

GENETICS

Potential Association of *DDR1* Genetic Variant with FEV₁ Decline by Aspirin Provocation in Asthmatics

JASON YONGHA KIM, M.S.,¹ JEONG-HYUN KIM, PH.D.,¹ BYUNG-LAE PARK, PH.D.,²
CHARISSE FLERIDA A. PASAJE, M.S.,¹ JOON SEOL BAE, PH.D.,¹ SOO-TAEK UH, M.D.,³
YONG-HOON KIM, M.D.,⁴ MI-KYEONG KIM, M.D.,⁵ INSEON S. CHOI, M.D.,^{6,7} SANG HEON CHO, M.D.,^{8,9}
BYOUNG WHUI CHOI, M.D.,¹⁰ JONG SOOK PARK, M.D.,³ CHOON-SIK PARK, M.D.,³ AND
HYOUNG DOO SHIN, PH.D.^{1,2,*}

¹Department of Life Science, Sogang University, Seoul, Republic of Korea.

²Department of Genetic Epidemiology, SNP Genetics, Inc., Seoul, Republic of Korea.

³Division of Allergy and Respiratory Medicine, Soonchunhyang University Bucheon Hospital, Bucheon, Republic of Korea.

⁴Division of Allergy and Respiratory Disease, Soonchunhyang University Cheonan Hospital, Cheonan, Republic of Korea.

⁵Division of Internal Medicine, College of Medicine, Chungbuk National University, Cheongju, Republic of Korea.

⁶Department of Allergy, Chonnam National University Medical School, Gwangju, Republic of Korea.

⁷Research Institute of Medical Sciences, Gwangju, Republic of Korea.

⁸Department of Internal Medicine, College of Medicine, Seoul National University, Seoul, Republic of Korea.

⁹Institute of Allergy and Clinical Immunology, College of Medicine, Seoul National University, Seoul, Republic of Korea.

¹⁰Division of Pulmonology and Allergy, Department of Internal Medicine, Chung-Ang University Yongsan Hospital, Seoul, Republic of Korea.

Background. The discoidin domain receptor tyrosine kinase 1 (*DDR1*) is positioned within the major histocompatibility complex (MHC) region which plays an important role in the immune system. In addition, *DDR1* has been elucidated to be downregulated during the epithelial–mesenchymal transition of bronchial epithelium. **Objective.** To investigate the potential genetic associations between *DDR1* and aspirin-exacerbated respiratory disease (AERD), this study conducted association studies of *DDR1* single nucleotide polymorphisms (SNPs) with AERD and the obstructive symptom of forced expiratory volume in 1 s (FEV₁) decline after aspirin provocation. **Methods.** Nine common SNPs were genotyped in 93 AERD patients and 96 aspirin-tolerant asthma (ATA) controls. The genotype distributions of all loci were in Hardy–Weinberg equilibrium (HWE; $p > .05$). **Results.** In the results of logistic analyses using age, sex, smoking status, and atopy as covariates, *DDR1* rs1264320 in the intronic region showed a potent association signal with FEV₁ decline by aspirin provocation in asthmatics of this study even after corrections for multiple testing ($p = .003$ and corrected $p = .01$). However, the variants of *DDR1* were not significantly associated with the AERD development (corrected $p > .05$). On further comparison of FEV₁ decline by aspirin provocation between AERD and ATA, the variant rs1264320 was found to be associated with the FEV₁ decline of ATA rather than AERD. **Conclusion.** Despite the need for further functional evaluations and replications, we conclude that *DDR1* polymorphisms are not likely to contribute to predispositions of AERD, but may be potentially associated with FEV₁ decline by aspirin provocation in asthmatics.

Keywords aspirin-exacerbated respiratory disease (AERD), *DDR1*, haplotype, single nucleotide polymorphism

INTRODUCTION

About 300 million patients worldwide are currently suffering from asthma (1), and about 20% of the asthma patients have aspirin-exacerbated respiratory disease (AERD) (2–4). AERD is characterized by symptoms such as lung inflammation and difficulty of breathing upon ingestion of non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin (5, 6). The clinical characteristics of AERD include a combination of aspirin sensitivity, bronchial asthma, and chronic rhinosinusitis with nasal polyposis (7–9). Aspirin, also known as acetylsalicylic acid, is primarily used to relieve pain, fever, and inflammation. Consistent

inflammation often results in an airway remodeling and an irreversible structure change of airway epithelium, leading to clinical symptoms of asthma (10, 11).

The discoidin domain receptor tyrosine kinase 1 (*DDR1*) is positioned on chromosome 6p21.3, an area known as the major histocompatibility complex (MHC) region. Although *DDR1* is expressed in several tissues including colon and mammary gland, the expression of *DDR1* is especially high at the basolateral surface of the bronchial epithelium in human, and the role of *DDR1* is critical in leukocyte migration (12, 13). Furthermore, *DDR1*, which is related to cytokine production in human macrophage (14), has been found to be a risk factor in the development of lung inflammation and fibrosis (15). In particular, *Ddr1*-deficient mice showed protection against bleomycin-induced lung fibrosis, indicating that the expression of *DDR1* and its interaction with

Jason Yongha Kim and Jeong-Hyun Kim contributed equally to this article.

*Corresponding author: Dr. Hyoung Doo Shin, Department of Life Science, Sogang University, Shinsu-dong, Seoul, Republic of Korea; Tel: 82-2-705-8615; Fax: 82-2-2026-4299; E-mail: hdshin@sogang.ac.kr

p38 mitogen-activated protein kinase (MAPK) activation may be one of the main factors for the development of lung inflammation and fibrosis (15).

In addition, the pathological conversion of epithelial cells into fibroblasts, also referred to as epithelial–mesenchymal transition (EMT), is thought to be one of the mechanisms for the increase of fibroblast, an event that is also closely associated with pathologies of airway diseases such as asthma (16). *DDR1* expression is downregulated during EMT of bronchial epithelium (16), suggesting that the expression of *DDR1* could affect the development of asthma and airway-related diseases. Based on these facts, this study aimed to investigate the potential genetic associations of *DDR1* polymorphisms with AERD development and the obstructive symptom of FEV₁.

SUBJECTS AND METHODS

Study Subjects

This study was conducted in compliance with the Global Initiative for Asthma (GINA)'s Global Strategy for Asthma Management and Prevention Study (17) and related guidelines (18). A total of 189 subjects were recruited from the Asthma Genome Research Center comprising hospitals of Soonchunhyang University, Chung-Ang University, Cheonnam University, Chungbuk University, and Seoul National University. 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 the inhalant bronchodilator-induced improvement with over 15% of forced expiratory volume in 1 s (FEV₁) and/or the airway hyperresponsiveness to the provocative concentration of P20 methacholine (19).

Twenty-four common inhalant allergens were used for a skin prick test (20). Total IgE was measured using the CAP system (Pharmacia Diagnostics, Uppsala, Sweden), and atopy was defined as having a wheal diameter with an inhalant allergen of at least 3 mm and equal to 60% of that induced by histamine without reaction of the negative control. Twenty-four common inhalant allergens (e.g., dust mites [*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*], cat fur, dog fur, cockroaches, grasses, trees, and ragweed pollens) (Bencard Co. Ltd, Brentford, UK) were used for a skin prick test. Oral aspirin challenge was performed with increasing doses of aspirin using methods slightly modified from those described previously (21, 22). Patients with history of aspirin hypersensitivity were given 30 mg and those having no history of aspirin hypersensitivity started with 100 mg of aspirin orally. Symptoms, external signs (urticaria and angioedema), blood pressure, and FEV₁ were documented every 30 min for a period of 2 h. In the absence of any symptoms or signs suggestive of adverse reaction after 2 h, 60 or 100 mg of aspirin was administered and the same measurements were repeated every 1 h, with doses of 450 mg until the patient developed a reaction. If no reaction occurred 4 h after the final dose, the test was deemed negative. Changes in the FEV₁ were followed for

4 h after the final aspirin dose. Subjects were categorized into two groups as follows: 15% or greater decreases in FEV₁ with naso-ocular or cutaneous reactions as AERD case group and less than 15% decreases in FEV₁ without naso-ocular or cutaneous reactions as aspirin-tolerant asthma (ATA) control group.

SNP Genotyping

We selected nine common single nucleotide polymorphisms (SNPs) (minor allele frequency [MAF] > 0.05) based on the Asian population database from the International HapMap database (<http://hapmap.ncbi.nlm.nih.gov/>) and then genotyped the SNPs in 93 AERD and 96 ATA subjects. Genotyping was performed using Taqman assay with the assessment of data quality by duplicate DNAs ($n = 10$). SNPs that could not satisfy the following criteria were excluded from the analysis: (i) a minimum call rate of 95%, (ii) no duplicate error, (iii) Hardy–Weinberg equilibrium (HWE) of $p > .05$. As a result, a total of nine SNPs from *DDR1* were successfully genotyped.

Statistics

We examined Lewontin's D' (ID') and the linkage disequilibrium (LD) coefficient r^2 between all pairs of biallelic loci to determine LD among the SNPs. LD was obtained using the *Haploview* v4.1 program downloaded from the Broad Institute (23). Haplotypes were estimated using PHASE software (24) and then computed by logistic analyses using the Statistical Analysis System. Associations were analyzed using logistic models with age, gender, smoking status, and atopy as covariates. To provide the multiple testing correction for the p -value, we used the SNPSpD software to calculate the effective number of independent marker loci (<http://gump.qimr.edu.au/general/daledN/SNPSpD/>).

RESULTS

Characteristics of Study Subject

A total of 189 subjects including 93 AERD patients and 96 ATA controls were recruited for the study. The clinical characteristics of subjects are summarized in Table 1. Study subjects showed the male to female ratio to be ~1:2 for AERD and ~1:3 for ATA, as expected of the sex differences in adult asthma according to the Global Allergy and Asthma European Network (GA2LEN). The FEV₁ decline by aspirin provocation of AERD patients ($23.70 \pm 14.48\%$) was significantly higher than that of ATA controls ($0.94 \pm 2.76\%$, $p < .001$). However, no significant differences in blood eosinophil, methacholine PC₂₀, total IgE, smoking status, and positive rate of skin test were found in the subgroups of this study ($p > .05$, Table 1).

Genotyping, LD, and Haplotype of *DDR1* Polymorphisms

Based on the Asian population database International HapMap, a total of nine common SNPs of *DDR1* were selected and genotyped in a Korean population. Among the SNPs, two synonymous polymorphisms (*rs2229933* and *rs1049623*), which, respectively, correspond to silent

TABLE 1.—Clinical profiles of aspirin-induced asthma patients and controls.

Clinical profiles	AERD	ATA	<i>p</i> Value
Number of subjects (<i>n</i>)	93	96	
Sex (male/female)	32/61	24/72	.156
Age [mean (range)]	44.4 (17–73)	45.8 (15–77)	.497
Blood eosinophil (%)	6.29 ± 5.80	4.88 ± 4.19	.060
PC ₂₀ , methacholine (mg/ml)	4.23 ± 7.18	3.04 ± 4.27	.193
FEV ₁ decline by aspirin provocation	23.61 ± 14.48	0.94 ± 2.76	.001
Total IgE (IU/ml)	321.65 ± 623.31	309.54 ± 426.04	.878
Ex-smoker/current smoker (%)	15.63/9.38	6.45/12.90	.219
Positive rate of skin test (%)	61.46	56.99	.532

Notes: Data of blood eosinophil, PC₂₀, methacholine, and total IgE are presented as mean ± SD. AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma. Values significant at 0.05 are shown in bold.

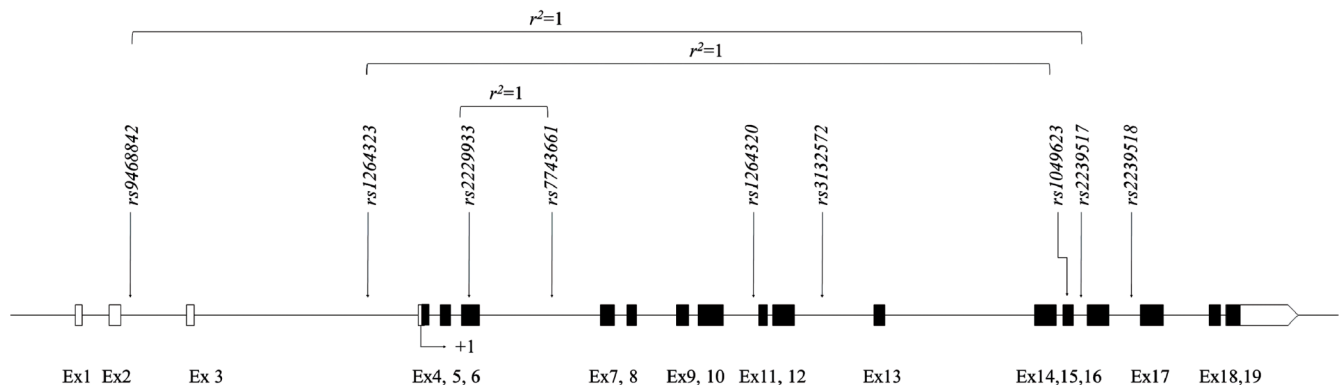
mutations of Leu94Leu and of Val599Val, are located in coding regions, while the other seven SNPs are positioned in intronic regions (Figure 1A and Supplementary Table 1; [http://informahealthcare.com/doi/suppl/\[doinumber\]](http://informahealthcare.com/doi/suppl/[doinumber])). All SNPs were in HWE ($p > .05$, Supplementary Table 1). Three complete LDs ($r^2 = 1$; *rs9468842* and *rs2239517*; *rs1264323* and *rs1049623*; *rs2229933* and *rs7743661*) were found among the SNPs genotyped in this study (Figure 1A). Pair-wise comparisons showed one tight LD block among the eight SNPs (Figure 1C), and six haplotypes were inferred using *PHASE* software (Figure 1B). Five

common haplotypes with frequency over 0.05 were used for the haplotype association analysis. In addition, haplotypes 1 and 2 were excluded from logistic analyses due to its equivalence to *rs2239518* and *rs1264320*, respectively.

Association Analyses of *DDR1* Polymorphisms with AERD Development and FEV₁ Decline

Regression analyses between *DDR1* variants and FEV₁ decline by aspirin provocation in asthmatics, adjusted to age, gender, smoking status, and atopy as covariates, were first conducted. Interestingly, *rs1264320* in the intronic

A. Map of *DDR1* (NM_001954) (*Discoidin domain receptor family member 1*) on chromosome 6p21.3



B. Haplotypes of *DDR1*

Hap.	<i>rs9468842</i>	<i>rs1264323</i>	<i>rs2229933</i>	<i>rs7743661</i>	<i>rs1264320</i>	<i>rs1049623</i>	<i>rs2239517</i>	<i>rs2239518</i>	Freq.
ht1	T	T	C	C	G	G	A	T	0.449
ht2	C	C	G	T	A	A	G	C	0.286
ht3	C	C	G	T	G	A	G	C	0.104
ht4	T	T	C	C	G	G	A	C	0.080
ht5	C	T	G	T	G	G	G	C	0.061
ht6	C	C	G	T	G	A	G	C	0.019

C. LDs among *DDR1* polymorphisms

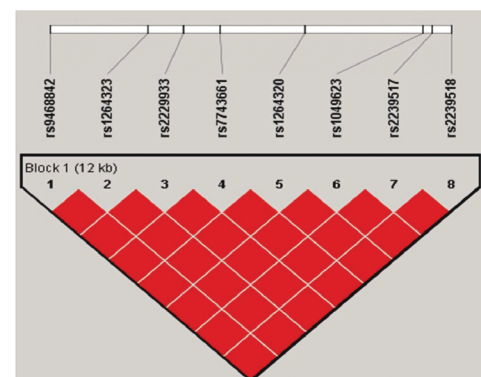


FIGURE 1.—Physical map, haplotypes, and LD of the *DDR1* gene. (A) Schematic gene map and SNPs in the *DDR1* gene on chromosome 6p21.3 (16kb). Black blocks represent coding exons and white blocks represent 5' and 3'UTR. The first base of translation site was denoted as nucleotide +1. (B) Haplotypes of *DDR1*. (C) LD coefficient (D') among *DDR1* SNPs in a Korean population. UTR, untranslated region.

TABLE 2.—Regression analyses of *DDR1* genetic variants and FEV₁ decline by aspirin provocation.

SNP/haplotype	Position	Amino acid change	C/C	C/R	R/R	<i>p_a</i>	<i>p_a^{cor.a}</i>	<i>p_b</i>	<i>p_b^{cor.a}</i>	<i>p_c</i>	<i>p_c^{cor.a}</i>
			[<i>n</i> (FEV ₁ decline)]	[<i>n</i> (FEV ₁ decline)]	[<i>n</i> (FEV ₁ decline)]						
<i>rs9468842</i>	Intron		55 (10.79 ± 13.55)	90 (13.99 ± 16.08)	44 (9.83 ± 15.79)	.57	1.00	.69	1.00	.16	.58
<i>rs1264323</i>	Intron		69 (10.34 ± 13.54)	84 (14.50 ± 16.11)	36 (9.83 ± 16.38)	.94	1.00	.41	1.00	.25	.92
<i>rs2229933</i>	Exon 6	Leu94Leu	55 (10.79 ± 13.55)	88 (13.87 ± 16.24)	44 (9.83 ± 15.79)	.56	1.00	.73	1.00	.17	.62
<i>rs7743661</i>	Intron		55 (10.79 ± 13.55)	89 (13.91 ± 16.16)	45 (10.07 ± 15.70)	.61	1.00	.69	1.00	.2	.71
<i>rs1264320</i>	Intron		96 (12.05 ± 15.08)	77 (14.11 ± 16.21)	15 (1.57 ± 7.16)	.16	.57	.84	1.00	.003	.01
<i>rs3132572</i>	Intron		180 (11.74 ± 14.87)	7 (19.14 ± 26.50)	—	.22	.79	.22	.79	—	—
<i>rs1049623</i>	Exon 14	Val599Val	69 (10.34 ± 13.54)	83 (14.46 ± 16.21)	35 (9.51 ± 16.51)	.87	1.00	.44	1.00	.22	.79
<i>rs2239517</i>	Intron		55 (10.79 ± 13.55)	88 (13.87 ± 16.24)	44 (9.83 ± 15.79)	.56	1.00	.73	1.00	.17	.62
<i>rs2239518</i>	Intron		61 (11.48 ± 15.96)	87 (13.14 ± 15.36)	41 (10.79 ± 14.60)	.8	1.00	.48	1.00	.71	1.00
<i>DDR1_h13</i>			150 (11.11 ± 14.82)	34 (16.04 ± 18.00)	2 (10.00 ± 9.90)	.19	.69	.13	.48	.72	1.00
<i>DDR1_h14</i>			157 (11.70 ± 15.72)	28 (13.94 ± 14.21)	1 (4.00)	.6	1.00	.52	1.00	.67	1.00
<i>DDR1_h15</i>			164 (12.36 ± 15.67)	22 (9.26 ± 13.70)	—	.37	1.00	.37	1.00	—	—

Notes: C/C is common homozygote; C/R heterozygote; R/R rare homozygote. *DDR1_h11* and *DDR1_h12* are not listed due to the equivalence to *rs2239518* and *rs1264320*, respectively.

p_a, *p_b* and *p_c* indicate *p*-value of co-dominant, dominant, and recessive models, respectively.

^aEffective number of independent marker loci = 3.6038.

Values significant at 0.05 are shown in bold.

region showed a significant association with FEV₁ decline after aspirin challenge in asthmatics even after the corrections for multiple testing ($p = .003$, $p^{\text{cor.}} = .01$, Table 2). In particular, the rare homozygote (AA) of *rs1264320* revealed about eightfold and ninefold decreases in FEV₁ by the aspirin provocation compared with the common homozygote (GG) and heterozygote (GA), respectively (Figure 2), indicating that *rs1264320* could have a protective effect on FEV₁ decline after aspirin challenge in the asthmatics. In the logistic analyses which were adjusted for age, sex, smoking status, and atopy as covariates, the *rs1264320* showed a nominal evidence of association with

AERD development under a recessive model (OR = 0.20, $p = .02$, Supplementary Table 2). However, the signal disappeared after correcting the data for the multiple testing ($p^{\text{cor.}} > .05$). Although haplotype *DDR1_h15*, which was infrequent in AERD patients compared with ATA controls, also showed correlation with AERD under co-dominant and dominant models (OR = 0.35, $p = .04$), the significance disappeared after correction ($p^{\text{cor.}} > .05$, Supplementary Table 2). In further comparison of FEV₁ decline by the aspirin provocation between AERD and ATA, the strength of association with *rs1264320* was increased in the ATA group ($p = .02-.04$), whereas no signal for association was found in the AERD group (Supplementary Table 3).

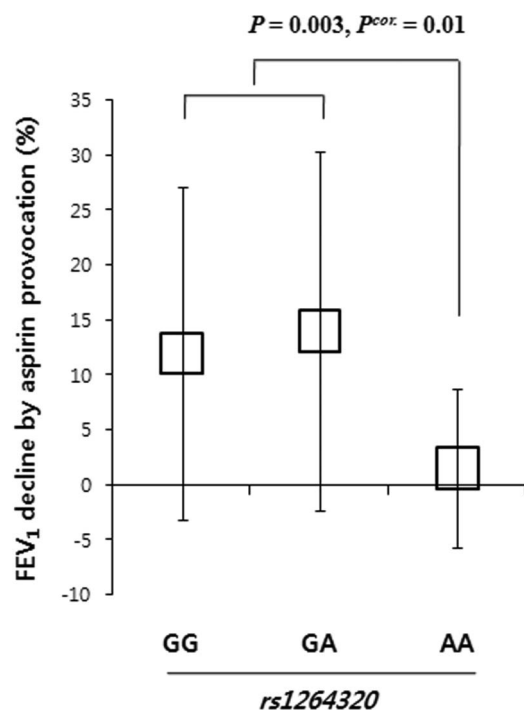


FIGURE 2.—FEV₁ decline by aspirin provocation comparisons among *rs1264320* alleles. The rare homozygote (AA) showed approximately eightfold and ninefold decrease when compared with the common homozygote (GG) and heterozygote (GA), respectively.

DISCUSSION

The MHC region contains many genes that are responsible for immunological functions. It has been previously reported that SNPs of the genes in the MHC region, such as *HLA-DRB1*, *DQA1*, *NOTCH4*, and *TNXB*, have shown significant associations with immune-mediated diseases including multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus (25). Furthermore, several genes within the MHC region are also shown to be associated with asthma (26). On the other hand, genetic variants of *DDR1* that is also positioned at the MHC region have been reported to induce the development of autoimmune diseases (27, 28), whereas no association studies between *DDR1* polymorphisms and bronchial hypersensitivity in asthma have been performed. Despite the failure to find robust associations of *DDR1* variants with AERD, our preliminary results provide additional information that *DDR1* may be potentially associated with the obstructive symptoms in asthma.

The overproduction of pro-inflammatory cysteinyl leukotrienes (CysLTs) has been implicated in aspirin hypersensitivity (29). However, many studies have recently reported that genetic variants of non-leukotriene genes such as *FSIP1* and *TBX21* might be associated with

AERD (30–32). In particular, *rs7179742* of *FSIP1* was found to be significantly associated with AERD development, suggesting the possible relations to amyloid beta precursor protein and *α disintegrin and metalloproteinase 33* (*ADAM33*), which are known to play a role in the development of aspirin hypersensitivity (31). On the other hand, a functional SNP in the promoter region of *TBX21* has been elucidated to affect transcriptional activity of the gene and thus increase the risk for AERD (32). Therefore, genetic variants of genes on other pathways, rather than leukotriene-related genes, may be related with the development of aspirin hypersensitivity in asthma.

It has been previously revealed that *DDR1* plays a potential role in the bronchial tissue and lung. High expressions of *DDR1* mRNA in the human bronchial epithelial cells and *DDR1* at the basolateral cell surface of the bronchial epithelium have also been reported (13). In addition, *DDR1* has been shown to be associated with lung inflammation and fibrosis (15). In a previous report, *Ddr1* knock-out mice showed a resistance to the lung inflammation and fibrosis induced by bleomycin (15), an antibiotic agent which causes pulmonary fibrosis as a side effect. These previous results suggest that *DDR1* might have an important role in the respiratory inflammation process via activations of important regulators such as p38 MAPK (14, 33). On the other hand, possible associations between the polymorphisms of *DDR1* and non-segmental vitiligo (NSV) in a Korean population have also been investigated (34). Although initial results in the study showed associations of five polymorphisms in the coding, 3'-untranslated and 3'-near regions with NSV, significances disappeared after multiple testing corrections.

More recently, the understanding and repair of defects in the EMT process have been explored as a new therapeutic approach for respiratory diseases such as asthma, chronic obstructive pulmonary disease, and pulmonary fibrosis (16). Although expression of *DDR2* was not altered, *DDR1* was downregulated during EMT of bronchial epithelial cells (16), indicating that *DDR1* might be involved in the trans-differentiation of bronchial epithelial cells. In addition, interplay between *DDR1* and N-cadherin has been recently reported (35). Considering that N-cadherin is upregulated during human lung development (36), it is suggested that the *DDR1* interaction with other mediators of bronchospasm may play a role in pulmonary and/or respiratory responses.

Among the nine SNPs genotyped in this study, *rs3132572* was not included during determinations of LD and haplotype due to the low MAF (< 0.05) of the SNP. Although no robust associations between *DDR1* polymorphisms and AERD were found, the *rs1264320* variant in the intronic region showed a significant signal in the association with FEV₁ decline by aspirin provocation in asthmatics even after the correction for multiple testing under a recessive model ($p = .003$ and $p^{\text{cor}} = .01$), with the increased strength of association with the variant in ATA group rather than in AERD group. Previous studies have shown that SNPs located in intronic regions could be associated with various diseases. For example, an intronic

SNP *rs13293564* in *protein unc-13 homolog B (UNC13B)* was found to be significantly associated with nephropathy in patients with type 1 diabetes (37). In addition, *rs2267716* in the intron of *corticosterone-releasing hormone receptor 2 (CRHR2)* was shown to have a risk in the development of hepatocellular carcinoma among chronic hepatitis B patients (38). Due to the role of intronic SNPs in affecting transcription efficiency or alternative splicing (39, 40), *in silico* analysis was further performed to predict whether *rs1264320* could be a potential branch point (BP) site for alternative splicing using EMBL-EBI splice site prediction (<http://www.ebi.ac.uk/asd-srv/wb.cgi?method=2>). Although *rs1264320* variant was not predicted as a BP site, there is a need for additional functional studies for the variant.

CONCLUSIONS

In summary, we conducted association studies between nine SNPs of *DDR1* and AERD to find that only *rs1264320* showed a minor association with AERD, but the association disappeared after correcting for multiple testing. However, regression analyses between *DDR1* SNPs and the fall rate of FEV₁ revealed that *rs1264320* was significantly associated with the phenotype. Although further replications in other cohorts and functional evaluations are required, the significant association between *rs1264320* variant in *DDR1* and FEV₁ decline by aspirin provocation in asthmatics may provide information that *DDR1* could have a role in the obstructive phenotype of asthma.

DECLARATION OF INTEREST

This work was supported by a grant from the Korea Health 21 R&D Project (A010249); a grant M1-0302-00-0073 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 (2009-0093822).

REFERENCES

1. Fanta CH. Asthma. *N Engl J Med* 2009; 360:1002–1014.
2. Lee RU, Stevenson DD. Aspirin-exacerbated respiratory disease: evaluation and management. *Allergy Asthma Immunol Res* 2011; 3:3–10.
3. Jenkins C, Costello J, Hodge L. Systematic review of prevalence of aspirin induced asthma and its implications for clinical practice. *BMJ* 2004; 328:434.
4. Stevenson DD, Szczeklik A. Clinical and pathologic perspectives on aspirin sensitivity and asthma. *J Allergy Clin Immunol* 2006; 118: 773–786.
5. Widal F, Abrami P, Lermoyez J. Anaphylaxie et idiosyncrasie. 1992 [Anaphylaxis and idiosyncrasy. 1992]. *Allergy Proc* 1993; 14:373–376.
6. Babu KS, Salvi SS. Aspirin and asthma. *Chest* 2000; 118:1470–1476.
7. Kim SH, Hur GY, Choi JH, Park HS. Pharmacogenetics of aspirin-intolerant asthma. *Pharmacogenomics* 2008; 9:85–91.
8. Kim SH, Kim YK, Park HW, Kim SH, Ye YM, Min KU, Park HS. Adenosine deaminase and adenosine receptor polymorphisms in aspirin-intolerant asthma. *Respir Med* 2009; 103:356–363.

9. Kim SH, Ye YM, Hur GY, Lee SK, Sampson AP, Lee HY, Park HS. CysLTR1 promoter polymorphism and requirement for leukotriene receptor antagonist in aspirin-intolerant asthma patients. *Pharmacogenomics* 2007; 8:1143–1150.
10. Holgate ST, Polosa R. The mechanisms, diagnosis, and management of severe asthma in adults. *Lancet* 2006; 368:780–793.
11. Djukanovic R, Roche WR, Wilson JW, Beasley CR, Twentyman OP, Howarth RH, Holgate ST. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990; 142:434–457.
12. Kamohara H, Yamashiro S, Galligan C, Yoshimura T. Discoidin domain receptor 1 isoform-a (DDR1alpha) promotes migration of leukocytes in three-dimensional collagen lattices. *Faseb J* 2001; 15:2724–2726.
13. Sakamoto O, Suga M, Suda T, Ando M. Expression of discoidin domain receptor 1 tyrosine kinase on the human bronchial epithelium. *Eur Respir J* 2001; 17:969–974.
14. Matsuyama W, Wang L, Farrar WL, Faure M, Yoshimura T. Activation of discoidin domain receptor 1 isoform b with collagen up-regulates chemokine production in human macrophages: role of p38 mitogen-activated protein kinase and NF-kappa B. *J Immunol* 2004; 172:2332–2340.
15. Avivi-Green C, Singal M, Vogel WF. Discoidin domain receptor 1-deficient mice are resistant to bleomycin-induced lung fibrosis. *Am J Respir Crit Care Med* 2006; 174:420–427.
16. Camara J, Jarai G. Epithelial-mesenchymal transition in primary human bronchial epithelial cells is Smad-dependent and enhanced by fibronectin and TNF-alpha. *Fibrogenesis Tissue Repair* 2010; 3:2.
17. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE, Zar HJ. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008; 31:143–178.
18. Macy E, Bernstein JA, Castells MC, Gawchik SM, Lee TH, Settipane RA, Simon RA, Wald J, Woessner KM. Aspirin challenge and desensitization for aspirin-exacerbated respiratory disease: a practice paper. *Ann Allergy Asthma Immunol* 2007; 98:172–174.
19. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, Cockcroft DW, Fish JE, Sterk PJ. Guidelines for methacholine and exercise challenge testing-1999. *Am J Respir Crit Care Med* 2000; 161:309–329.
20. Kim TH, Chang HS, Park SM, Nam BY, Park JS, Rhim T, Park HS, Kim MK, Choi IS, Cho SH, Chung IY, Park BL, Park CS, Shin HD. Association of angiotensin I-converting enzyme gene polymorphisms with aspirin intolerance in asthmatics. *Clin Exp Allergy* 2008; 38:1727–1737.
21. Cormican LJ, Farooque S, Altmann DR, Lee TH. Improvements in an oral aspirin challenge protocol for the diagnosis of aspirin hypersensitivity. *Clin Exp Allergy* 2005; 35:717–722.
22. Kim JH, Cha JY, Cheong HS, Park JS, Jang AS, Uh ST, Kim MK, Choi IS, Cho SH, Park BL, Bae JS, Park CS, Shin HD. KIF3A, a Cilia Structural Gene on Chromosome 5q31, and Its Polymorphisms Show an Association with Aspirin Hypersensitivity in Asthma. *J Clin Immunol* 2011; 31:112–121.
23. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21:263–265.
24. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001; 68:978–989.
25. Rioux JD, Goyette P, Vyse TJ, Hammarstrom L, Fernando MM, Green T, De Jager PL, Foisy S, Wang J, de Bakker PI, Leslie S, McVean G, Padyukov L, Alfredsson L, Annese V, Hafler DA, Pan-Hammarstrom Q, Matell R, Sawcer SJ, Compston AD, Cree BA, Mirel DB, Daly MJ, Behrens TW, Klareskog L, Gregersen PK, Oksenberg JR, Hauser SL. Mapping of multiple susceptibility variants within the MHC region for 7 immune-mediated diseases. *Proc Natl Acad Sci U S A* 2009; 106:18680–18685.
26. Shiina T, Inoko H, Kulski JK. An update of the HLA genomic region, locus information and disease associations: 2004. *Tissue Antigens* 2004; 64:631–649.
27. Santiago JL, Li W, Lee A, Martinez A, Chandrasekaran A, Fernandez-Arquero M, Khalili H, de la Concha EG, Urcelay E, Gregersen PK. Localization of Type 1 Diabetes susceptibility in the ancestral haplotype 18.2 by high density SNP mapping. *Genomics* 2009; 94:228–232.
28. Silva de Castro CC, do Nascimento LM, Walker G, Werneck RI, Nogoceke E, Mira MT. Genetic variants of the DDR1 gene are associated with vitiligo in two independent Brazilian population samples. *J Invest Dermatol* 2010; 130:1813–1818.
29. Tantisira KG, Drazen JM. Genetics and pharmacogenetics of the leukotriene pathway. *J Allergy Clin Immunol* 2009; 124:422–427.
30. Park JS, Chang HS, Park CS, Lee JH, Lee YM, Choi JH, Park HS, Kim LH, Park BL, Choi YH, Shin HD. Association analysis of cysteinyl-leukotriene receptor 2 (CYSLTR2) polymorphisms with aspirin intolerance in asthmatics. *Pharmacogenet Genomics* 2005; 15:483–492.
31. Kim JY, Kim JH, Park TJ, Bae JS, Lee JS, Pasaje CF, Park BL, Cheong HS, Park JS, Park SW, Uh ST, Kim MK, Choi IS, Cho SH, Choi BW, Park CS, Shin HD. Positive association between aspirin-intolerant asthma and genetic polymorphisms of FSIP1: a case-case study. *BMC Pulm Med* 2010; 10:34.
32. Akahoshi M, Obara K, Hirota T, Matsuda A, Hasegawa K, Takahashi N, Shimizu M, Nakashima K, Cheng L, Doi S, Fujiwara H, Miyatake A, Fujita K, Higashi N, Taniguchi M, Enomoto T, Mao XQ, Nakashima H, Adra CN, Nakamura Y, Tamari M, Shirakawa T. Functional promoter polymorphism in the TBX21 gene associated with aspirin-induced asthma. *Hum Genet* 2005; 117:16–26.
33. Vogel W, Gish GD, Alves F, Pawson T. The discoidin domain receptor tyrosine kinases are activated by collagen. *Mol Cell* 1997; 1:13–23.
34. Kim H, Uhm Y, Yun J, Im S, Yim S, Chung J, Shin M, Lee M. Association between polymorphisms of discoidin domain receptor tyrosine kinase 1 (DDR1) and non-segmental vitiligo in the Korean population. *Eur J Dermatol* 2010; 20:231–232.
35. Shintani Y, Fukumoto Y, Chaika N, Svoboda R, Wheelock MJ, Johnson KR. Collagen I-mediated up-regulation of N-cadherin requires cooperative signals from integrins and discoidin domain receptor 1. *J Cell Biol* 2008; 180:1277–1289.
36. Kaarteenaho R, Lappi-Blanco E, Lehtonen S. Epithelial N-cadherin and nuclear beta-catenin are up-regulated during early development of human lung. *BMC Dev Biol* 2010; 10:113.
37. Tregouet DA, Groop PH, McGinn S, Forsblom C, Hadjadj S, Marre M, Parving HH, Tarnow L, Telgmann R, Godefroy T, Nicaud V, Rousseau R, Parkkonen M, Hoverfalt A, Gut I, Heath S, Matsuda F, Cox R, Kazeem G, Farrall M, Gauguier D, Brand-Herrmann SM, Cambien F, Lathrop M, Vionnet N. G/T substitution in intron 1 of the UNC13B gene is associated with increased risk of nephropathy in patients with type 1 diabetes. *Diabetes* 2008; 57:2843–28450.
38. Gu X, Qi P, Zhou F, Ji Q, Wang H, Dou T, Zhao Y, Gao C. An intronic polymorphism in the corticotropin-releasing hormone receptor 2 gene increases susceptibility to HBV-related hepatocellular carcinoma in Chinese population. *Hum Genet* 2010; 127:75–81.
39. Pagani F, Baralle FE. Genomic variants in exons and introns: identifying the splicing spoilers. *Nat Rev Genet* 2004; 5:389–396.
40. Brinster RL, Allen JM, Behringer RR, Gelinas RE, Palmiter RD. Introns increase transcriptional efficiency in transgenic mice. *Proc Natl Acad Sci U S A* 1988; 85:836–840.

Copyright of Journal of Asthma is the property of Taylor & Francis Ltd and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.