (81)	2.3% (6)	100% (265)					
rs1530273	N	28.5% (200)	49.8% (349)	21.7% (152)	100% (701)	.779	1.03[0.84–1.26]	.731	1.06[0.77–1.45]	.918
	Y	30.6% (81)	47.9% (127)	21.5% (57)	100% (265)					
rs2241883	N	59.1% (413)	37.3% (261)	3.6% (25)	100% (699)	.68	0.95[0.74–1.22]	.595	0.92[0.69–1.24]	.882
	Y	58% (153)	38.6% (102)	3.4% (9)	100% (264)					
rs1545224	N	26.8% (188)	49.9% (350)	23.4% (164)	100% (702)	.455	1.08[0.88–1.33]	.6	1.09[0.79–1.5]	.482
	Y	29.3% (78)	49.2% (131)	21.4% (57)	100% (266)					
rs1545223	N	58.2% (409)	37.7% (265)	4.1% (29)	100% (703)	.89	0.98[0.77–1.26]	.694	0.94[0.7–1.26]	.565
	Y	57.5% (153)	39.1% (104)	3.4% (9)	100% (266)					
rs2197076	N	26.8% (188)	49.5% (347)	23.7% (166)	100% (701)	.495	1.07[0.88–1.32]	.688	1.07[0.77–1.48]	.469
	Y	28.9% (77)	49.2% (131)	21.8% (58)	100% (266)					
		+/+	–/+	–/–	Total	P*	OR [95% CI]	P*	OR [95% CI]	P*
BL1ht3c	N	85.6% (602)	13.7% (96)	0.7% (5)	100% (703)	.045	1.58[1.01–2.48]	.999		.068
	Y	89.8% (239)	9.8% (26)	0.4% (1)	100% (266)					
BL2ht1c	N	24.2% (170)	49.6% (349)	26.2% (184)	100% (703)	.471	0.93[0.76–1.14]	.626	0.92[0.67–1.28]	.486
	Y	22.2% (59)	49.2% (131)	28.6% (76)	100% (266)					
BL2ht2c	N	54.5% (383)	40.1% (282)	5.4% (38)	100% (703)	.362	1.12[0.88–1.44]	.254	1.55[0.73–3.29]	.556
	Y	57.1% (152)	38.7% (103)	4.1% (11)	100% (266)					
BL2ht3c	N	61.7% (434)	35.4% (249)	2.8% (20)	100% (703)	.512	0.92[0.71–1.19]	.723	0.87[0.39–1.93]	.536
	Y	60.2% (160)	36.5% (97)	3.4% (9)	100% (266)					

Note. NP, nasal polyposis (N, negative; Y, positive); A1, common allele; A2, minor allele; OR, odds ratio; 95% CI, 95% confidence interval

*P values were obtained using logistic regression analysis controlling for age, sex, smoke status, atopy, and BMI as covariate.

high degree of genetic homogeneity [31], we consider that such a stratification bias is unlikely in our sample.

In conclusion, we evaluated the association of polymorphisms of *FABP1*, which is a candidate gene demonstrated by our proteomic approach in the nasal polyps of patients with AERD, with the risk and phenotypes of AERD in patients with asthma. Although we did not find a significant association between the *FABP1* polymorphisms and AERD, our data suggest that SNPs are not associated with the increased expression of FABP1 in asthmatic patients with AERD and that the search for other genetic factors should be extended.

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