ORIGINAL ARTICLE

A Possible Association Between *ZNRD1* and Aspirin-Induced Airway Bronchoconstriction in a Korean Population

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Abstract

Background: The etiology of aspirin-exacerbated respiratory disease (AERD) has been attributed to the combination of environmental and genetic risk factors. Although widely investigated in various diseases associated with immune dysfunction, the human zinc ribbon domain containing 1 (*ZNRD1*) gene is thought to play a role in the pathogenesis of AERD by altering the mechanisms involved in disease development.
Methods: We selected 6 single-nucleotide polymorphisms (SNPs) for genotyping from the International HapMap database in order to analyze the association between polymorphisms in *ZNRD1* and AERD in a Korean asthma cohort. Genotyping was carried out using the TaqMan assay, and differences in genotype frequency distributions were analyzed using logistic regression models.
Results: Nominal associations were found between *ZNRD1 rs1150740* and risk of AERD via codominant and dominant genetic inheritance (P=.03; odds ratio, 1.14 [1.14-10.16]). The same polymorphism was found to be significantly associated with a decrease in forced expiratory volume in the first second of expiration, an important diagnostic marker of AERD, even after multiple testing corrections (P=.006, P^{corr}=.03 in codominant and dominant models).
Conclusions: These preliminary findings suggest a possible relationship between *ZNRD1* and aspirin-induced respiratory dysfunctions in a Korean population and provide essential information on the etiology of AERD.

Key words: Aspirin exacerbated respiratory disease. FEV_1. Haplotype. Single-nucleotide polymorphism. *ZNRD1*.

Resumen

Antecedentes: La etiología de la enfermedad respiratoria exacerbada por AAS (EREA) ha sido atribuida a la combinación de factores de riesgo ambientales y genéticos. Aunque ha sido ampliamente estudiado en varias enfermedades asociadas con trastornos inmunitarios, se considera que el gen *ZNRD1* humano desempeña un papel importante en la patogenia de la EREA al afectar a los mecanismos que intervienen en el desarrollo de la enfermedad.
Métodos: Se seleccionaron 6 polimorfismos de un solo nucleótido (SNP) para genotipado de la base de datos International HapMap, con el objeto de analizar la asociación entre las variaciones de *ZNRD1* y la EREA en una cohorte de personas de origen coreano con asma. El genotipado se llevó a cabo mediante el ensayo TaqMan, y las diferencias en las distribuciones de frecuencia de los genotipos se analizaron mediante modelos de regresión logística.
Resultados: Se observaron asociaciones nominales entre *ZNRD1 rs1150740* y el riesgo de EREA por medio de la herencia genética dominante y codominante (p=0,03; OR: 1,14 [1,14-10,16]). Se observó que el mismo polimorfismo estaba significativamente asociado con una disminución del volumen espiratorio máximo en el primer segundo de espiración, un marcador diagnóstico importante de EREA, incluso después de múltiples correcciones de análisis (p=0,006, p^{corr}=0,03 en modelos dominantes y codominantes).
Conclusiones: Estos hallazgos preliminares indican una posible relación entre ZNRD1 y las disfunciones respiratorias inducidas por AAS en una población coreana y proporcionan información esencial sobre la etiología de la EREA.

Palabras clave: Enfermedad respiratoria exacerbada por AAS. VEM_1. Haplotipo. Polimorfismo de un solo nucleótido. *ZNRD1*.

Introduction

Although noted for their medicinal functions, aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) can induce adverse effects. Aspirin-exacerbated respiratory disease (AERD) is a nonallergic asthma phenotype characterized by aspirin-induced bronchospasm, inflammatory infiltrate in nasal polyp cells, and chronic rhinitis. About 25% of patients with asthma admitted to hospital for mechanical ventilation are aspirin-intolerant [1,2], thus highlighting the role of aspirin in near-fatal asthma attacks. We recently demonstrated an association between the risk of AERD and the following genes: solute carrier family 6 (neurotransmitter transporter, betaine/GABA) member 12 (*SLC6A12*), emilin/multimerin domain–containing protein 2 (*EMID2*), and ubiquitin protein ligase E3C (UBE3C) [3-5]. Therefore, previously undiscovered mechanisms could be clinically relevant in aspirin hypersensitivity among asthmatics.

Human zinc ribbon domain containing 1 (*ZNRD1*; NM_014596) is a transcription-associated gene on chromosome 6p21.3 spanning more than 3.65 kilobases. This region contains potent markers of AERD, major histocompatibility complex class II DP beta 1 (*HLA-DPB1*) [6], and tumor necrosis factor (*TNF*) α [7], thus implicating the locus in the pathogenesis of AERD. ZNRD1 is a zinc finger-related protein that catalyzes the transcription of DNA into RNA and has been an attractive therapeutic target for various diseases [8,9]. Murine models of airway inflammation reveal decreased levels of zinc in the airway epithelium [10], and supplementation with zinc reduces levels of eosinophils and lymphocytes in bronchial cells [11]. In humans, a significant reduction in zinc levels has been observed in the serum and plasma of asthma patients [12], and zinc has been correlated with the incidence of wheezing among infants [13]. Although the underlying mechanisms remain unclear, *ZNRD1* is thought to play a role in respiratory diseases.

ZNRD1 is a gene of the major histocompatibility complex class 1, a family of molecules that governs immune responses to allergens. Although the immune system has primarily been implicated in allergic asthma, previous reports have revealed associations between components of the immune system and susceptibility to AERD [6,14], suggesting that the immune system may also mediate aspirin-induced bronchospasm in asthmatics.

ZNRD1 has been implicated in diseases associated with immune deficiency, including human immunodeficiency virus (HIV) infection, but not with AERD. By modifying the structure of the mechanisms involved in the development of nonallergic asthma and mapping these mechanisms to an asthma-related locus, *ZNRD1* is hypothesized to be a functional and positional marker of AERD. We performed a case-control analysis in a Korean population to investigate a possible association between *ZNRD1* and AERD.

Materials and Methods

Patients

Asthmatic patients were recruited in Korean hospitals listed in the Asthma Genome Research Center. The Institutional Review Board of each hospital approved the study protocol, and written informed consent was obtained from each patient before blood was drawn. Asthma was diagnosed according to the guidelines of the Global Initiative for Asthma (GINA), with special emphasis on symptoms of dyspnea and wheezing during the last year plus 1 of the following: *1)* airway reversibility measured by a positive bronchodilator response, namely, an increase of >15% in forced expiratory volume in the first second of expiration (FEV_1) or of >12% plus 200 mL following inhalation of a short-acting bronchodilator; *2)* airway hyperreactivity to <10 mg/mL of the provocative concentration of methacholine that causes a 20% fall in FEV_1 (PC_{20}); or *3)* >20% increase in FEV_1 following 2 weeks of treatment with inhaled corticosteroids and long-acting bronchodilators [15]. Pulmonary function tests were performed according to the procedures of the American Thoracic Society [16] using Vmax Series 2130 Autobox Spirometry (Sensor Medics). A skin prick test was performed using 24 common inhalant allergens, atopy was determined as a wheal reaction ≥3 mm in diameter, and total immunoglobulin (Ig) E was measured using the CAP system (Pharmacia Diagnostics). All patients underwent oral aspirin challenge (OAC) with increasing doses (10 450 mg) [17]. Patients were categorized according to individual reactions to OAC: those exhibiting a ≥20% decrease in FEV_1 or a 15% 19% decrease in FEV_1 with naso-ocular or cutaneous reactions were considered to have AERD, whereas those exhibiting a <15% decrease in FEV_1 with no naso-ocular or cutaneous reactions were considered to have aspirin-tolerant asthma (ATA, controls).

Single-Nucleotide Polymorphism Selection and Genotyping

Candidate single-nucleotide polymorphisms (SNP) were selected and screened from the International HapMap Project database according to linkage disequilibrium (LD) status in the Asian population (Chinese Han and Japanese) and minor allele frequencies (MAF >0.05). Genotyping was carried out using polymerase chain reaction (PCR)–based DNA typing in the ABI prism 7900HT sequence detection system (Applied Biosystems) following a TaqMan assay. The assay IDs (Applied Biosystems) and probe sequence of each SNP are described in Supplementary Table 1. Genotyping data quality was assessed by duplicate DNA testing (n=10; rate of concordance in duplicates >99%). Haplotypes were inferred from the successfully genotyped SNPs using the PHASE algorithm, version 2.0 [18].

Statistical Analysis

LD between all pairs of biallelic loci was determined using Lewontin's D' (|D'|), and the LD coefficient r^2 was examined using the Haploview algorithm [19]. In order to determine the association between *ZNRD1* and the risk of AERD, logistic regression analysis was carried out with the following covariates: adjusted age (continuous value), sex (male, 0; female, 1), smoking status (nonsmoker, 0; ex-smoker, 1; smoker, 2), atopy (absence, 0; presence, 1), and body mass index. Furthermore, differences in the decline in FEV_1 between the AERD and ATA groups were examined using a regression

model controlling for age, sex, smoking status, and atopy as covariates. Data were managed and analyzed using Statistical Analysis System version 9.1 (SAS Inc.). In order achieve optimal correction for multiple testing of markers representing SNPs in LD with each other, we calculated the effective number of independent marker loci (Meff) which accounts for the eigenvalue (spectral) decomposition of all the genotypes represented in the correlation matrix [20] using the SNPSpD program (http://genepi.qimr.edu.au/general/daleN/SNPSpD/). Furthermore, the statistical power of single associations was calculated using the Power for Genetic Association Analyses (PGA) software application [21]. Expression quantitative trait loci (eQTL) analysis was carried out using the eQTL browser (http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/).

Results

Clinical Characteristics of the Study Patients

The clinical profiles of the study patients are shown in Table 1. The study sample comprised 93 patients with AERD (males, 32; females, 61) and 96 controls with ATA (males, 24; females, 72). Significant differences were observed between patients and controls (P=.001) for fall in FEV_1 after OAC (AERD, 23.61% vs ATA, 0.94%), positive rate of aspirin intolerance (AERD, 26.67% vs ATA, 8.42%), and positive rate of nasal polyps (AERD, 63.86% vs ATA, 29.27%) (Table 1). No significant differences were observed between cases and controls for other diagnostic factors.

Table 1. Clinical Profiles of the Study Patients[a]

Clinical profile	AERD	ATA	*P* Value
Number of subjects, n	93	96	
Mean age, y (range)	44.39 (17-73)	45.79 (15-77)	.497
Mean age at onset, y (range)	38.01 (0-70)	37.99 (5-73)	.995
Sex (male/female)	32/61	24/72	.156
Body mass index, kg/m^2	23.47 (3.18)	24.41 (3.29)	.049
Ex-smoker/current smoker, %	15.63/9.38	6.45/12.90	.219
Blood eosinophils, %	6.29 (5.80)	4.88 (4.19)	.060
Predicted FVC, %	89.90 (14.74)	87.76 (12.80)	.293
Predicted FEV_1, %	86.63 (16.74)	88.26 (17.04)	.509
PC_{20} methacholine, mg/mL	4.23 (7.18)	3.04 (4.27)	.193
Total IgE, IU/mL	321.65 (623.31)	309.54 (426.04)	.878
Fall rate of FEV1 by aspirin provocation	23.61 (14.48)	0.94 (2.76)	.001
Positive rate of skin test, %	61.46	56.99	.532
Positive rate of aspirin intolerance, %	26.67	8.42	.001
Positive rate of nasal polyps, %	63.86	29.27	.001

Abbreviations: AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; FEV_1, forced expiratory volume in the first second of expiration; FVC, forced vital capacity; PC_{20}, provocative concentration of methacholine that causes a 20% fall in FEV_1.
[a]Values are expressed with SE unless otherwise indicated

Table 2. Genotype and Allele Distribution of *ZNRD1* Variants

				MAF				
Locus	Position	Allele	*P* Value[a]	Korean	Caucasian	Chinese	Japanese	African
rs3132129	5' near gene	C>T	.941	0.005	0.916	0.993	1.000	0.991
rs3757329	5' near gene	A>C	.648	0.254	0.049	0.147	0.212	0.272
rs7769930	5' near gene	A>C	.577	0.227	0.062	0.148	0.163	–
rs1150741	Intron 1	C>G	.867	0.278	–	–	–	–
rs1150740	Intron 3	G>T	.491	0.048	0.084	0.077	0.102	0.114
rs1150739	Intron 3	G>A	.292	0.471	0.363	0.438	0.597	0.404

Abbreviation: MAF, minor allele frequency.
[a]Values of deviation from Hardy-Weinberg Equilibrium in a Korean population.

Distribution of ZNRD1 Variants

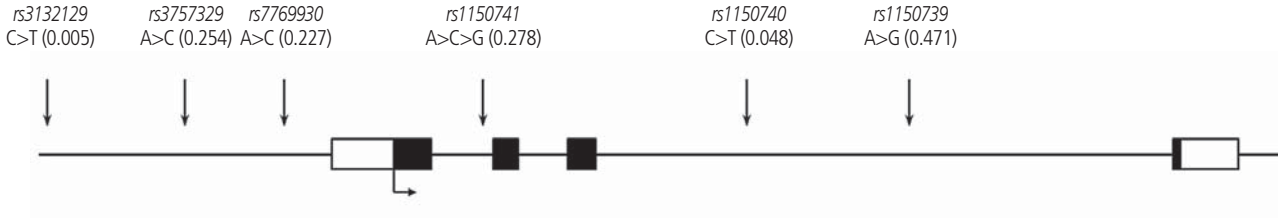
With an average call rate of 99.9%, we successfully genotyped 6 *ZNRD1* SNPs, including 3 polymorphisms in the 5′ near region (*rs3132129*, *rs3757329*, and *rs7769930*) and 3 in the noncoding intronic regions (*rs1150741*, *rs1150740*, and *rs1150739*) (Table 2 and Figure, A). The distribution of each locus was in Hardy-Weinberg Equilibrium (*P*>.05; Table 2). The LD plot revealed a strong linkage between *rs7769930* and *rs3757329* (r^2>0.8; Figure, B and Supplementary Table 2). This finding is of interest, since it may indicate disease susceptibility candidate regions that are unique to specific populations. Three major haplotypes with frequencies >0.05 (Figure, C) were obtained from 1 haplotype block using pairwise comparisons of the genotyped SNPs. *ZNRD1_ht2* is unique to the minor allele of *rs1150741*, while *ZNRD1_ht3* is unique to the minor allele of rs*7769930*.

Association Between ZNRD1 Variants and the Risk of Aspirin Intolerance Among Asthmatics

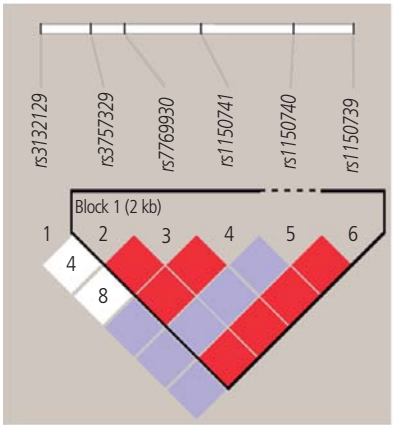
The results of logistic regression analysis initially revealed a significant association between *ZNRD1 rs1150740* and the risk of AERD via codominant and dominant mechanisms (*P*=.03; odds ratio, 1.14 [1.14-10.16]; Table 3). However, the significant value was not retained after performing multiple testing corrections (P^{corr}>.05, Table 3) with a Meff of 5.551 extracted from SNPSpD. Since the inferred haplotypes were found to be equivalent to the analyzed SNPs (*ht1* with *rs1150739*, *ht2* with *rs1150741*, and *ht3* with *rs7769930*; Figure, B), analysis of the 3 major haplotypes with frequencies >0.05 revealed the same effect as the polymorphisms tested.

In a further analysis, namely, of the relationship between *ZNRD1* and the fall in FEV_1 after OAC, an important diagnostic marker of AERD was determined using a regression model. The results revealed a significant association between rs1150740 and the decline in FEV_1, even after multiple comparisons (P=.006, P^{corr}=.03 in codominant and dominant models; Table 4).

A



B



C

Hap.	rs3757329	rs7769930	rs1150741	rs1150739	Frequency
ht1	A	A	C	A	0.468
ht2	A	A	G	G	0.278
ht3	C	C	C	G	0.227
ht4	C	A	C	G	0.024
ht5	C	A	C	A	0.003

Figure. Physical map, LD, and haplotypes of the *ZNRD1* gene. A, Schematic gene map and single-nucleotide polymorphisms of *ZNRD1* on chromosome 6p21.3 (3.65kb). Black blocks represent coding exons and white blocks represent the 5′ and 3′ untranslated regions. The first base of the translation site was denoted as nucleotide +1. Single-nucleotide polymorphisms in absolute linkage are indicated by brackets (r^2=1). B, Haplotypes of *ZNRD1*. C, Linkage disequilibrium coefficient (|D′|) among single-nucleotide polymorphisms of *ZNRD1* in a Korean population. Red blocks indicate |D′| = 1, LOD ≥2, blue blocks |D′| = 1, LOD <2 and white blocks |D′| <1, LOD <2.

Table 3. Association Analysis of *ZNRD1* Polymorphisms and Haplotypes With Aspirin-Exacerbated Respiratory Disease[a]

Locus	MAF		Co-dominant			Dominant			Recessive			Statistical Power
	AERD	ATA	OR (95% CI)	P^a	P^b	OR (95% CI)	P^a	P^{corr}	OR (95% CI)	P^a	P^{corr}	
rs3132129	0.958	0.959	1.06 (0.06-17.52)	.97	–	1.06 (0.06-17.52)	.97	–	–	–	–	11.46
rs3757329	0.414	0.906	0.79 (0.49-1.29)	.35	–	0.85 (0.48-1.54)	.60	–	0.42 (0.11-1.57)	.20	–	9.010
rs7769930	0.911	0.404	0.79 (0.48-1.29)	.34	–	0.84 (0.46-1.54)	.58	–	0.43 (0.12-1.59)	.20	–	5.760
rs1150741	0.940	0.872	1.04 (0.65-1.65)	.89	–	1.07 (0.59-1.91)	.83	–	0.97 (0.31-2.98)	.95	–	8.660
rs1150740	0.466	0.793	3.40 (1.14-10.16)	.03	NS	3.40 (1.14-10.16)	.03	NS	–	–	–	17.49
rs1150739	0.469	0.451	1.18 (0.79-1.77)	.43	–	1.28 (0.67-2.46)	.46	–	1.22 (0.61-2.42)	.58	–	50.44

Abbreviations: CI, confidence interval; NS, not significant; OR, odds ratio.
[a]Codominant, dominant, and recessive models of logistic regression analyses were used to calculate odds ratios (95% confidence interval) controlling for age, sex, smoking status, atopy, and body mass index. *ht1*, *ht2*, and *ht3* were equivalent to *rs1150739*, *rs1150741*, and *rs7769930*, respectively.
[b]$P<.05$.
[c]*P* Values after multiple testing corrections (Meff=5.551).

Table 4. Regression Analysis of *ZNRD1* Polymorphisms and Haplotypes With Fall Rate of FEV_1 After Oral Aspirin Provocation

Locus	C/C	C/R	R/R	Pa^a	Pa^{corrb}	Pb^a	Pb^{corrb}	Pc^a	Pc^{corrb}
rs3132129	185 (12.06 [15.47])	2 (7.50±10.61)	–	.71	–	.71	–	–	–
rs3757329	104 (11.55 [15.05])	74 (12.44±14.98)	11 (14.85±21.13)	.74	–	.80	–	.75	–
rs7769930	113 (11.72 [15.15])	63 (12.05±14.98)	11 (14.85±21.13)	.82	–	.90	–	.74	–
rs1150741	97 (12.93 [16.39])	76 (11.33±14.98)	14 (9.33±10.18)	.35	–	.49	–	.37	–
rs1150740	170 (11.07 [14.70])	18 (21.23±18.82)	0	.006	.03	.006	.03	–	–
rs1150739	56 (11.77 [15.89])	86 (11.81±14.31)	45 (12.70±17.10)	.56	–	.67	–	.60	–

Abbreviations: C/C, major homozygote; C/R, heterozygote; R/R minor homozygote.
[a]Codominant, dominant, and recessive models was used for multiple linear regression analyses controlling for age, sex, smoking status, atopy and body mass index. Mean (SD) of the natural logarithmic ratio of each genotype is shown in parenthesis.
ht1, *ht2*, and *ht3* were equivalent with rs*1150739*, *rs1150741*, and *rs7769930*, respectively.
[b]*P* Values after multiple testing corrections (Meff=5.551).

Discussion

AERD has been attributed to overproduction of cysteinyl-leukotrienes resulting from inhibition of the cyclooxygenase-2 pathway and diversion of arachidonic acid metabolites towards the 5-lipoxygenase pathway. Aside from the traditional theory of disease development, case-control studies have shown that the mechanisms of immune inflammatory response are implicated in the risk of AERD [6,7,22]. However, the exact role of the immune system in aspirin-induced bronchospasm remains controversial, owing to the lack of known antigen during disease onset.

The *ZNRD1* gene has been analyzed extensively in studies on the pathophysiology of HIV infection [23], a disease that is associated with immune system dysfunction. Furthermore, immunochemical analysis has revealed increased expression of *ZNRD1* in multidrug-resistant gastric cancer cells [24]. Since aspirin is increasingly used as a means of reducing the risk of gastric cancer [25], a possible link between *ZNRD1* and AERD could be established. Decreased levels of zinc in the airways of mice and humans with chronic inflammation provide further evidence that *ZNRD1* may be involved in bronchial hyperresponsiveness.

In line with previous reports [26], we observed a female predominance among the AERD patients enrolled in this study. The etiology of airway inflammation has demonstrated relationships between the development of nasal polyps, asthma, and aspirin-intolerance [26]. The clinical history of the study patients revealed significantly more nasal polyps in the AERD group than in the ATA group. Furthermore, it is worth noting that the fall in FEV_1 after OAC was significantly greater in AERD patients than in ATA patients, thus supporting reports that FEV_1 is an important diagnostic marker of the disease.

To our knowledge, this is the first study to present the results of an association analysis of *ZNRD1* and the risk of AERD. We found that *rs1150740* was nominally associated with the risk of AERD, suggesting that this polymorphism could play a role in aspirin-induced bronchoconstriction among Korean asthmatics. However, since we detected a low association signal and the effect of polymorphisms varies according to ethnicity and geographic location, further investigations in different cohorts are warranted.

FEV_1 is the most common parameter in assessing bronchodilation. A low FEV_1 rate has been consistently associated with AERD and other types of airway disease. Proper interpretation of the decrease in FEV_1 is crucial in the management of airway obstruction. In this study, *ZNRD1 rs1150740*, which was observed to be marginally associated with the risk of AERD, was also found to be significantly associated with the decline in FEV_1. Patients who were heterozygous for the rare allele (C/R genotype) of the polymorphism were at a higher risk of airway obstruction than patients with other genotypes (C/C and R/R). Since the association signal remained significant after correction, *rs1150740* is expected to facilitate exacerbation of airway inflammation after ingestion of aspirin.

Coordinated regulation of transcription and splicing is key to the development and progression of various disorders. A previous report revealed that polymorphisms in the intronic regions of the gene may induce exon skipping, activate cryptic spliced sites, or affect efficient intron splicing processivity [27], resulting in synthesis of proteins that influence disease pathophysiology. In an attempt to understand whether any of the variants studied can serve as eQTLs and regulate expression levels of *ZNRD1*, further analysis was performed using the eQTL browser. Although the results reveal that *rs1150741* may be a cis-acting regulatory element to LOC347981 (eQTL score, 9.45) and Hs.519979 (eQTL score, 14.22), none of the SNPs tested function directly as potential regulators for *ZNRD1* expression (data not shown).

Supplementary Table 1. Assay Information of *ZNRD1* Single-Nucleotide Polymorphisms

Locus	Assay IDa and Probe Sequence[a]	Probe Sequence
rs3132129	C__27463053_10	–
rs3757329	C__27477758_10	–
rs7769930	C__25924715_20	–
rs1150741	GGCGGTTGTACATTTGGTCT	Forward
	TCAAAGTCTGCACAGGCAAG	Reverse
	GTCAGCTCTCTCTGGT	Probe-1 (VIC)
	GTCAGCTGTCTCTGG	Probe-2 (FAM)
rs1150740	TTGACTGCGGTTGAAGACTG	Forward
	TCCCAAAGTGCTGGGATTAC	Reverse
	CTTATGTTGTTTTTTT	Probe-1 (VIC)
	CTTATGTTTTTTTTT	Probe-2 (FAM)
rs1150739	CAGCAGCATCAGACACACAA	Forward
	TTCCATGTCACTAATTCTGTCTTCA	Reverse
	AGAATTATGAGACATA	Probe-1 (VIC)
	AGAATTATAAGACAT	Probe-2 (FAM)

[a]Taqman assay IDs from Applied Biosystems.

Supplementary Table 2. Measures of Linkage Disequilibrium Among the Tested *ZNRD1* Single-Nucleotide Polymorphisms

		rs3132129	rs3757329	rs7769930	rs1150741	rs1150740	rs1150739
r^2	rs3132129	–	0.049	0.084	1	1	1
	rs3757329	0	–	1	1	1	1
	rs7769930	0	0.864	–	1	1	1
	rs1150741	0.002	0.131	0.113	–	1	1
	rs1150740	0	0.016	0.014	0.018	–	1
	rs1150739	0.006	0.303	0.261	0.342	0.054	–

Findings from power calculations of single associations showed that the average statistical power to detect effect sizes with the current sample size is 17.37% (Table 3). Furthermore, the possibility that the signal detected could only be a marker for the region and that genes flanking *ZNRD1* could be responsible for the association effect cannot be ruled out. In order to address these limitations, further analyses using larger sample sizes are warranted.

To conclude, findings from this study indicate a possible role of *ZNRD1* in aspirin-induced respiratory dysfunctions in a Korean population. Although future replications and exact functional analyses are needed to confirm the function of *ZNRD1* in the pathogenesis of AERD, these preliminary results can provide crucial supporting information on the genetic etiology of aspirin-hypersensitive airway inflammation.

Acknowledgments

This work was supported by Korea Science and Engineering Foundation (KOSEF), which is funded by the Korean government (MEST) (No. 2009-0080157), and a grant from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A010249). The DNA samples were generously provided by Soonchunhyang University, Bucheon Hospital Biobank, a member of the National Biobank of Korea, which is supported by the Ministry of Health, Welfare and Family Affairs, Republic of Korea.

The authors declare no competing interests.

References

1. Picado C, Castillo JA, Montserrat JM, Agusti-Vidal A. Aspirin-intolerance as a precipitating factor of life-threatening attacks of asthma requiring mechanical ventilation. Eur Respir J. 1989;2(2):127-9.
2. Marquette CH, Saulnier F, Leroy O, Wallaert B, Chopin C, Demarcq JM, Durocher A, Tonnel AB. Long-term prognosis of near-fatal asthma. A 6-year follow-up study of 145 asthmatic patients who underwent mechanical ventilation for a near-fatal attack of asthma. Am Rev Respir Dis. 1992;146(1):76-81.
3. Pasaje CF, Kim JH, Park BL, Cheong HS, Chun JY, Park TJ, Lee JS, Kim Y, Bae JS, Park JS, Yoon SH, Uh ST, Choi JS, Kim YH, Kim MK, Choi IS, Cho SH, Choi BW, Park CS, Shin HD. Association of SLC6A12 variants with aspirin-intolerant asthma in a Korean population. Ann Hum Genet. 2010;74(4):326-34.
4. Pasaje CF, Kim JH, Park BL, Cheong HS, Kim MK, Choi IS, Cho SH, Hong CS, Lee YW, Lee JY, Koh IS, Park TJ, Lee JS, Kim Y, Bae JS, Park CS, Shin HD. A possible association of EMID2 polymorphisms with aspirin hypersensitivity in asthma. Immunogenetics. 2010;63(1):13-21.
5. Lee JS, Kim JH, Bae JS, Kim JY, Park TJ, Pasaje CF, Park BL, Cheong HS, Park JS, Uh ST, Kim MK, Choi IS, Cho SH, Choi BW, Park CS, Shin HD. Association analysis of UBE3C polymorphisms in Korean aspirin-intolerant asthmatic patients. Ann Allergy Asthma Immunol. 2010;105(4):307-12.
6. Choi JH, Lee KW, Oh HB, Lee KJ, Suh YJ, Park CS, Park HS. HLA association in aspirin-intolerant asthma: DPB1*0301 as a strong marker in a Korean population. J Allergy Clin Immunol. 2004;113(3):562-4.
7. Kim SH, Ye YM, Lee SK, Choi JH, Holloway JW, Park CS, Park HS. Association of TNF-alpha genetic polymorphism with HLA DPB1*0301. Clin Exp Allergy. 2006;36(10):1247-53.
8. Guo W, Zhao YP, Jiang YG, Wang RW, Hong L, Fan DM. Upregulation of ZNRD1 enhances cisplatin resistance in human esophageal cancer cells by regulation of ERCC1 and Bcl-2. Tumour Biol. 2008;29(3):188-94.
9. Hong L, Ning X, Shi Y, Shen H, Zhang Y, Lan M, Liang S, Wang J, Fan D. Reversal of multidrug resistance of gastric cancer cells by down-regulation of ZNRD1 with ZNRD1 siRNA. Br J Biomed Sci. 2004;61(4):206-10.
10. Truong-Tran AQ, Ruffin RE, Foster PS, Koskinen AM, Coyle P, Philcox JC, Rofe AM, Zalewski PD. Altered zinc homeostasis and caspase-3 activity in murine allergic airway inflammation. Am J Respir Cell Mol Biol. 2002;27(3):286-96.
11. Lang C, Murgia C, Leong M, Tan LW, Perozzi G, Knight D, Ruffin R, Zalewski P. Anti-inflammatory effects of zinc and alterations in zinc transporter mRNA in mouse models of allergic inflammation. Am J Physiol Lung Cell Mol Physiol. 2007;292(2):L577-84.
12. Murgia N, Muzi G, Dell' Omo M, Montuschi P, Melchiorri D, Ciabattoni G, Abbritti EP, Orazi N, Sapia IE, Abbritti G. Induced sputum, exhaled breath condensate and nasal lavage fluid in electroplating workers exposed to chromium. Int J Immunopathol Pharmacol. 2006;19(4 Suppl):67-71.
13. Tahan F, Karakukcu C. Zinc status in infantile wheezing. Pediatr Pulmonol. 2006;41(7):630-4.

14. Lee SH, Rhim T, Choi YS, Min JW, Kim SH, Cho SY, Paik YK, Park CS. Complement C3a and C4a increased in plasma of patients with aspirin-induced asthma. Am J Respir Crit Care Med. 2006;173(4):370-8.
15. 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. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med. 2000;161(1):309-29.
16. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152(3):1107-36.
17. 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(6):717-22.
18. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. 2001;68(4):978-89.
19. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21(2):263-5.
20. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet. 2004;74(4):765-9.
21. Menashe I, Rosenberg PS, Chen BE. PGA: power calculator for case-control genetic association analyses. BMC Genet. 2008;9:36.
22. Berce V, Repnik K, Potocnik U. Association of CCR5-delta32 mutation with reduced risk of nonatopic asthma in Slovenian children. J Asthma. 2008;45(9):780-4.
23. Holt N, Wang J, Kim K, Friedman G, Wang X, Taupin V, Crooks GM, Kohn DB, Gregory PD, Holmes MC, Cannon PM. Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nat Biotechnol. 2010;28(8):839-47.
24. Zhang YM, Zhao YQ, Pan YL, Shi YQ, Jin XH, Yi H, Fan DM. Effect of ZNRD1 gene antisense RNA on drug resistant gastric cancer cells. World J Gastroenterol. 2003;9(5):894-8.
25. Yang P, Zhou Y, Chen B, Wan HW, Jia GQ, Bai HL, Wu XT. Aspirin use and the risk of gastric cancer: a meta-analysis. Dig Dis Sci. 2010;55(6):1533-9.
26. Samter M, Beers RF, Jr. Intolerance to aspirin. Clinical studies and consideration of its pathogenesis. Ann Intern Med. 1968;68(5):975-83.
27. Pagani F, Baralle FE. Genomic variants in exons and introns: identifying the splicing spoilers. Nat Rev Genet. 2004;5(5):389-96.

Manuscript received September 17, 2011; accepted for publication November 30, 2011.

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