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Potential Association of *DCBLD2* Polymorphisms with Fall Rates of FEV_1 by Aspirin Provocation in Korean Asthmatics

Tae-Joon Park[1], Jeong-Hyun Kim[1], Byung-Lae Park[2], Hyun Sub Cheong[2], Joon Seol Bae[1], Charisse F. Pasaje[1], Jong-Sook Park[3], Soo-Taek Uh[3], Mi-Kyeong Kim[4], Inseon S. Choi[5], Choon-Sik Park[3], and Hyoung Doo Shin[1,2]

[1]Department of Life Science, Sogang University, Seoul; [2]Department of Genetic Epidemiology, SNP Genetics Inc., Seoul; [3]Genome Research Center for Allergy and Respiratory Diseases, Division of Allergy and Respiratory Medicine, Soonchunhyang University Bucheon Hospital, Bucheon; [4]Department of Internal Medicine, Chungbuk National University College of Medicine, Cheongju; [5]Department of Allergy, Chonnam National University Medical School and Research Institute of Medical Sciences, Gwangju, Korea

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Address for Correspondence:
Hyoung Doo Shin, PhD
Department of Life Science, Sogang University,
35 Baekbeom-ro, Mapo-gu, Seoul 121-742, Korea
Tel: +82.2-3273-1671, Fax: +82.2-3273-1680
E-mail: hdshin@sogang.ac.kr

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Aspirin exacerbated respiratory disease (AERD) is a clinical syndrome characterized by chronic rhinosinusitis with nasal polyposis and aspirin hypersensitivity. The aspirin-induced bronchospasm is mediated by mast cell and eosinophilic inflammation. Recently, it has been reported that the expression of *discoidin, CUB* and *LCCL domain-containing protein 2 (DCBLD2)* is up-regulated in lung cancers and is regulated by transcription factor AP-2 alpha (TFAP2A), a component of activator protein-2 (AP-2) that is known to regulate IL-8 production in human lung fibroblasts and epithelial cells. To investigate the associations between AERD and *DCBLD2* polymorphisms, 12 common variants were genotyped in 163 AERD subjects and 429 aspirin tolerant asthma (ATA) controls. Among these variants, seven SNPs (*rs1371687*, *rs7615856*, *rs828621*, *rs828618*, *rs828616*, *rs1062196*, and *rs8833*) and one haplotype (*DCBLD2-ht1*) show associations with susceptibility to AERD. In further analysis, this study reveals significant associations between the SNPs or haplotypes and the percentage of forced expiratory volume in one second (FEV_1) decline following aspirin challenge using multiple linear regression analysis. Furthermore, a non-synonymous SNP *rs16840208* (*Asp723Asn*) shows a strong association with FEV_1 decline in AERD patients. Although further studies for the non-synonymous *Asp723Asn* variation are needed, our findings suggest that *DCBLD2* could be related to FEV_1-related phenotypes in asthmatics.

Key Words: DCBLD2; Aspirin Exacerbated Respiratory Diseases; Single Nucleotide Polymorphism (SNP); Haplotype

INTRODUCTION

Aspirin-exacerbated respiratory disease (AERD), is a clinical syndrome with inflammatory responses in both the upper and lower respiratory tracts (1). It is characterized by chronic rhinosinusitis with nasal polyposis which is followed by asthma and hypersensitivity to aspirin (2). Aspirin-induced bronchospasm in asthma patients are mediated by mast cell and eosinophilic inflammation which is already ongoing before the first aspirin ingestion (2, 3). The mechanisms for pathogenesis of AERD are not completely explained, however, it has been suggested that over-production of cysteinyl leukotriene (CysLT) and its receptor on inflammatory cells occurs in the respiratory tract of AERD patients (4).

The transmembrane protein encoded by *discoidin, CUB* and *LCCL domain-containing protein 2* (*DCBLD2*) gene, also abbreviated as *CLCP1* or *ESDN*, is regarded as a contributor for regulation of cell proliferation. Our previous genome-wide and follow-up studies showed nominal associations between *DCBLD2* polymorphisms and AERD (5). Despite little information on the function of *DCBLD2*, this gene has also been elucidated to be up-regulated in the development and metastasis of lung cancers (6). In addition, the transcription factor TFAP2A induces an inhibitory effect on *DCBLD2* transcription (7). The TFAP2A, an important transcription factor, is a component of activator protein-2 (AP-2) that regulates IL-8 expression in human lung fibroblasts and epithelial cells (8). On the other hand, previous studies have also proposed that the up-regulation of *DCBLD2*

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could be involved in pathways related to immune and injury-mediated remodeling (9, 10). In addition, *neuropilin-1*, whose domains are structurally similar with *DCBLD2* domain proteins and has been identified as an isoform of a specific vascular endothelial growth factor receptor in human airway epithelial cells, showed a higher expression in patients who have chronic rhinosinusitis and/or nasal polyposis (9, 11).

Based on the possible relations of *DCBLD2* to airway remodeling, we investigate further the associations of *DCBLD2* single nucleotide polymorphisms (SNPs) with the fall rates of forced expiratory volume by aspirin provocation as well as AERD development under various genetic models.

MATERIALS AND METHODS

Study subjects

Asthmatic subjects were recruited from the Asthma Genome Research Center comprising hospitals of Soonchunhyang University in Seoul and Bucheon, Chungnam National University and Chungbuk National University in Korea. Patients met the definition of asthma based on the Global Initiative for Asthma guidelines 2010 (http://www.ginasthma.org/guidelines-gina-report-global-strategy-for-asthma.html). All subjects had a history of dyspnea and wheezing during the past year plus one of the following: 1) > 15% increase in forced expiratory volume in one second (FEV_1) or > 12% increase plus 200 mL following inhalation of a short-acting bronchodilator, 2) < 10 mg/mL PC_{20} methacholine, or 3) > 20% increase in FEV_1 following 2 weeks of treatment with inhaled steroids and long-acting bronchodilators. Twenty-four common inhalant allergens (e.g., dust mites, cat fur, dog fur, cockroaches, grasses, trees, ragweed pollen; Bencard Co. Ltd., Brentford, UK) were used in a skin-prick test. Total IgE was measured by the CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Atopy was defined as a wheal reaction equal to or greater than histamine or 3 mm in diameter.

The oral aspirin provocation test was performed with slight modifications in increasing doses of aspirin (12), following the guideline of EAACI/GA2LEN (13). Briefly, the patient having history of aspirin hypersensitivity was given 30 mg and those having no history started 100 mg of aspirin orally. Symptoms, external signs (urticaria and angioedema), blood pressure and FEV_1 were documented every 30 min for a period of 2 hr. In the absence of any symptoms or signs suggestive of adverse reaction after 2 hr, 60 mg or 100 mg of aspirin was administered and the same measurements were repeated every 1 hr, with doses of 450 mg until the patient developed a reaction. If no reaction occurred 5 hr after the final dose, the test was deemed negative. Changes in the FEV_1 were followed for 5 hr after the final aspirin dose. Aspirin-induced bronchospasm, reflected by the rate (%) of decline in FEV_1, was calculated as the pre challenge FEV_1 minus the post challenge FEV_1 divided by the pre-challenge FEV_1. Subjects were categorized into two groups based on OAC reactions: ≥ 20% decrease in FEV_1 or a 15%-19% decrease in FEV_1 with naso-ocular reactions as AERD patients group, whereas a < 15% decrease in FEV_1 without naso-ocular reactions as ATA controls group.

SNP selection and genotyping

We selected common SNPs based on the frequencies in Asian population from the International HapMap Project database (http://hapmap.ncbi.nlm.gov/index.html.en). The selected 12 SNPs were genotyped in a total of 592 asthmatic subjects composed of 163 AERD and 429 ATA subjects. Genotyping was carried out using TaqMan assay in the ABI prism 7900HT sequence detection system (Applied Biosystems, Carlsbad, California, USA) with the assessment of data quality by duplicate DNAs (n = 10). Genotype data were obtained using the ABI-PRISM sequence detection system (SDS) software version 2.3. SNPs that did not match the following standards were excluded from the study: 1) a minimum call rate of 95%; 2) no duplicate error; and 3) *P* values of Hardy-Weinberg Equilibrium more than 0.05. All the 12 SNPs of *DCBLD2* were successfully genotyped.

Statistics

The association of SNPs and haplotypes of *DCBLD2* with AERD was carried out with logistic analyses controlling for age, sex, smoking status, atopy and body mass index (BMI) as covariates using the Statistical Analysis System (SAS). The FEV_1 change induced by aspirin provocation which was considered as a continuous variable was subjected to a simple linear regression analysis, and the differences in the values among the genotypes or haplotypes were examined using a linear regression model that controlled for age, sex, atopy and smoking status as covariates. For linkage disequilibrium (LD), we examined Lewontin's D´ (|D´|) and the LD coefficient r^2 between all pairs of biallelic loci using the Haploview v4.1 software downloaded from the Broad Institute (http://www.broadinstitute.org/mpg/haploview) (14). Haplotypes were first estimated using PHASE software (15), and then computed by logistic analyses using SAS.

Ethics statement

The institutional review board approved the protocol (SCHBC_IRB_05_02), and all subjects provided informed consent.

RESULTS

Characteristics of study subjects

The clinical profiles of the study subjects are summarized in Table 1. All of the subjects were asthma patients and were divided into two groups, AERD patients and ATA control groups, according to degree of aspirin sensitivity. Overall, a decrease in FEV_1 of -15% to 68% induced by aspirin provocation was ob-

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Table 1. Clinical profiles of study subjects

Clinical profiles	AERD	ATA	*P* value
Number of subjects	163	429	
Age of first medical examination (mean [range])	43.13 (17.22-72.73)	47.30 (15.40-77.88)	0.001
Body mass index (kg/m^2)	23.39 ± 3.25	24.58 ± 3.39	0.001
Fall rate of FEV_1 (%)	24.63 ± 16.11	3.54 ± 4.85	< 0.001
Blood eosinophil (%)	5.96 ± 5.21	6.03 ± 5.92	0.88
FEV_1 (% predicted)	87.58 ± 16.94	91.66 ± 16.87	0.009
PC_{20} methacholine (mg/mL)	5.02 ± 7.83	6.91 ± 8.90	0.02
Total IgE (IU/mL)	348.60 ± 596.44	361.00 ± 607.56	0.83
Sex (male/female)	59/104	147/282	0.66
Current smoker (%)	21.47	30.07	0.02
Positive rate of skin test (%)	52.76	57.81	0.27

Clinical profile of AERD was compared to ATA controls. AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma.

Table 2. Single nucleotide polymorphisms and minor allele frequencies of human *DCBLD2* in Korean subjects

SNP/Haplotype	Allele	Position	Amino acid change	HWE*	MAF
rs2439224	T > C	Intron1		0.482	0.158
rs1371687	C > T	Intron1		0.800	0.316
rs9838238	T > C	Exon2	I144M	0.459	0.029
rs17278047	G > C	Intron2		0.102	0.064
rs7615856	T > C	Intron2		0.962	0.288
rs828621	A > T	Intron4		0.852	0.288
rs828618	G > A	Intron4		0.440	0.428
rs828616	A > G	Exon6	I262I	0.892	0.318
rs16840208	C > T	Exon16	D723N	0.107	0.055
rs17270986	A > G	3'UTR		0.955	0.156
rs1062196	A > G	3'UTR		0.952	0.193
rs8833	T > C	3'UTR		0.939	0.284
DCBLD2-ht1				0.482	0.427
DCBLD2-ht2				0.581	0.254
DCBLD2-ht3				0.554	0.101
DCBLD2-ht4				0.107	0.055
DCBLD2-ht5				0.893	0.059

**P* values of deviation from Hardy-Weinberg Equilibrium (HWE) in Korean population. MAF, minor allele frequency.

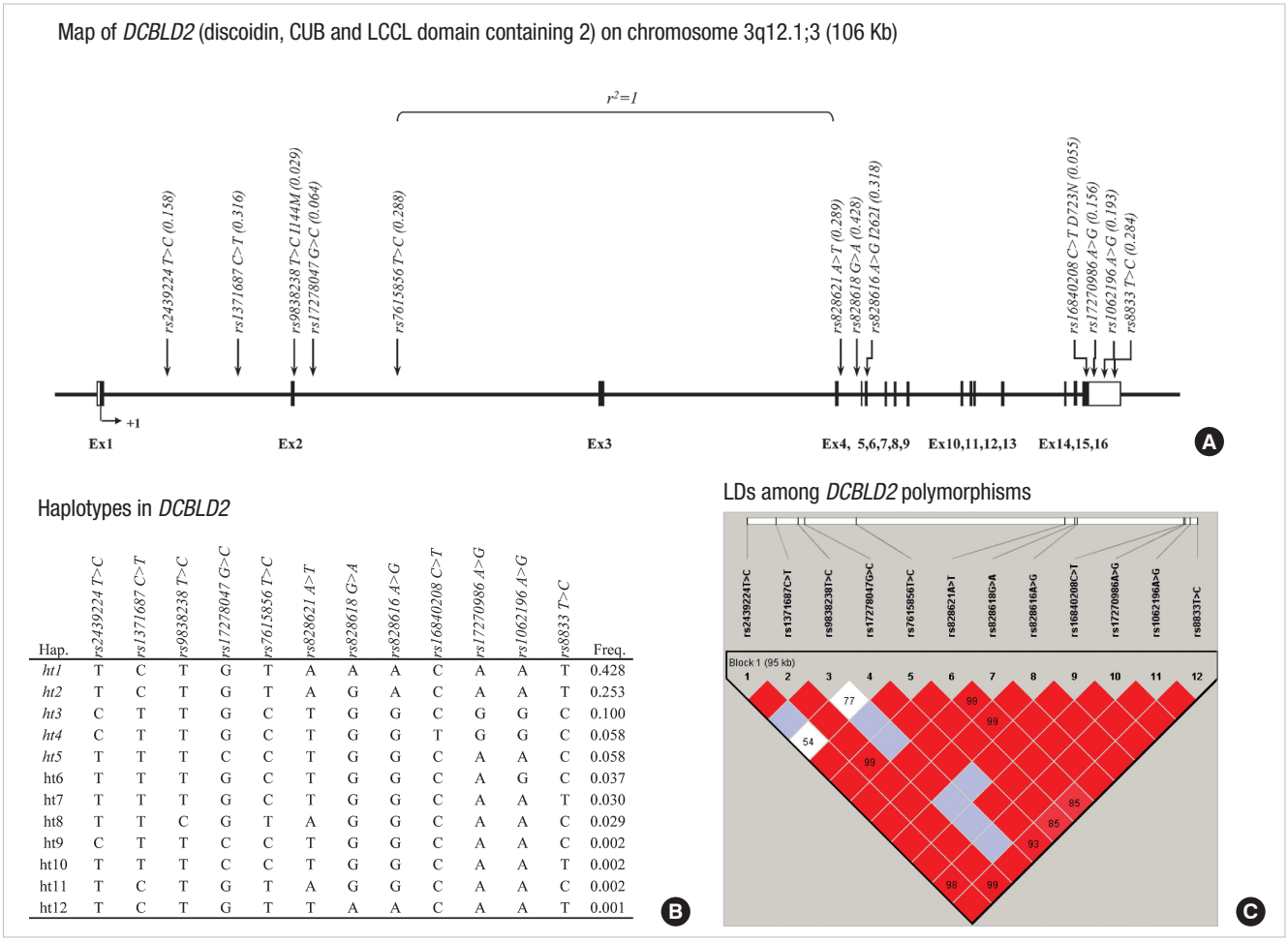
served. The percentage of decrease in FEV_1 by aspirin provocation in AERD patients (24.6%) were significantly higher compared to ATA controls (3.5%; $P < 0.001$). The values of predicted FEV_1 %, PC_{20} methacholine and body mass index in AERD patients were significantly lower than those of ATA controls (P = 0.009, 0.02 and 0. 001, respectively). In addition, the mean age of a first medical examination in AERD was significantly lower than ATA ($P = 0.001$). Percentage of smoking status in AERD patients was also lower compared to ATA controls ($P = 0.02$).

Genotyping and haplotypes of *DCBLD2* polymorphisms

We selected 12 common polymorphisms of *DCBLD2*, six in exon regions and six in introns, based on the frequencies on Asian population from the International HapMap Project (Table 2, Fig. 1). The minor allele frequencies (MAFs) of these 12 SNPs in the Korean asthmatics (n = 592) are shown in Table 2. The genotype distributions of all loci are in Hardy-Weinberg equilibrium (Table 2). The linkage disequilibrium coefficients (|D´|) among the SNPs were calculated for all of the study subjects (Fig. 1). Complete LDs were observed between two SNPs; *rs7615856* and *rs828621* ($r^2 = 1$). Twelve haplotypes were constructed and five of them with frequencies over 0.05 (*DCBLD-ht1* to *DCBLD-ht5*) were included in the association analysis (Fig. 1).

Associations of *DCBLD2* polymorphisms with FEV_1 decline and AERD development

Initially, this study investigated the associations between genotypes or haplotypes and the percentage of FEV_1 decline following aspirin challenge using multiple linear regression analysis (Table 3). Interestingly, nine SNPs (*rs2439224, rs1371687, rs7615856, rs828621, rs828618, rs828616, rs16840208, rs17270986* and *rs8833*) were significantly associated with the percentage FEV_1 decline induced by aspirin challenge in the asthmatics ($P < 0.05$, Table 3). Furthermore, among the significantly associated SNPs, a non-synonymous SNP *rs16840208* (*D723N* or *Asp723Asn*) was found to induce a strong genetic effect ($P = 0.02$ under co-dominant; $P = 0.009$ in dominant model). In the haplotype analysis, *DCBLD2-ht1* and *DCBLD2-ht4* were also associated with the



Hap.	rs2439224 T>C	rs1371687 C>T	rs9838238 T>C	rs17278047 G>C	rs7615856 T>C	rs828621 A>T	rs828618 G>A	rs828616 A>G	rs16840208 C>T	rs17270986 A>G	rs1062196 A>G	rs8833 T>C	Freq.
ht1	T	C	T	G	T	A	A	A	C	A	A	T	0.428
ht2	T	C	T	G	T	A	G	A	C	A	A	T	0.253
ht3	C	T	T	G	C	T	G	G	C	G	G	C	0.100
ht4	C	T	T	G	C	T	G	G	T	G	G	C	0.058
ht5	T	T	T	C	C	T	G	G	C	A	A	C	0.058
ht6	T	T	T	G	C	T	G	G	C	A	G	C	0.037
ht7	T	T	T	G	C	T	G	G	C	A	A	T	0.030
ht8	T	T	C	G	T	A	G	G	C	A	A	C	0.029
ht9	C	T	T	C	C	T	G	G	C	A	A	C	0.002
ht10	T	T	T	C	C	T	G	G	C	A	A	T	0.002
ht11	T	C	T	G	T	A	G	G	C	A	A	C	0.002
ht12	T	C	T	G	T	T	A	A	C	A	A	T	0.001

Fig. 1. Gene maps and haplotypes of *DCBLD2*. (**A**) Polymorphisms of *DCBLD2* investigated in this study. Coding exons are marked by shaded blocks; 5´- and 3´-untranslated region (UTR) by white blocks. Two SNPs (*rs7615856* and *rs828621*) are in complete LD ($r^2 = 1$). (**B**) Haplotypes of *DCBLD2* in the Korean population. Only those with frequencies over 0.05 are analyzed for associations. (**C**) LD blocks and correlation coefficients among *DCBLD2* polymorphisms.

Table 3. Association of SNPs and haplotypes of *DCBLD2* with FEV_1 decline by aspirin provocation in asthmatics

SNP/Haplotype	Allele	Genotype			*P* value		
		C/C	C/R	R/R	Co-dominant	Dominant	Recessive
rs2439224	T > C	423 (10.03 ± 14.10)	152 (7.30 ± 10.88)	17 (6.71 ± 5.62)	**0.02**	**0.02**	0.45
rs1371687	C > T	281 (10.15 ± 14.30)	251 (9.17 ± 12.89)	60 (5.23 ± 7.48)	**0.03**	0.16	**0.02**
rs9838238	T > C	558 (9.34 ± 13.36)	34 (7.52 ± 10.75)	-	0.59	0.59	-
rs17278047	G > C	522 (9.47 ± 13.51)	65 (7.56 ± 10.94)	5 (6.38 ± 7.34)	0.39	0.40	0.69
rs7615856	T > C	304 (10.03 ± 14.09)	239 (9.04 ± 12.91)	49 (5.21 ± 7.14)	**0.04**	0.17	**0.03**
rs828621	A > T	304 (10.03 ± 14.09)	238 (9.10 ± 12.91)	50 (5.05 ± 7.16)	**0.04**	0.17	**0.02**
rs828618	G > A	190 (7.02 ± 9.20)	294 (10.06 ± 14.48)	108 (10.87 ± 15.12)	**0.02**	**0.01**	0.23
rs828616	A > G	279 (10.25 ± 14.30)	253 (9.07 ± 12.89)	60 (5.23 ± 7.48)	**0.02**	0.12	**0.02**
rs16840208	C > T	531 (9.71 ± 13.54)	57 (4.91 ± 9.31)	4 (7.68 ± 4.71)	**0.02**	**0.009**	0.80
rs17270986	A > G	423 (10.03 ± 14.10)	155 (7.22 ± 10.80)	14 (7.41 ± 5.86)	**0.03**	**0.02**	0.66
rs1062196	A > G	386 (9.75 ± 13.45)	184 (8.53 ± 13.31)	21 (6.24 ± 6.07)	0.15	0.19	0.33
rs8833	T > C	305 (10.17 ± 14.02)	240 (8.82 ± 12.90)	47 (5.29 ± 7.74)	**0.04**	0.12	0.05
		-/-	+/-	+/+			
DCBLD2-ht1		191 (6.97 ± 9.20)	293 (10.11 ± 14.48)	108 (10.87 ± 15.12)	**0.02**	**0.01**	0.23
DCBLD2-ht2		332 (9.34 ± 13.64)	219 (9.23 ± 13.25)	41 (8.33 ± 9.16)	0.81	0.90	0.74
DCBLD2-ht3		479 (9.45 ± 13.72)	108 (8.29 ± 11.06)	5 (9.08 ± 6.22)	0.46	0.44	0.95
DCBLD2-ht4		531 (9.71 ± 13.54)	57 (4.91 ± 9.31)	4 (7.68 ± 4.71)	**0.02**	**0.009**	0.80
DCBLD2-ht5		525 (9.43 ± 13.49)	65 (7.76 ± 11.03)	2 (4.45 ± 1.63)	0.47	0.51	0.64

Genotype distribution of each SNP is presented as the number of subjects (percentage of FEV_1 decline by aspirin provocation, mean ± SE). *P* values for linear regression analysis controlling age, sex, smoking status and atopy as covariates. C/C, Major homozygote; C/R, Heterozygote; R/R, Minor homozygote.

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Table 4. Analyses of association of *DCBLD2* polymorphisms with risk of AERD

SNP/Haplotype	Allele	Position	MAF		Co-dominant		Dominant		Recessive	
			AERD (n = 163)	ATA (n = 429)	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
rs2439224	T > C	Intron1	0.129	0.170	0.74 (0.51-1.07)	0.11	0.71 (0.47-1.09)	0.11	0.61 (0.17-2.20)	0.45
rs1371687	C > T	Intron1	0.267	0.334	0.75 (0.56-1.00)	0.05	0.72 (0.50-1.04)	0.08	0.61 (0.31-1.23)	0.17
rs9838238	T > C	Exon2	0.025	0.030	0.83 (0.36-1.91)	0.66	0.83 (0.36-1.91)	0.66	-	-
rs17278047	G > C	Intron2	0.052	0.068	0.89 (0.51-1.55)	0.68	0.88 (0.48-1.62)	0.69	0.81 (0.08-7.87)	0.86
rs7615856	T > C	Intron2	0.242	0.304	0.75 (0.56-1.02)	0.06	0.73 (0.50-1.05)	0.09	0.62 (0.29-1.32)	0.21
rs828621	A > T	Intron4	0.242	0.305	0.75 (0.56-1.01)	0.06	0.73 (0.50-1.05)	0.09	0.60 (0.28-1.29)	0.19
rs828618	G > A	Intron4	0.482	0.409	1.31 (1.00-1.71)	**0.05**	1.32 (0.88-1.99)	0.18	1.57 (0.99-2.48)	0.05
rs828616	A > G	Exon6	0.267	0.337	0.74 (0.55-0.99)	**0.04**	0.71 (0.49-1.02)	0.07	0.61 (0.31-1.23)	0.17
rs16840208	C > T	Exon16	0.040	0.062	0.66 (0.35-1.23)	0.19	0.61 (0.31-1.20)	0.15	0.99 (0.10-9.79)	0.99
rs17270986	A > G	Exon16	0.129	0.167	0.75 (0.51-1.09)	0.13	0.71 (0.47-1.09)	0.11	0.79 (0.21-2.93)	0.73
rs1062196	A > G	Exon16	0.163	0.204	0.76 (0.54-1.08)	0.13	0.68 (0.46-1.02)	0.06	1.16 (0.43-3.10)	0.77
rs8833	T > C	Exon16	0.236	0.302	0.75 (0.56-1.02)	0.07	0.71 (0.49-1.03)	0.07	0.71 (0.33-1.53)	0.39
DCBLD2-ht1			0.482	0.408	1.32 (1.01-1.72)	**0.05**	1.33 (0.89-2.01)	0.17	1.57 (0.99-2.48)	0.05
DCBLD2-ht2			0.252	0.254	0.99 (0.74-1.34)	0.95	0.95 (0.65-1.37)	0.77	1.17 (0.57-2.40)	0.67
DCBLD2-ht3			0.089	0.105	0.83 (0.53-1.31)	0.43	0.84 (0.52-1.35)	0.46	0.59 (0.06-5.50)	0.64
DCBLD2-ht4			0.040	0.062	0.66 (0.35-1.23)	0.19	0.61 (0.31-1.20)	0.15	0.99 (0.10-9.79)	0.99
DCBLD2-ht5			0.049	0.062	0.92 (0.51-1.67)	0.79	0.95 (0.52-1.74)	0.86	-	-

Logistic analyses controlling for age, sex, smoking status, atopy and body mass index (BMI) as covariates are performed using the Statistical Analysis System (SAS). OR (95% CI) and *P* values of co-dominant model are also reported in the supporting information of our previous report (Kim et al. 2010b). MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

percentage FEV$_1$ decline induced by aspirin challenge in the asthmatics (*P* = 0.009 for *DCBLD2-ht4* under dominant model to *P* = 0.02 for *DCBLD2-ht1* and *DCBLD2-ht4* under co-dominant, Table 3).

Furthermore, results from regression analysis comparing AERD and ATA groups revealed that nine SNPs (*P* = 0.001 for *rs828618* under dominant model to *P* = 0.05 for *rs2439224*, *rs828618*, *rs17270986* under co-dominant) and two haplotypes (*P* = 0.001 for *DCBLD2-ht1* under dominant model to *P* = 0.05 for *DCBLD2-ht1* under dominant; *P* = 0.03 for *DCBLD2-ht2* under recessive) were significantly associated with the percentage FEV$_1$ decline induced by aspirin challenge in AERD patients (Supplementary Table 1). In contrast, findings from regression analysis of ATA group showed no significant association between *DCBLD2* SNPs and FEV$_1$ decline induced by aspirin provocation, except for *DCBLD2-ht1* haplotype (*P* = 0.02 in recessive model, Supplementary Table 1).

In further association analysis between *DCBLD2* SNPs/haplotypes and the risk of AERD using multiple logistic models, two SNPs (*rs828618* and *rs828616*; *P* = 0.05 and *P* = 0.04, respectively) and one haplotype (*DCBLD2-ht1*; *P* = 0.05) showed nominal signals with the risk of AERD (Table 4).

DISCUSSION

To our knowledge, this study is the first to investigate the association between *DCBLD2* and AERD. In this study, data from logistic and linear regression analyses showed significant associations of *DCBLD2* polymorphisms with FEV$_1$ decline by aspirin provocation (*P* = 0.009 for *rs16840208* under dominant model to *P* = 0.04 for *rs7615856*, *rs828621* and *rs8833* under co-dominant, Table 3) and nominal signals to AERD development (*P* = 0.04 for *rs828616* to *P* = 0.05 for *rs828618* under co-dominant model, Table 4). Furthermore, the strength of association with the fall rate of FEV$_1$ by aspirin provocation was increased in the AERD subgroup (*P* = 0.001 for *rs828618* under dominant model to *P* = 0.05 for *rs2439224*, *rs828618*, *rs17270986* under co-dominant, Supplementary Table 1) compared to ATA (*P* > 0.05), suggesting that genetic variations of *DCBLD2* may affect decline of FEV$_1$ by aspirin provocation in AERD patients.

Recently, a significant up-regulation of *DCBLD2*, previously identified as *CLCP1*, has been elucidated in the metastasis of lung cancer cell line (6), suggesting that *DCBLD2* potentially plays a role in lung-related functions. In addition, *DCBLD2*, also known as ESDN, is a key factor in the modulation of vascular smooth muscle cell (VSMC) growth and regulation of VSMC proliferation processes that are involved in vascular remodeling. DCBLD2 is up-regulated in remodeling arteries, and its down-regulation by RNA interference (RNAi) has been found to significantly enhance VSMC DNA synthesis and migration through induction of platelet-derived growth factor (PDGF), a prototypic growth factor for VSMCs (10, 16).

The transcription factor TFAP2A, a member of the AP-2 family, plays an important role in regulation of *DCBLD2* transcription. It has been shown that TFAP2A binding to *DCBLD2* promoter region induces a decrease in promoter activity measured by luciferase assay in this gene. Conversely, mutations in TFAP2A binding site in *DCBLD2* promoter lead to the increase in tran-

scription activity (7). AP-2 is a regulator for IL-8 production that is associated with the destructive pulmonary inflammation and the recruitment of neutrophils. Deletion of AP-2 binding site in the upstream region of *IL-8* promoter significantly decreased *IL-8* transcription activity (8), suggesting that DCBLD2 might be related to the production of interleukins in human pulmonary diseases through interaction with AP-2. This possibility is supported by the facts that discodin domain affects immune responses in the involvement of interleukins (17, 18).

Although the functional properties of DCBLD2 in tumor development and vascular remodeling have been reported, to date no previous study has proposed the functional or genetic effects of *DCBLD2* on asthma and aspirin-induced hypersensitivity. However, neuropilin-1, which is structurally similar to DCBLD2 (19) has been found to be related to the suppressor function of $CD4^+CD25^+$ T cells in airway inflammation and hyper-responsiveness (20). In addition, neuropilin-1 is associated with the development of nasal polyposis, one of the symptoms of AERD. Distribution of neuropilin-1, as a co-receptor of vascular endothelial growth factor (VEGF), was shown to be 7-fold higher in nasal lavage from patients with polyposis than control subjects. Moreover, blockade of VEGF, which is suppressed by neuropilin-1, results in increased apoptosis and inhibition of autocrine epithelial VGEF production (11). Therefore, with the structural similarity to neuropilin-1, there is a possibility DCBLD2 may play a similar role in aspirin sensitivity to asthma.

The bronchoalveolar lavage (BAL) in asthma has been implicated in asparagine levels. The BAL fluid from asthmatic patients showed higher level of asparagine (Asn, N) than from normal controls (21). Other studies have reported that a polymorphism in the human *neuropeptide S receptor* (*NPSR*) which produces an amino acid substitution from Asn to isoleusine (Ile) (*Asn107Ile*) is associated with susceptibility to asthma. The *Asn107Ile* polymorphism of *NPSR* was found to be highly expressed in human asthmatic airway tissue (22, 23). In the present study, *rs16840208* (*Asp723Asn*) is significantly associated with FEV_1 decline by aspirin provocation ($P = 0.02$ under co-dominant and 0.009 under dominant model), suggesting that the amino acid change from aspartic acid (Asp) to asparagine of *rs16840208* may be a genetic factor affecting the risk of pulmonary diseases including asthma.

Among the SNPs associated with FEV_1 decline by aspirin provocation and/or AERD, 5 SNPs (*rs2439224, rs1371687, rs7615856, rs828621*, and *rs828618*) were located in intronic region of *DCBLD2*. Despite difficulty in assessing the functions of SNPs that are not positioned at the exon and promoter regions, previous studies have suggested that intronic SNPs may play important roles in gene transcription rate and abnormal splicing events such as exon skipping, activation of cryptic splice sites and production of alternatively spliced isoforms in human disease phenotypes (24, 25). Furthermore, it has also been reported that intronic SNPs in *solute carrier family 6, member 7* (*SLC6A7*) (26), *solute carrier family 6, member 12* (*SLC6A12*) (27), *kinesin family number 3A* (*KIF3A*) (28), *calcium channel, voltage-dependent, gamma subunit 6* (*CACNG6*) (29) and *fibrous sheath interacting protein 1* (*FSIP1*) (30) are associated with asthma and/or decline in FEV_1 by aspirin challenge.

In summary, findings from this study provide evidences that genetic polymorphisms of *DCBLD2* including a non-synonymous *rs16840208* (*Asp723Asn*) might induce decline in FEV_1 by aspirin ingestion in Korean asthma patients. Furthermore, considering that 5-lipoxygenase, whose pathway is one of the central mechanisms to produce CysLTs from arachidonic acid (31), plays a role in VSMC that is modulated by DCBLD2 (10, 32), these findings suggest that genetic variants of *DCBLD2* could provide a new strategy for the control of aspirin intolerance.

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Supplementary Table 1. Association of SNPs and haplotypes of *DCBLD2* with FEV_1 decline by aspirin provocation in AERD and in ATA group

	SNP/ Haplotype	Allele	Genotype			*P* value		
			C/C	C/R	R/R	Co-dominant	Dominant	Recessive
In AERD	*rs2439224*	T > C	123 (25.78 ± 16.67)	34 (21.51 ± 13.92)	3 (12.80 ± 5.94)	**0.05**	0.08	0.19
	rs1371687	C > T	87 (25.22 ± 16.56)	62 (25.57 ± 15.96)	11 (14.66 ± 9.85)	0.18	0.55	**0.03**
	rs9838238	T > C	152 (24.90 ± 16.14)	8 (19.51 ± 15.67)	0 (.)	0.47	0.47	-
	rs17278047	G > C	144 (24.82 ± 16.59)	15 (23.21 ± 11.46)	1 (18)	0.63	0.67	0.73
	rs7615856	T > C	93 (24.87 ± 16.55)	58 (25.92 ± 15.81)	9 (13.81 ± 9.15)	0.26	0.71	**0.03**
	rs828621	A > T	93 (24.87 ± 16.55)	58 (25.92 ± 15.81)	9 (13.81 ± 9.15)	0.26	0.71	**0.03**
	rs828618	G > A	43 (17.72 ± 12.43)	79 (28.51 ± 15.84)	38 (24.36 ± 17.97)	**0.05**	**0.001**	0.95
	rs828616	A > G	87 (25.22 ± 16.56)	62 (25.57 ± 15.96)	11 (14.66 ± 9.85)	0.18	0.55	**0.03**
	rs16840208	C > T	149 (25.15 ± 16.28)	10 (18.71 ± 12.06)	1 (6)	0.07	0.10	0.22
	rs17270986	A > G	123 (25.78 ± 16.67)	34 (21.51 ± 13.92)	3 (12.80 ± 5.94)	**0.05**	0.08	0.19
	rs1062196	A > G	115 (24.38 ± 15.67)	39 (27.60 ± 17.26)	6 (9.88 ± 7.59)	0.45	0.94	**0.02**
	rs8833	T > C	94 (24.99 ± 16.24)	57 (25.70 ± 16.20)	9 (14.03 ± 10.90)	0.25	0.65	**0.04**
	DCBLD2-ht1		43 (17.72 ± 12.43)	79 (28.51 ± 15.84)	38 (24.36 ± 17.97)	**0.05**	**0.001**	0.95
	DCBLD2-ht2		91 (24.70 ± 17.18)	57 (26.59 ± 14.04)	12 (14.72 ± 14.32)	0.38	0.99	**0.03**
	DCBLD2-ht3		133 (25.17 ± 16.50)	26 (22.12 ± 14.24)	1 (17)	0.31	0.31	0.72
	DCBLD2-ht4		149 (25.15 ± 16.28)	10 (18.71 ± 12.06)	1 (6)	0.07	0.10	0.22
	DCBLD2-ht5		144 (24.82 ± 16.59)	16 (22.89 ± 11.14)	0 (.)	0.67	0.67	-
In ATA	*rs2439224*	T > C	297 (3.59 ± 4.88)	118 (3.20 ± 4.75)	14 (5.40 ± 4.80)	0.86	0.75	0.17
	rs1371687	C > T	191 (3.41 ± 5.16)	189 (3.79 ± 4.53)	49 (3.11 ± 4.85)	0.94	0.55	0.44
	rs9838238	T > C	403 (3.52 ± 4.85)	26 (3.83 ± 4.87)	0 (.)	0.77	0.77	-
	rs17278047	G > C	375 (3.63 ± 4.89)	50 (2.86 ± 4.61)	4 (3.48 ± 3.96)	0.37	0.33	0.91
	rs7615856	T > C	208 (3.51 ± 5.15)	1 81 (3.64 ± 4.48)	40 (3.28 ± 4.96)	0.98	0.79	0.62
	rs828621	A > T	208 (3.51 ± 5.15)	180 (3.68 ± 4.46)	41 (3.12 ± 5.00)	0.92	0.79	0.50
	rs828618	G > A	147 (3.89 ± 4.64)	213 (3.29 ± 4.88)	69 (3.58 ± 5.21)	0.51	0.27	0.84
	rs828616	A > G	189 (3.48 ± 5.13)	191 (3.71 ± 4.57)	49 (3.11 ± 4.85)	0.89	0.75	0.44
	rs16840208	C > T	379 (3.70 ± 4.76)	47 (1.98 ± 5.18)	3 (8.23 ± 5.60)	0.23	0.08	0.10
	rs17270986	A > G	297 (3.59 ± 4.88)	121 (3.21 ± 4.71)	11 (5.94 ± 5.15)	0.85	0.75	0.11
	rs1062196	A > G	268 (3.56 ± 4.97)	145 (3.40 ± 4.64)	15 (4.79 ± 4.92)	0.75	1.00	0.34
	rs8833	T > C	208 (3.59 ± 5.09)	183 (3.56 ± 4.53)	38 (3.22 ± 5.08)	0.77	0.95	0.57
	DCBLD2-ht1		148 (3.85 ± 4.66)	212 (3.32 ± 4.87)	69 (3.58 ± 5.21)	0.56	0.32	0.84
	DCBLD2-ht2		240 (3.56 ± 4.79)	160 (3.13 ± 5.03)	29 (5.69 ± 3.81)	0.39	0.89	**0.02**
	DCBLD2-ht3		343 (3.42 ± 4.97)	82 (3.90 ± 4.25)	4 (7.10 ± 5.05)	0.23	0.34	0.14
	DCBLD2-ht4		379 (3.70 ± 4.76)	47 (1.98 ± 5.18)	3 (8.23 ± 5.60)	0.23	0.08	0.10
	DCBLD2-ht5		378 (3.63 ± 4.88)	49 (2.81 ± 4.70)	2 (4.45 ± 1.63)	0.40	0.33	0.74

Genotype distribution of each SNP is presented as the number of subjects (percentage of FEV_1 decline by aspirin provocation, mean ± SE). *P* values for linear regression analysis controlling age, sex, smoking status and atopy as covariates. C/C, Major allele homozygote; C/R, Heterozygote; R/R, Minor allele homozygote.