ORIGINAL ARTICLE

Genetic association analysis of *TAP1* and *TAP2* polymorphisms with aspirin exacerbated respiratory disease and its FEV1 decline

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Aspirin exacerbated respiratory disease (AERD) induces bronchoconstriction in asthmatic patients characterized with a clinical condition of severe decline in forced expiratory volume in one second (FEV1) after ingestion of aspirin. Two genes consisting a heterodimer, *transporter 1* and *2*, *ATP-binding cassette, sub-family B (MDR/TAP) (TAP1 and TAP2)* within the major histocompatibility complex (MHC) region, have been implicated in immunodeficiency and bronchiectasis development. To investigate the associations of *TAP1* and *TAP2* genetic polymorphisms with AERD and phenotypic FEV1 decline, a total of 43 common single-nucleotide polymorphisms (SNPs) including 12 SNPs of *TAP1* and 31 SNPs of *TAP2* were genotyped in 93 AERD patients and 96 aspirin-tolerant asthma controls. Interestingly, regression analysis revealed that polymorphisms and haplotypes of *TAP2* were associated with FEV1 decline by aspirin provocation ($P=0.002-0.04$), with about twofold decline rate of FEV1 in most of minor homozygotes compared with major homozygotes. In addition, nominal evidences of association between *TAP2* and AERD development were observed ($P=0.02-0.04$). However, *TAP1* polymorphisms showed no relations to both AERD and FEV1 decline after aspirin challenge ($P>0.05$). Although further functional evaluations and replications are required, our preliminary findings provide supporting information that variants of *TAP2* might be predisposing factors for FEV1 decline-related symptoms.

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INTRODUCTION

Aspirin exacerbated respiratory disease (AERD) is characterized by distinct clinical symptoms associated with severe bronchoconstriction after ingestion of non-steroidal anti-inflammatory drugs including aspirin. Estimates of AERD prevalence are about 0.6–2.5% in the general population and 10–20% in asthmatic patients.^{1,2} The disease often accompanies by other symptoms referred to aspirin triad, such as bronchial asthma and chronic rhinosinusitis with nasal polyps.^{2,3} Forced expiratory volume in one second (FEV1), as one of criteria for total lung capacity, can determine severe decline of lung function. Although comprehensive mechanisms have not been known, changes in the structure and function of the airways cause bronchospasms. Although pathophysiology of AERD has been closely linked to the overproduction of cysteinyl-leukotrienes (CysLTs),⁴ recent studies have

also reported that genes on other pathways, such as the structural gene *protocadherin-1 (PCDH1)* and the transporter gene *solute carrier family 6 (neurotransmitter transporter, betaine/GABA) member 12 (SLC6A12)*, could be involved in the development of aspirin hypersensitivity in asthmatics.^{5,6}

Both *transporter 1* and *2*, *ATP-binding cassette, sub-family B (MDR/TAP) (TAP1 and TAP2)*, also referred as *transporter associated with antigen processing*, are located within the major histocompatibility complex (MHC) class II region of the leukocyte antigen (HLA) locus on human chromosome 6. TAP complex is a heterodimer consisted of two subunits encoded by *TAP1* and *TAP2*. Mutations in either *TAP1* or *TAP2* have been found to impair the translocation of peptides from the cytosol into the endoplasmic reticulum.⁷ The TAP deficiency syndrome characterized by immunodeficiency usually shows recurrent

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Table 1 Clinical profiles of study subjects

Clinical profiles	AERD (n=93)	ATA (n=96)	P-value
Age year, mean (range)	44.4 (17–73)	45.8 (15–77)	0.497
Sex (male/female)	32/61	24/72	0.156
Ex-smoker/Current smoker (%)	15.63/9.38	6.45/12.90	0.219
Blood eosinophil (%)	6.29 ± 5.80	4.88 ± 4.19	0.060
PC20, methacholine (mg ml ⁻¹)	4.23 ± 7.18	3.04 ± 4.27	0.193
Total IgE (IU ml ⁻¹)	321.65 ± 623.31	309.54 ± 426.04	0.878
Decline of FEV1 by aspirin provocation (%)	23.61 ± 14.48	0.94 ± 2.76	0.001
Positive rate of skin test (%)	61.46	56.99	0.532

Abbreviations: AERD, aspirin exacerbated respiratory disease; ATA, aspirin-tolerant asthma; FEV1, forced expiratory volume in one second.
Value significant at $P < 0.05$ is shown in bold.

Table 2 Polymorphisms of TAP1 and TAP2 investigated in this study

Gene	SNP ID	Polymorphism	Position	Amino-acid change	Genotype (n)			MAF	HWE*
TAP1	rs2071481	A/G	Intron	Ile393Val	AA (109)	AG (60)	GG (10)	0.223	0.648
	rs2284190	T/C	Intron		TT (132)	TC (50)	CC (3)	0.151	0.479
	rs4148880	A/G	Exon		AA (124)	AG (55)	GG (8)	0.190	0.549
	rs2395269	T/G	Intron		TT (125)	TG (52)	GG (8)	0.184	0.391
	rs12529313	A/G	Intron	Asp697Gly	AA (124)	AG (55)	GG (8)	0.190	0.549
	rs2071482	G/T	Intron		GG (125)	GT (53)	TT (7)	0.181	0.643
	rs735883	C/T	Intron		CC (78)	CT (85)	TT (22)	0.349	0.875
	rs1800453	A/G	Exon		AA (130)	AG (48)	GG (7)	0.168	0.341
	rs4711312	A/G	Intron	Val379Ile	AA (130)	AG (48)	GG (7)	0.168	0.341
	rs1057373	G/T	3'UTR		GG (132)	GT (46)	TT (7)	0.162	0.248
	rs2071540	G/A	3'near		GG (61)	GA (94)	AA (30)	0.416	0.535
	rs2071541	T/C	3'near		TT (127)	TC (50)	CC (8)	0.178	0.289
TAP2	rs3763366	C/G	5'near	Synonymous (Gly386Gly)	CC (51)	CG (94)	GG (44)	0.481	0.957
	rs4148870	G/A	Intron		GG (55)	GA (89)	AA (43)	0.468	0.546
	rs2071544	G/A	Intron		GG (50)	GA (95)	AA (44)	0.484	0.931
	rs2071465	G/C	Intron		GG (89)	GC (78)	CC (19)	0.312	0.755
	rs2239701	A/G	Intron	Synonymous (Asn436Asn)	AA (44)	GA (101)	GG (42)	0.495	0.272
	rs241424	C/T	Intron		CC (49)	CT (94)	TT (44)	0.487	0.934
	rs3819721	G/A	Intron		GG (101)	GA (72)	AA (14)	0.267	0.814
	rs241426	T/A	Intron		TT (74)	TA (91)	AA (22)	0.361	0.453
	rs3819714	G/A	Intron	Arg651Cys	GG (63)	GA (98)	AA (26)	0.401	0.214
	rs241429	C/T	Intron		CC (74)	CT (86)	TT (27)	0.374	0.804
	rs4148871	C/T	Intron		CC (122)	CT (62)	TT (3)	0.182	0.118
	rs241430	G/A	Intron		GG (68)	GA (95)	AA (25)	0.386	0.362
	rs241432	A/C	Intron	Synonymous	AA (66)	AC (95)	CC (28)	0.399	0.512
	rs4148873	G/A	Exon		GG (141)	GA (43)	AA (3)	0.131	0.893
	rs2228397	G/T	Exon		GG (90)	GT (78)	TT (19)	0.310	0.730
	rs241433	T/G	Intron		TT (63)	TG (96)	GG (28)	0.406	0.381
	rs1015166	C/T	Intron	Arg651Cys	CC (99)	CT (75)	TT (13)	0.270	0.813
	rs4576294	G/A	Exon		GG (177)	GA (10)	AA (0)	0.027	0.707
	rs241436	C/T	Intron		CC (57)	CT (82)	TT (48)	0.476	0.098
	rs241437	C/T	Intron		CC (52)	CT (92)	TT (43)	0.476	0.851
	rs241438	G/A	Intron	Arg651Cys	GG (49)	GA (91)	AA (48)	0.497	0.662
	rs241439	C/A	Intron		CC (52)	CA (91)	AA (44)	0.479	0.733
	rs4148876	C/T	Exon/intron		CC (132)	CT (52)	TT (3)	0.155	0.403
	rs241454	T/C	3'UTR/intron		TT (71)	TC (92)	CC (24)	0.374	0.492
	rs10484565	G/A	3'UTR/intron	Arg651Cys	GG (146)	GA (38)	AA (3)	0.118	0.772
	rs2857101	A/G	3'UTR/intron		AA (71)	AG (94)	GG (24)	0.376	0.407
	rs13501	G/A	3'UTR/intron		GG (56)	GA (96)	AA (35)	0.444	0.586
	rs1894411	A/G	3'near/intron		AA (141)	AG (42)	GG (5)	0.138	0.390
	rs2856993	C/G	3'near/intron	Arg651Cys	CC (89)	CG (83)	GG (15)	0.302	0.473
	rs2857103	G/T	3'near/intron		GG (56)	GT (96)	TT (35)	0.444	0.586
	rs2621321	T/C	3'near		TT (81)	TC (88)	CC (18)	0.332	0.399

Abbreviations: HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; SNP, single-nucleotide polymorphism; UTR, untranslated region.
*P-value of deviation from HWE.

bacterial infection in the upper respiratory tract and bronchiectasis in the lower respiratory tract.⁸ Furthermore, polymorphisms in *TAP1* and *TAP2* have been implicated in idiopathic bronchiectasis in children,⁹ and a polymorphism in *TAP1* has been found to induce the risk of atopy.¹⁰

Because of the associations between the TAP complex and immune responses in the respiratory tracts, we hypothesized that genetic variations of *TAP1* and *TAP2* might be associated with AERD and/or its related symptoms.

MATERIALS AND METHODS

Study subjects

Study subjects were recruited from hospitals of Soonchunhyang, Chungbuk National, Chonnam National, Seoul National and Chung-Ang Universities in Korea. Study protocols were approved by the Institutional Review Board of the hospitals where all subjects were recruited, and all subjects provided written informed consent. All patients who meet the guidelines of Global Initiative for Asthma (GINA) for the clinical symptoms of asthma showed histories of dyspnea and wheezing during the previous 12 months, along with one of the following: (1) >15% increase in FEV1 or >12% increase plus 200 ml following inhalation of a short-acting bronchodilator, (2) <10 mg ml⁻¹ PC20 methacholine and (3) >20% increase in FEV1 following 2 weeks of treatment with inhaled steroids and long-acting bronchodilators. Total IgE was determined using the UniCAP system (Pharmacia Diagnostics, Uppsala, Sweden). Oral aspirin challenge was performed with a slight modification in increasing doses of aspirin,¹¹ following the guidelines of EAACI/GA2LEN.¹² Changes in FEV1 were followed for 5 h after the last aspirin challenge dose. Aspirin-induced

bronchospasms, as reflected by rate (%) of FEV1 decline, were calculated as the pre-challenge FEV1 minus the post-challenge FEV1 divided by the pre-challenge FEV1. On the basis of the oral aspirin provocation test, subjects were stratified into two groups as follows: patients who showed 20% or greater decrease in FEV1 or 15 to 19% decrease in FEV1 with naso-ocular or cutaneous reactions were classified as AERD cases, whereas asthmatics who showed less than 15% decreases in FEV1 without naso-ocular or cutaneous reactions were grouped as aspirin-tolerant asthma (ATA) controls.

Single-nucleotide polymorphism (SNP) selection and genotyping

Based on the minor allele frequency of Asian populations from the International HapMap Project database (<http://hapmap.ncbi.nlm.nih.gov/index.html>), a total of 43 common single-nucleotide polymorphisms (SNPs) (minor allele frequency >0.05) composed of 12 *TAP1* SNPs and 31 *TAP2* SNPs were selected for genotyping. Genotyping was performed in a total of 189 asthma patients including 93 AERD patients and 96 ATA controls using TaqMan assay on the ABI prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA), along with assessment of data qualities by duplicate DNAs ($n=10$). SNPs that did not meet the following criteria were excluded from association analyses: (1) a minimum call rate of 95%; (2) no duplicate error; (3) Hardy-Weinberg equilibrium of $P>0.05$.

Statistics

Linkage disequilibrium (LD) of *TAP1* and *TAP2* polymorphisms was analyzed using the Haploview v4.1. software downloaded from the Broad Institute (<http://www.broadinstitute.org/mpg/haploview>).¹³ LD coefficients ($|D'|$ and r^2) between all pairs of biallelic loci were used to determine LD among the SNPs.

a

TAP2 on chromosome 6p21.3

b

Haplotypes of TAP2

BL1						BL2								BL3							BL4									
Hap.	rs3763366	rs4148870	rs2071544	rs2071465	Freq.	Hap.	rs4148871	rs241430	rs241432	rs4148873	rs2228397	rs241433	rs1015166	Freq.	Hap.	rs241437	rs241438	rs241439	rs4148876	rs241454	rs10484565	Freq.	Hap.	rs2857101	rs13501	rs1894411	rs2856993	rs2857103	rs2621321	Freq.
ht1	G	G	A	G	0.481	ht1	C	G	A	G	T	T	C	0.302	ht1	C	A	C	C	C	G	0.348	ht1	A	G	A	C	G	T	0.417
ht2	C	A	G	C	0.313	ht2	C	G	A	G	G	T	T	0.270	ht2	T	G	A	C	T	G	0.203	ht2	G	A	A	G	T	C	0.302
ht3	C	A	G	G	0.155	ht3	T	A	C	G	G	G	C	0.182	ht3	T	G	A	T	T	G	0.152	ht3	A	G	G	C	G	T	0.139
ht4	C	G	G	G	0.048	ht4	C	A	C	A	G	G	C	0.118	ht4	C	A	C	C	T	G	0.150	ht4	A	A	A	C	T	T	0.070
ht5	C	G	A	G	0.003	ht5	C	A	C	G	G	G	C	0.086	ht5	T	G	A	C	T	A	0.115	ht5	G	A	A	C	T	T	0.043
						ht6	C	G	A	G	G	T	C	0.021	ht6	C	G	C	C	C	G	0.021	ht6	G	A	A	C	T	C	0.029
						Others	.	.	.	.	.	.	.	0.021	Others	.	.	.	.	.	.	0.012								

Figure 1 Physical map and haplotypes of *TAP2*. (a) Physical map of *TAP2* and its SNPs genotyped in this study. This gene has two isoforms, NM_000544 and NM_018833, which are different in 3' untranslated region (UTR), especially in exon 12. The coding exons are represented by black blocks, and 5'UTR and 3'UTR by white blocks. (b) Haplotypes of *TAP2*. Haplotypes with frequency >0.05 (denoted as italics) are used for association analyses. BL, haplotype block.

Haplotypes were inferred from the PHASE algorithm.¹⁴ Associations of each SNP and haplotype of the genes with AERD and its related symptoms were assessed using the statistical analysis system (SAS), with adjusting age, gender, smoking status and atopy as covariates.

RESULTS

Characteristics of study subjects

Clinical and demographic characteristics of 189 study subjects including 93 AERD cases and 96 ATA controls are presented in Table 1. The male-female ratio of the study subjects was at ~1:2 for AERD and ~1:3 for ATA, as expected of the differential gender ratio in adult asthma according to the Global Allergy and Asthma European Network (GA2LEN). Although comparisons of total IgE level and skin test showed no significant differences between the subgroups ($P>0.05$), about 1.3-fold nominal increase in blood eosinophils of AERD patients compared with ATA controls was observed (Table 1). The FEV1 decline by aspirin provocation in the patient group was significantly higher than that in the control group ($P<0.001$).

Polymorphisms, LD and haplotypes of TAP1 and TAP2

A total of 43 common SNPs (minor allele frequency >0.05), 12 SNPs in *TAP1* and 31 SNPs in *TAP2*, were selected and successfully genotyped in 93 AERD patients and 96 ATA controls (Table 2). Four non-synonymous SNPs in coding regions (Ile393Val and Asp697Gly in *TAP1*; Val379Ile and Arg651Cys in *TAP2*) were included. In the case of *TAP2*, positions of eight SNPs are different depending on two isoforms, NM_000544 and NM_018833 (Figure 1a and Table 2). All SNPs showed no departure from Hardy-Weinberg equilibrium ($P>0.05$, Table 2).

Pairwise comparisons of the selected SNPs revealed that, although *TAP1* rs2071481 was excluded from the LD plot, SNPs in *TAP1* were in one tight LD block (Supplementary Figure 1), whereas polymorphisms of *TAP2* encompassed four LD blocks (Figure 2). Among the inferred haplotypes in *TAP1* (Supplementary Figure 1) and *TAP2* (Figure 1b), only common haplotypes with frequency over 0.05 were used for the haplotype association analysis. Because of the equivalences of haplotypes to SNPs (*ht1* to rs2071540, *ht3* to rs1057373, *ht4* to rs2284190 in *TAP1* as shown in Supplementary Figure 1; *BL1-ht1* to rs3763366, *BL1-ht2* to rs2071465, *BL2-ht1* to rs2228397, *BL2-ht2* to rs1015166, *BL2-ht3* to rs4148871, *BL3-ht3* to rs4148876, *BL3-ht5* to rs10484565; *BL4-ht2* to rs2856993, *BL4-ht3* to rs1894411 in *TAP2* as shown in Figure 1b), some haplotypes were also excluded from the haplotype association analysis.

Association analysis of polymorphisms in TAP1 and TAP2 with AERD and its related symptoms

Logistic analyses that were adjusted by age, gender, smoking status and atopy as covariates using multiple models showed that none of the *TAP1* polymorphisms/haplotypes were associated with both FEV1 decline by aspirin provocation and AERD development ($P>0.05$, Supplementary Tables 1 and 2). However, regression analysis revealed significant associations of seven polymorphisms and two haplotypes in *TAP2* with FEV1 decline by aspirin provocation ($P=0.002-0.04$, Table 3). Except rs241437 in block 3 of *TAP2*, most of minor homozygotes showed about twofold decline rate of FeV1 compared with major homozygotes. In particular, most significant polymorphisms were focused on the distinctive region between two transcripts of

Figure 2 LD plot of *TAP2* polymorphisms investigated in this study. *TAP2* polymorphisms investigated in this study compose of four haplotype blocks. LD number in blocks represent values of multiallelic D' .

NM_000544 and NM_018833 (Figure 1a). Although significances were not high as the values in the association with FEV1 decline, nominal evidences in the association of two polymorphisms and one haplotype of *TAP2* with AERD development were observed (OR=0.44–1.94, $P=0.02$ – 0.04 , Table 4).

DISCUSSION

Although the exact mechanism underlying aspirin hypersensitivity has not been elucidated, AERD, also referred to aspirin-intolerant asthma, is often severe and even can be life threatening. Blockage of cyclooxygenase (COX) pathway, which converts arachidonic acid into prostaglandins, by aspirin eventually results in the diversion of arachidonic

acid metabolites to CysLTs via the 5-lipoxygenase pathway.^{2,4} Overproduction of CysLTs and deficiency of prostaglandin E_2 in bronchial fibroblasts by COX inhibition have been proposed to have crucial roles in AERD development.^{4,15} However, along with conflicting results for the associations between polymorphisms of genes in leukotriene and COX pathways^{16–18} genes on other pathways including bronchial hyperresponsiveness by MHC or structural genes have provided new insights for AERD pathogenesis.^{5,19} Therefore, although further replications and functional evaluations are required, our findings on the associations of *TAP2* with AERD-related symptoms, particularly with FEV1 decline by aspirin provocation, might provide evidences for the role of the gene in respiratory deficiencies.

Table 3 Associations of *TAP2* polymorphisms and haplotypes with decline of FEV1 by aspirin provocation

Haplotype block	SNP or haplotype	Position	C/C	C/R	R/R	Pa	Pb	Pc
Block 1	<i>rs3763366</i>	5'near	51 (12.05 ± 15.52)	94 (12.43 ± 16.33)	44 (11.41 ± 13.15)	0.97	0.88	0.82
	<i>rs4148870</i>	Intron	55 (10.90 ± 12.92)	89 (12.39 ± 16.94)	43 (12.66 ± 15.29)	0.65	0.60	0.82
	<i>rs2071544</i>	Intron	50 (12.34 ± 15.54)	95 (12.28 ± 16.31)	44 (11.41 ± 13.15)	0.90	0.99	0.82
	<i>rs2071465</i>	Intron	89 (12.35 ± 16.45)	78 (11.50 ± 14.84)	19 (12.19 ± 13.64)	0.84	0.78	0.98
	<i>TAP2-BL1-ht3</i>		134 (11.36 ± 14.05)	46 (13.16 ± 17.90)	6 (17.30 ± 25.32)	0.40	0.48	0.47
	<i>rs2239701</i>	Intron	42 (10.11 ± 12.71)	101 (13.44 ± 16.57)	44 (10.55 ± 14.99)	0.79	0.33	0.59
	<i>rs241424</i>	Intron	49 (9.63 ± 15.77)	94 (13.59 ± 16.14)	44 (11.30 ± 13.20)	0.53	0.22	0.81
	<i>rs3819721</i>	Intron	101 (12.50 ± 15.18)	72 (10.96 ± 14.00)	14 (13.95 ± 23.34)	0.84	0.61	0.62
	<i>rs241426</i>	Intron	74 (11.83 ± 15.99)	91 (12.02 ± 14.33)	22 (12.60 ± 18.27)	0.73	0.76	0.80
	<i>rs3819714</i>	Intron	63 (10.66 ± 14.01)	98 (12.34 ± 15.48)	26 (14.04 ± 18.48)	0.35	0.41	0.51
	<i>rs241429</i>	Intron	74 (13.69 ± 17.06)	86 (10.97 ± 13.18)	27 (10.75 ± 17.34)	0.18	0.12	0.64
	<i>rs4148871</i>	Intron	122 (12.47 ± 16.30)	62 (11.70 ± 13.75)	3 (–0.33 ± 1.53)	0.28	0.43	0.14
	<i>rs241430</i>	Intron	68 (13.86 ± 17.16)	95 (11.29 ± 14.08)	25 (9.99 ± 15.11)	0.14	0.10	0.51
	<i>rs241432</i>	Intron	66 (14.28 ± 17.23)	95 (11.33 ± 14.11)	28 (9.52 ± 14.58)	0.07	0.06	0.34
Block 2	<i>rs4148873</i>	Exon	141 (13.04 ± 16.38)	43 (7.86 ± 10.62)	3 (23.23 ± 17.72)	0.22	0.09	0.27
	<i>rs2228397</i>	Exon	90 (10.73 ± 15.26)	78 (12.26 ± 15.05)	19 (17.09 ± 17.29)	0.13	0.27	0.13
	<i>rs241433</i>	Intron	63 (13.59 ± 16.92)	96 (11.70 ± 14.64)	28 (9.52 ± 14.58)	0.14	0.16	0.35
	<i>rs1015166</i>	Intron	99 (11.31 ± 14.23)	75 (13.87 ± 16.90)	13 (6.67 ± 14.55)	0.85	0.46	0.32
	<i>TAP2-BL2-ht4</i>		145 (12.69 ± 16.31)	38 (8.45 ± 10.82)	3 (23.23 ± 17.72)	0.43	0.23	0.27
	<i>TAP2-BL2-ht5</i>		156 (12.31 ± 15.12)	28 (8.98 ± 15.68)	2 (29.50 ± 34.65)	0.83	0.48	0.09
	<i>rs4576294</i>	Exon	177 (11.86 ± 14.96)	10 (14.77 ± 22.84)	.	0.51	0.51	.
	<i>rs241436</i>	Intron	57 (13.24 ± 15.99)	82 (13.45 ± 15.93)	48 (8.11 ± 13.33)	0.13	0.55	0.05
	<i>rs241437</i>	Intron	52 (14.24 ± 16.71)	92 (12.76 ± 15.39)	43 (7.72 ± 13.18)	0.04	0.22	0.04
	<i>rs241438</i>	Intron	49 (9.11 ± 14.28)	91 (12.17 ± 14.85)	48 (14.80 ± 17.14)	0.07	0.12	0.16
	<i>rs241439</i>	Intron	52 (14.62 ± 16.63)	91 (12.24 ± 15.21)	44 (8.46 ± 13.92)	0.05	0.14	0.07
	<i>rs4148876</i>	Exon/intron	132 (12.76 ± 16.31)	52 (10.84 ± 13.07)	3 (–0.33 ± 1.53)	0.15	0.25	0.14
	<i>rs241454</i>	3'UTR/intron	71 (9.10 ± 14.09)	92 (12.71 ± 15.00)	24 (17.93 ± 19.01)	0.007	0.03	0.03
	<i>rs10484565</i>	3'UTR/intron	146 (11.61 ± 15.33)	38 (14.63 ± 15.83)	3 (–1.57 ± 2.10)	0.78	0.44	0.12
Block 3	<i>TAP2-BL3-ht1</i>		79 (10.12 ± 14.46)	86 (11.91 ± 14.78)	21 (19.38 ± 19.84)	0.02	0.13	0.01
	<i>TAP2-BL3-ht2</i>		118 (13.48 ± 15.75)	60 (9.45 ± 14.14)	8 (9.14 ± 19.45)	0.09	0.07	0.67
	<i>TAP2-BL3-ht4</i>		135 (12.27 ± 15.93)	46 (11.02 ± 13.94)	5 (13.42 ± 18.52)	0.62	0.61	0.87
	<i>rs2857101</i>	3'UTR/intron	71 (9.10 ± 14.09)	94 (12.86 ± 14.88)	24 (17.93 ± 19.01)	0.006	0.03	0.03
	<i>rs13501</i>	3'UTR/intron	56 (9.89 ± 14.61)	96 (10.89 ± 14.03)	35 (18.48 ± 18.72)	0.007	0.17	0.002
	<i>rs1894411</i>	3'near/intron	141 (12.32 ± 15.86)	42 (10.89 ± 13.67)	5 (13.94 ± 17.92)	0.65	0.59	0.98
	<i>rs2856993</i>	3'near/intron	89 (9.34 ± 14.28)	83 (13.57 ± 15.12)	15 (19.22 ± 20.48)	0.004	0.02	0.03
	<i>rs2857103</i>	3'near/intron	56 (9.89 ± 14.61)	96 (10.89 ± 14.03)	35 (18.48 ± 18.72)	0.007	0.17	0.002
	<i>rs2621321</i>	3'near	81 (9.39 ± 14.72)	88 (12.88 ± 14.46)	18 (19.58 ± 20.31)	0.007	0.04	0.02
	<i>TAP2-BL4-ht1</i>		64 (15.56 ± 17.49)	88 (10.38 ± 13.34)	34 (9.47 ± 15.73)	0.02	0.01	0.25
	<i>TAP2-BL4-ht4</i>		161 (11.95 ± 15.32)	24 (12.79 ± 16.77)	1 (0.80)	0.90	0.79	0.55

Abbreviations: SNP, single-nucleotide polymorphism; UTR, untranslated region.

C/C, C/R and R/R indicate the homozygote of common allele, the heterozygote and the homozygote of rare allele, respectively.

P -value is adjusted for age, sex, smoking and atopy as co-variables.

P_a , P_b , and P_c indicate the P -value of co-dominant, dominant, and recessive models, respectively.

BL1-ht1 and *BL1-ht2* are equivalent to *rs3763366* and *rs2071465*, respectively.

BL2-ht1, *BL2-ht2* and *BL2-ht3* are equivalent to *rs2228397*, *rs1015166* and *rs4148871*, respectively.

BL3-ht3 and *BL3-ht5* are equivalent to *rs4148876* and *rs10484565*, respectively.

BL4-ht2 and *BL4-ht3* are equivalent to *rs2856993* and *rs1894411*, respectively.

Values significant at $P < 0.05$ are shown in bold.

The HLA system, also described as MHC, is a key feature of the immune system. Although important roles of TAP molecules encoded within the MHC class II region have been elucidated in many immune processes,²⁰ few studies on the relations of TAP to asthma and its related phenotypes have been published. According to the clinical manifestations in patients with TAP deficiency syndrome, almost all the patients show ear/nose/throat diseases (for example, chronic sinusitis and nasal polyps), and ~80% of the patients exhibit lung diseases (for example, chronic spastic bronchitis and recurrent bacterial infection).⁸ Our preliminary results on the significant associations

between *TAP2* variants and the FEV1-related phenotype suggest that dysfunctions of *TAP2* could lead deficits in lower airways.

Polymorphisms in the *TAP1* and *TAP2* have shown discriminative association results depending on the nature of the allergic diseases. Non-synonymous SNPs and haplotypes of *TAP1* have been revealed to be associated with allergic rhinitis, whereas those of *TAP2* showed no associations.²¹ In addition, association analysis of *TAP1* polymorphisms, but not tested for *TAP2*, has provided a strong relation to atopy.¹⁰ Meanwhile, polymorphisms of both *TAP1* and *TAP2*, particularly with a portion of the same non-synonymous SNPs investigated

Table 4 Associations of *TAP2* polymorphisms and haplotypes with the risk of AERD

Haplotype block	SNP or haplotype	Position	MAF		Co-dominant		Dominant		Recessive	
			AERD (n=93)	ATA (n=96)	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Block 1	rs3763366	5'near	0.484	0.479	1.05 (0.70–1.59)	0.80	1.02 (0.53–1.97)	0.94	1.13 (0.56–2.27)	0.73
	rs4148870	Intron	0.473	0.464	1.00 (0.67–1.50)	0.98	1.00 (0.53–1.92)	0.99	1.01 (0.50–2.02)	0.98
	rs2071544	Intron	0.484	0.484	1.04 (0.69–1.56)	0.87	0.98 (0.51–1.89)	0.95	1.13 (0.56–2.27)	0.73
	rs2071465	Intron	0.322	0.302	1.09 (0.70–1.70)	0.70	1.10 (0.61–1.98)	0.76	1.17 (0.45–3.07)	0.75
	TAP2-BL1-ht3		0.150	0.161	0.88 (0.50–1.53)	0.64	0.92 (0.48–1.77)	0.80	0.50 (0.09–2.94)	0.45
	rs2239701	Intron	0.495	0.516	0.95 (0.62–1.45)	0.80	1.09 (0.54–2.18)	0.81	0.80 (0.40–1.59)	0.52
	rs241424	Intron	0.516	0.458	1.29 (0.85–1.95)	0.23	1.55 (0.79–3.03)	0.20	1.26 (0.64–2.50)	0.51
	rs3819721	Intron	0.231	0.302	0.68 (0.42–1.09)	0.11	0.69 (0.38–1.24)	0.21	0.40 (0.12–1.35)	0.14
	rs241426	Intron	0.374	0.349	1.18 (0.75–1.84)	0.48	1.41 (0.77–2.58)	0.27	0.90 (0.36–2.24)	0.81
	rs3819714	Intron	0.401	0.401	0.99 (0.63–1.53)	0.95	0.96 (0.52–1.78)	0.90	1.02 (0.44–2.38)	0.96
	rs241429	Intron	0.346	0.401	0.75 (0.49–1.15)	0.19	0.77 (0.42–1.42)	0.40	0.54 (0.23–1.28)	0.16
Block 2	rs4148871	Intron	0.176	0.188	0.87 (0.49–1.54)	0.62	0.98 (0.53–1.82)	0.95	.	.
	rs241430	Intron	0.359	0.411	0.74 (0.47–1.16)	0.18	0.70 (0.37–1.29)	0.25	0.64 (0.27–1.54)	0.32
	rs241432	Intron	0.366	0.432	0.70 (0.45–1.08)	0.11	0.64 (0.34–1.20)	0.16	0.61 (0.26–1.39)	0.24
	rs4148873	Exon	0.126	0.135	0.88 (0.47–1.63)	0.68	0.82 (0.42–1.63)	0.57	1.57 (0.13–18.53)	0.72
	rs2228397	Exon	0.346	0.276	1.40 (0.89–2.19)	0.14	1.40 (0.78–2.52)	0.26	1.97 (0.72–5.41)	0.19
	rs241433	Intron	0.374	0.438	0.70 (0.45–1.10)	0.12	0.64 (0.34–1.22)	0.17	0.62 (0.27–1.42)	0.26
	rs1015166	Intron	0.275	0.266	1.11 (0.69–1.77)	0.67	1.26 (0.70–2.26)	0.44	0.75 (0.23–2.43)	0.63
	TAP2-BL2-ht4		0.122	0.115	1.03 (0.54–1.94)	0.94	0.99 (0.49–2.01)	0.98	1.61 (0.14–18.90)	0.71
	TAP2-BL2-ht5		0.061	0.109	0.53 (0.24–1.14)	0.10	0.46 (0.20–1.05)	0.07	1.30 (0.08–21.36)	0.86
	rs4576294	Exon	0.022	0.031	0.67 (0.17–2.60)	0.57	0.67 (0.17–2.60)	0.57	.	.
	rs241436	Intron	0.434	0.516	0.77 (0.52–1.14)	0.19	1.02 (0.54–1.93)	0.95	0.44 (0.22–0.89)	0.02
Block 3	rs241437	Intron	0.434	0.516	0.74 (0.49–1.12)	0.15	0.85 (0.44–1.62)	0.61	0.50 (0.24–1.03)	0.06
	rs241438	Intron	0.543	0.453	1.40 (0.93–2.12)	0.11	1.92 (0.97–3.81)	0.06	1.31 (0.67–2.56)	0.43
	rs241439	Intron	0.434	0.521	0.72 (0.47–1.08)	0.11	0.75 (0.39–1.44)	0.39	0.53 (0.26–1.08)	0.08
	rs4148876	Exon/intron	0.143	0.167	0.80 (0.44–1.44)	0.45	0.90 (0.47–1.71)	0.74	.	.
	rs241454	3'UTR/intron	0.418	0.333	1.52 (0.97–2.39)	0.07	1.87 (1.01–3.47)	0.05	1.39 (0.58–3.37)	0.46
	rs10484565	3'UTR/intron	0.126	0.109	1.26 (0.67–2.39)	0.47	1.58 (0.78–3.22)	0.21	.	.
	TAP2-BL3-ht1		0.383	0.307	1.46 (0.93–2.28)	0.10	1.60 (0.88–2.91)	0.13	1.65 (0.64–4.23)	0.30
	TAP2-BL3-ht2		0.161	0.245	0.59 (0.34–1.00)	0.05	0.50 (0.27–0.94)	0.03	0.72 (0.16–3.21)	0.67
	TAP2-BL3-ht4		0.156	0.146	1.02 (0.58–1.80)	0.95	1.13 (0.59–2.17)	0.72	0.46 (0.07–3.02)	0.42
Block 4	rs2857101	3'UTR/intron	0.419	0.333	1.54 (0.98–2.43)	0.06	1.94 (1.05–3.60)	0.04	1.36 (0.56–3.27)	0.50
	rs13501	3'UTR/intron	0.484	0.406	1.47 (0.95–2.28)	0.08	1.45 (0.76–2.77)	0.26	1.98 (0.92–4.28)	0.08
	rs1894411	3'near/intron	0.130	0.146	0.83 (0.46–1.48)	0.53	0.86 (0.44–1.68)	0.65	0.49 (0.08–3.19)	0.46
	rs2856993	3'near/intron	0.341	0.266	1.51 (0.94–2.45)	0.09	1.74 (0.96–3.17)	0.07	1.37 (0.45–4.12)	0.58
	rs2857103	3'near/intron	0.484	0.406	1.47 (0.95–2.28)	0.08	1.45 (0.76–2.77)	0.26	1.98 (0.92–4.28)	0.08
	rs2621321	3'near	0.374	0.292	1.51 (0.95–2.41)	0.08	1.75 (0.96–3.21)	0.07	1.43 (0.52–3.93)	0.49
	TAP2-BL4-ht1		0.389	0.448	0.77 (0.51–1.18)	0.23	0.72 (0.39–1.34)	0.30	0.69 (0.32–1.50)	0.35
	TAP2-BL4-ht4		0.067	0.073	0.99 (0.44–2.24)	0.98	1.07 (0.45–2.54)	0.87	.	.

Abbreviations: AERD, aspirin exacerbated respiratory disease; ATA, aspirin-tolerant asthma; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism; UTR, untranslated region.

P-value is adjusted for age, sex, smoking and atopy as co-variables.

BL1-ht1 and BL1-ht2 are equivalent to rs3763366 and rs2071465, respectively.

BL2-ht1, BL2-ht2 and BL2-ht3 are equivalent to rs2228397, rs1015166 and rs4148871, respectively.

BL3-ht3 and BL3-ht5 are equivalent to rs4148876 and rs10484565, respectively.

BL4-ht2 and BL4-ht3 are equivalent to rs2856993 and rs1894411, respectively.

Values significant at $P < 0.05$ are shown in bold.

in the association with allergic rhinitis, have been found to induce susceptibility to bronchiectasis in children.⁹ On the other hand, only the variants in *TAP2* have been revealed to be a potential risk factor for systemic lupus erythematosus.²² Therefore, results from previous studies and the current report suggest that there might be complex and/or differential *TAP* functions in the transport process and immune response regulation.

In this study, the strength of association was increased in the 3'-region of *TAP2* isoform 1 (NM_000544) and isoform 2 (NM_018833), suggesting that SNPs in the region might have a role in the regulation of the gene. The *TAP2* isoform 2 (NM_018833; also termed as *TAP2iso*) derived from alternative splicing has been found to be normally co-expressed with isoform 1 (NM_000544), but has exhibited distinct functions.²³ Furthermore, functional polymorphisms in the peptide C-terminal residue of *TAP2* induce a change in substrate selectivity.²⁴ Therefore, although functional evaluations are needed, it is suggested that several polymorphisms of *TAP2* showing significant associations with FEV1 decline by aspirin provocation might contribute to additional immune diversity, especially with a possible effect on the selection process of antigen peptides that have a role in the pathogenesis of related conditions.

As expression quantitative trait loci are regions of the genome that regulate expression levels of mRNAs or proteins, we used the expression quantitative trait loci browser (<http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/>) to confirm whether the risk SNPs of *TAP2* could have an effect on the expression of the protein and/or mRNA. As a result, among six polymorphisms of expression quantitative trait loci for *TAP2* (Supplementary Figure 2), *rs13501* was found to act as a potential cis-regulator for *TAP2* expression, with an expression quantitative trait loci score of 5.706 (Supplementary Table 3). To investigate potential polymorphisms with significant functions in the gene, additional *in silico* analyses of potential branch point site for alternative splicing using EMBL-EBI splice-site prediction (<http://www.ebi.ac.uk/asd-srv/wb.cgi?method=2>) and putative binding site for regulators using the Signal Scan program (<http://www.bimas.cit.nih.gov/molbio/signal/>) were performed. Nevertheless, none of the significantly associated *TAP2* polymorphisms were predicted as a functional variant.

Because of the association of *HLA-DPB1*0301* with AERD in Koreans,²⁵ further analysis investigating the LD between *TAP2* and *HLA-DPB1* was performed using data from Asian populations (Japanese and Chinese) provided by the International HapMap Project database (<http://hapmap.ncbi.nlm.nih.gov/index.html.en>). However, *TAP2* is not in LD with *HLA-DPB1* (Supplementary Figure 3), suggesting that the potential association between *TAP2* polymorphisms and obstructive diseases may not be related with *HLA-DPB1*. Also, in additional analysis of LD near *TAP2* in Asian populations from the International HapMap Project, *TAP2* showed no LD with other genes in the nearby region (Supplementary Figure 4).

Recent studies have reported associations of the genetic variations in many genes on the arachidonate, immune response and inflammation pathways, such as *leukotriene C4 synthase (LTC4S)*,^{26,27} *arachidonate 5-lipoxygenase (ALOX5)*¹⁶ and *MHC class II, DP beta 1 (HLA-DPB1)*,²⁵ with aspirin hypersensitivity in asthmatics. However, conflicting results of the associations between polymorphisms of the genes and AERD have been reported, suggesting that underlying mechanisms of AERD pathogenesis may be more complicated than previously thought. Therefore, further replications and investigations with other candidate genes are required in order to comprehensively identify the exact genetic mechanisms of AERD etiology and its related phenotypes.

Genetic predisposition of *TAP2* to the diffuse panbronchiolitis, a chronic inflammatory lung disease (as classified by the GOLD guidelines; <http://www.goldcopd.org/>) that affects the upper and lower respiratory tracts, has been observed.²⁸ In addition, considering that *TAP* located on the endoplasmic reticulum membrane transports peptides from the cytoplasm into the endoplasmic reticulum and that endoplasmic reticulum stress (for example, by the affected translocation of peptides) contributes to the pathogenesis of chronic obstructive pulmonary disease,^{29,30} it is suggested that genetic variations of *TAP2* could be risk factors of pulmonary functional deficiencies. Although limitations of this study include insufficient sample size, considering that FEV1 decline is a criterion of total pulmonary function and is associated with bronchospasm by aspirin provocation, our preliminary results might provide clinically relevant information for the potential involvement of *TAP2*, rather than *TAP1*, in respiratory deficiencies.

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