Possible Association of *SLC22A2* Polymorphisms with Aspirin-Intolerant Asthma

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Key Words

Solute carrier family 22, member 2 · Aspirin-intolerant
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Abstract

Background: Aspirin-intolerant asthma (AIA) is a clinical syndrome characterized by acute bronchoconstriction following the ingestion of aspirin. Solute carrier family 22, member 2 (*SLC22A2*), also known as organic cation transporter 2 (OCT2), is predominantly expressed in the luminal membrane of airway epithelial cells and has been shown to mediate the transport of prostaglandins on the cyclooxygenase pathway which is regulated by aspirin blockage. Recently, *SLC22A2*-mediated uptake inhibition by several non-steroidal anti-inflammatory drugs and decreased *SLC22A2* transport activity by its genetic variants have been elucidated in asthma. **Methods:** To investigate the associations between AIA and genetic polymorphisms of the *SLC22A2* gene,

18 variants were genotyped in 163 AIA subjects and 429 aspirin-tolerant asthma (ATA) controls. Logistic analyses were used to evaluate p values for the associations of *SLC22A2* polymorphisms with AIA. **Results:** One common polymorphism in intron 5, i.e. *rs316021*, was significantly associated with susceptibility to AIA ($p = 0.004$, $P^{corr} = 0.05$, OR = 0.60, 95% CI = 0.43–0.85 in a codominant model). The minor allele frequency of *rs316021* in the AIA group was significantly lower than that in the ATA controls. In addition, a polymorphism in intron 4 (*rs3912161*) and a haplotype (*SLC22A2-ht3*) showed significantly stronger association signals with the FEV₁ fall rate induced by aspirin provocation in AIA subjects compared with ATA controls ($p = 0.004$, $P^{corr} = 0.05$). **Conclusion:** Our findings suggest that *SLC22A2* could be a susceptibility gene for aspirin intolerance in asthmatics.

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Introduction

Aspirin-intolerant asthma (AIA) is a unique clinical syndrome characterized by acute bronchoconstriction following the ingestion of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs). This syndrome is characterized by the triad of aspirin hypersensitivity, bronchial asthma, and chronic rhinosinusitis with nasal polyposis [1, 2]. AIA affects 5–15% of asthmatics and occurs more frequently in women [3, 4]. A 2-compartment model that is composed of the augmentation of cysteinyl leukotriene (CysLT) production and the overexpression of the CysLT receptor on inflammatory cells within the respiratory tract has been proposed as a factor in the development of AIA. However, to date, the pathogenesis of AIA has not been clearly understood [5].

Human polyspecific solute carrier family 22 (organic cation transporter), member 2 (SLC22A2; MIM No. 602608), encoded by *SLC22A2* is a member of the solute carrier family 22 superfamily, which constructs a structure consisting of 12 transmembrane domains. In previous studies, the transport of prostaglandins, which is converted from arachidonic acid in the cyclooxygenase pathway that is regulated by aspirin blockage, has been shown to be mediated by human organic cation transporters including SLC22A2 in the basolateral membrane of the proximal tubule [6]. Recently, it has also been revealed that polyspecific organic cation transports are involved in the transportation of acetylcholine (ACh) as a novel regulator of airway remodeling [7, 8]. Furthermore, SLC22A2 has been elucidated to modulate ACh release in human bronchial epithelia [9], suggesting that SLC22A2 might mediate the luminal release of ACh in human airways and that ingestion of NSAIDs could inhibit SLC22A2 functions in the treatment of asthma.

Based on these properties, we investigated whether single nucleotide polymorphisms (SNPs) of the *SLC22A2* gene are associated with the risk of AIA.

Materials and Methods

Study Subjects

This study was conducted in compliance with the Global Initiative for Asthma (GINA) Global Strategy for Asthma Management and Prevention Study. Subjects were recruited from the Asthma Genome Research Center comprising the hospitals of Soonchunhyang, Chungnam, Chungbuk, and Seoul National Universities in Korea. All subjects provided their informed consent, and the protocols were approved by the Institutional Re-

view Board of each hospital. Each patient showed airway reversibility measured by an inhalant bronchodilator-induced improvement of more than 15% in the forced expiratory volume in 1 s (FEV₁) and/or a hyperresponsiveness of less than 10 mg/ml of PC20 methacholine [10]. A methacholine challenge test was performed 1 week before aspirin challenge. Oral aspirin challenge was performed in all asthmatic subjects with increasing doses of aspirin using methods that were slightly modified from those described previously [11]. Aspirin-induced bronchospasms presented by the rate (%) of FEV₁ decline were evaluated as the scores of the prechallenge FEV₁ minus the postchallenge FEV₁ divided by the prechallenge FEV₁. Subjects showing a rate of FEV₁ decline of less than 15% without naso-ocular or cutaneous reactions were included in the aspirin-tolerant asthma (ATA) group. Subjects with hypertension/diabetes or those taking angiotensin-converting enzyme inhibitors were excluded from this study. The clinical parameters are summarized in table 1.

SNP Selection and Genotyping

We selected candidate SNPs that were reported to be non-monomorphic in the National Center for Biotechnology Information (build 36), and then genotyped in 163 AIA and 429 ATA subjects. Genotyping was carried out using the Illumina GoldenGate genotyping system at a multiplex level [12], and data quality was evaluated via duplicate DNAs (n = 10). The genotype quality score for retaining data was set to 0.25. SNPs that were below the following standards were excluded: (i) a minimum call rate of 90%, (ii) no duplicate error, and (iii) p values of Hardy-Weinberg equilibrium greater than 0.001. All 18 SNPs on *SLC22A2* were successfully genotyped.

Statistics

Comparisons of genotype distributions between AIA and ATA subjects were carried out using logistic regression controlling for sex, smoking status, and atopy as covariates using HelixTree® software (Golden Helix, Bozeman, Mont., USA). To carry out the association analysis of the polymorphisms in *SLC22A2*, we examined Lewontin's D' ($|D'|$) and the linkage disequilibrium (LD) coefficient r^2 between all pairs of biallelic loci. LD was inferred using the algorithm developed by the Broad Institute (using the program Haploview) [13]. Haplotypes were first estimated using PHASE software [14] and then computed by logistic analyses using Statistical Analysis System (SAS). Subjects harboring missing genotypes were omitted in the analysis of individual SNPs and haplotypes. The genotyping success rate was >99%, with 0.7% missing genotypes; it is unlikely that omitting a small number of individuals introduced any bias into the analysis. Significant associations are shown in bold face (p < 0.05). The common alleles were used as the referent genotype to the heterozygote and homozygote of the minor allele in referent analysis. The effective numbers of independent marker loci in each gene were calculated to correct for multiple testing using the software SNPSpD (<http://genepi.qimr.edu.au/general/daleN/SNPSpD/>), which is based on the spectral decomposition (SpD) of the matrices of pair-wise LD between SNPs [15].

Table 1. Clinical profiles of AIA patients and controls

Clinical profile	Asthmatics (all subjects)	AIA	ATA
n	592	163	429
Mean age of first medical examination (range), years	46.15 (15.40–77.88)	43.13 (17.22–72.73)*	47.30 (15.40–77.88)
Height, cm	160.78 ± 8.63	161.72 ± 8.69	160.42 ± 8.39
Weight, kg	62.81 ± 10.84	61.25 ± 10.38*	63.40 ± 10.97
BMI	24.24 ± 3.39	23.39 ± 3.25*	24.58 ± 3.39
Fall rate, %	9.27 ± 13.24	24.63 ± 16.11**	3.54 ± 4.85
Blood eosinophil, %	6.01 ± 5.73	5.96 ± 5.21	6.03 ± 5.92
FVC % predicted	88.54 ± 14.08	90.35 ± 14.04	87.85 ± 14.05
FEV ₁ % predicted	90.54 ± 16.97	87.58 ± 16.94*	91.66 ± 16.87
PC20, methacholine, mg/ml	6.43 ± 8.67	5.02 ± 7.83*	6.91 ± 8.90
Total IgE, IU/ml	357.65 ± 604.09	348.60 ± 596.44	361.00 ± 607.56
Male/female ratio	206/386	59/104	147/282
Current smoker, %	27.70	21.47*	30.07
Positive rate of skin test, %	56.42	52.76	57.81
Positive rate of specific IgE, D.f., %	36.38	38.30	35.75
Positive rate of specific IgE, D.p., %	44.56	45.77	44.16

Each clinical profile of AIA was compared to the respective ATA control. BMI = Body mass index; FVC = forced expiratory vital capacity; FEV₁ = forced expiratory volume in 1 s; D.f. = *Dermatophagoides farinae*; D.p. = *Dermatophagoides pteronyssinus*.

* $p < 0.05$, ** $p < 0.0001$ compared to ATA control.

Results

Characteristics of the Study Subjects

The clinical profiles of the study subjects are summarized in table 1. The asthmatic subjects were divided into 2 groups (AIA and ATA groups) according to their sensitivity to aspirin. A decrease in FEV₁ of –15 to 68% induced by aspirin provocation was observed. The percentage of decrease in FEV₁ by aspirin provocation in AIA patients was significantly higher compared to ATA controls ($p < 0.0001$). The values of FEV₁ % predicted and PC20 methacholine were significantly lower in AIA patients than in ATA controls ($p < 0.05$). The percentage of current smokers was significantly lower in the AIA group than in the ATA group ($p < 0.05$). In addition, the mean age of patients who had their first medical examination was significantly lower in AIA patients than in ATA controls ($p < 0.05$), indicating that the symptomatic outcome of AIA patients may occur earlier depending on the sensitivity to aspirin.

Genotyping and Haplotypes Inferred

We selected 18 nonmonomorphic sequence variants in the *SLC22A2* gene: 1 in the promoter (*rs3127573*), 1 in the coding region of exon 4 (*rs316019*), 3 in 3'-untranslated

region (*rs9364550*, *rs3127594*, and *rs694812*), and 13 in introns (*rs316008*, *rs3798156*, *rs316012*, *rs316013*, *rs316018*, *rs3912161*, *rs2279463*, *rs316021*, *rs315983*, *rs10945657*, *rs3798158*, *rs10755577*, and *rs13195185*) (online suppl. table 1, www.karger.com/doi/10.1159/000321267; fig. 1a). Eighteen SNPs were genotyped for the genetic association study with AIA. The minor allele frequencies of these 18 SNPs in a Korean population ($n = 592$) including 163 AIA and 429 ATA subjects are shown in online supplementary table 1. The genotype distributions of all loci were in Hardy-Weinberg equilibrium ($p > 0.05$). The LD coefficients ($|D'|$) among the SNPs were calculated for all of the study subjects (fig. 1c). Complete LDs were observed between SNPs, *rs316012* and *rs316013*, *rs316013* and *rs316018*, and *rs13195185* and *rs694812* ($r^2 = 1$). Ten haplotypes were constructed, and 4 of these with frequencies over 0.05 (*SLC22A2-ht1*, *SLC22A2-ht2*, *SLC22A2-ht3*, and *SLC22A2-ht4*) were analyzed for the association study (table 2; fig. 1b).

Associations of Polymorphisms and Haplotypes in *SLC22A2* with AIA

Eighteen SNPs and 4 haplotypes were analyzed for association with the risk of AIA using multiple logistic models in asthmatics (table 2). The association

Fig. 1. Gene maps and haplotypes of the *SLC22A2* gene. **a** Polymorphisms identified in *SLC22A2*. Coding exons are marked by black blocks and 3'-UTR by white blocks. The LD coefficients (r^2) are based on the genotypes of Korean samples. **b** Haplotypes of *SLC22A2* in the Korean population. Only those with frequencies over 0.01 are shown. **c** LD blocks and correlation coefficients among *SLC22A2* polymorphisms. Hap. = Haplotype; Freq. = frequency.

analyses showed that 1 SNP (*rs3912161*) and 1 haplotype (*SLC22A2-ht3*) were frequent in AIA subjects with a significant association of susceptibility with the risk of AIA, whereas 9 SNPs (*rs316012*, *rs316013*, *rs316018*, *rs316021*, *rs9364550*, *rs315983*, *rs10755577*, *rs13195185*, and *rs694812*) and 1 haplotype (*SLC22A2-ht4*) in AIA subjects showed considerably lower major allele frequencies (MAFs) than those in ATA controls. Specifically, *rs316021* had the strongest genetic effect among the SNPs and showed an association with the risk of AIA after multiple testing corrections ($p = 0.004$, $P^{corr} = 0.05$, OR = 0.60, 95% CI =

0.43–0.85 in the codominant model) with a lower MAF in AIA patients compared to ATA controls (MAF = 0.172 for AIA vs. 0.245 for ATA). When running multiple testing corrections on p values, the effective number of independent marker loci in *SLC22A2* calculated by SNPSpD was 13.00.

Association of Polymorphisms and Haplotypes in *SLC22A2* with a Decline in FEV_1

In addition, we investigated the associations of the percentages of maximum FEV_1 fall following aspirin

Table 2. Analyses of association of *SLC22A2* polymorphisms with risk of aspirin intolerance in asthma

SNP/ haplotype	Allele	Position	MAF		Codominant			Dominant			Recessive		
			AIA (n = 163)	ATA (n = 429)	OR (95% CI)	p	<i>P</i> ^{corr} *	OR (95%CI)	p	<i>P</i> ^{corr} *	OR (95% CI)	p	<i>P</i> ^{corr} *
<i>rs3127573</i>	T>C	Promoter	0.087	0.097	0.91 (0.57–1.43)	0.67	–	0.91 (0.56–1.48)	0.71	–	0.67 (0.07–6.15)	0.72	–
<i>rs316008</i>	A>C	Intron2	0.114	0.142	0.73 (0.49–1.10)	0.14	–	0.70 (0.45–1.08)	0.10	–	1.23 (0.20–7.57)	0.83	–
<i>rs3798156</i>	G>A	Intron2	0.060	0.083	0.69 (0.40–1.17)	0.16	–	0.70 (0.41–1.21)	0.20	–	–	0.98	–
<i>rs316012</i>	G>A	Intron2	0.163	0.221	0.66 (0.47–0.93)	0.02	0.23	0.63 (0.43–0.93)	0.02	0.27	0.52 (0.17–1.57)	0.25	–
<i>rs316013</i>	T>C	Intron2	0.163	0.222	0.66 (0.47–0.93)	0.02	0.22	0.63 (0.43–0.93)	0.02	0.25	0.52 (0.17–1.57)	0.24	–
<i>rs316018</i>	C>T	Intron3	0.163	0.221	0.66 (0.47–0.93)	0.02	0.23	0.63 (0.43–0.93)	0.02	0.27	0.52 (0.17–1.57)	0.25	–
<i>rs316019</i>	G>T	Exon4	0.102	0.139	0.70 (0.46–1.05)	0.08	–	0.71 (0.45–1.11)	0.13	–	0.27 (0.03–2.09)	0.21	–
<i>rs3912161</i>	A>G	Intron4	0.109	0.069	1.74 (1.10–2.75)	0.02	0.22	1.76 (1.10–2.82)	0.02	0.25	3.11 (0.17–57.14)	0.44	–
<i>rs2279463</i>	T>C	Intron4	0.063	0.083	0.72 (0.43–1.22)	0.22	–	0.74 (0.43–1.26)	0.27	–	–	0.98	–
<i>rs316021</i>	A>G	Intron5	0.172	0.245	0.60 (0.43–0.85)	0.004	0.05	0.57 (0.39–0.84)	0.005	0.06	0.45 (0.15–1.35)	0.15	–
<i>rs9364550</i>	G>T	3'-UTR	0.036	0.075	0.49 (0.26–0.92)	0.03	0.34	0.49 (0.26–0.94)	0.03	0.42	–	0.98	–
<i>rs315983</i>	C>T	Intron8	0.139	0.192	0.63 (0.43–0.91)	0.01	0.19	0.59 (0.39–0.89)	0.01	0.14	0.70 (0.19–2.59)	0.59	–
<i>rs10945657</i>	T>G	Intron8	0.054	0.066	0.74 (0.42–1.32)	0.31	–	0.74 (0.42–1.32)	0.31	–	–	–	–
<i>rs3798158</i>	A>C	Intron8	0.262	0.245	1.10 (0.82–1.48)	0.52	–	1.09 (0.75–1.57)	0.66	–	1.31 (0.65–2.65)	0.46	–
<i>rs10755577</i>	C>T	Intron9	0.063	0.103	0.59 (0.36–0.98)	0.04	0.54	0.61 (0.36–1.03)	0.06	0.82	–	0.98	–
<i>rs13195185</i>	T>G	Intron9	0.084	0.124	0.63 (0.40–0.99)	0.04	0.57	0.61 (0.38–0.99)	0.04	0.58	0.47 (0.06–3.92)	0.48	–
<i>rs3127594</i>	T>A	3'-UTR	0.027	0.029	0.90 (0.41–2.00)	0.80	–	0.90 (0.41–2.00)	0.80	–	–	–	–
<i>rs694812</i>	A>G	3'-UTR	0.084	0.121	0.64 (0.41–1.00)	0.05	0.67	0.62 (0.39–1.00)	0.05	0.65	0.57 (0.07–4.92)	0.61	–
<i>SLC22A2-ht1</i>			0.443	0.452	0.96 (0.75–1.25)	0.78	–	0.88 (0.60–1.30)	0.53	–	1.06 (0.67–1.65)	0.81	–
<i>SLC22A2-ht2</i>			0.232	0.210	1.15 (0.84–1.57)	0.39	–	1.13 (0.78–1.65)	0.51	–	1.45 (0.61–3.41)	0.40	–
<i>SLC22A2-ht3</i>			0.102	0.063	1.79 (1.12–2.87)	0.01	0.19	1.81 (1.12–2.95)	0.02	0.21	3.13 (0.17–57.20)	0.44	–
<i>SLC22A2-ht4</i>			0.033	0.065	0.50 (0.26–0.97)	0.04	0.51	0.50 (0.25–0.99)	0.05	0.62	–	0.99	–

Logistic models adjusted for age, sex, smoking status, and atopy as covariates were used. Bold values indicate the case of $p < 0.05$.

* p values after correction for multiple testing.

provocation with genotypes or haplotypes using multiple linear regression analyses (table 3). Ten SNPs (*rs316012*, *rs316013*, *rs316018*, *rs3912161*, *rs316021*, *rs9364550*, *rs315983*, *rs10755577*, *rs13195185*, and *rs694812*) were significantly associated with the percentage of maximum FEV₁ fall induced by aspirin provocation in asthmatics before multiple testing corrections, with the strongest significance in *rs3912161*. However, no SNP showed a nominal association signal after multiple testing corrections among these 10 SNPs. Asthmatics who carried minor allele *rs3912161* exhibited a significantly higher fall rate of FEV₁ induced by aspirin provocation than that of those who carried the major allele. In the haplotype analysis, *SLC22A2-ht3* containing the minor allele *rs3912161* was also significantly associated with the percentage of maximum FEV₁ fall induced by aspirin challenge in the asthmatics ($p = 0.004$, $P^{corr} = 0.05$ in the codominant model). The asthmatics with *SLC22A2-ht3* homozygotes had the highest responses to aspirin challenge, whereas those with *SLC22A2-ht4* homozygotes showed the lowest responses.

Discussion

AIA is an allergic reaction caused by the administration of aspirin [4]. It is characterized by acetyl salicylic acid hypersensitivity, bronchial asthma, and chronic rhinosinusitis with nasal polypsis [16]. Although many AIA susceptible genetic variants have been reported on 5-lipoxygenase and leukotriene synthetic pathways, a comprehensive etiology of AIA has not been clearly identified to date; thus, there is a need for new links to airway remodeling in the pathogenesis of AIA. Therefore, our findings on the associations between genetic polymorphisms and their haplotypes in the *SLC22A2* gene and AIA provide a new link between organic cation transportation and aspirin hypersensitivity in asthmatics, with a possible involvement of ACh as a novel regulator of airway remodeling.

Among the 10 SNPs and 2 haplotypes associated with AIA in this study, *rs316021*, which is located in the intronic region, showed a potential association signal with the risk of AIA after multiple testing corrections ($p = 0.004$, $P^{corr} = 0.05$, OR = 0.60, 95% CI = 0.43–0.85). The result is meaningful since introns can affect the transcription efficiency of genes, which in turn may affect gene expres-

Table 3. Regression analysis for the association of SNPs and haplotypes of the *SLC22A2* gene with the maximum fall rate of FEV₁ by aspirin provocation in asthmatics

SNP/ haplotype	Allele	Genotype			Codominant		Dominant		Recessive	
		C/C	C/R	R/R	p*	p ^{corr**}	p*	p ^{corr**}	p*	p ^{corr**}
<i>rs3127573</i>	T>C	487 (9.48 ± 13.40)	101 (8.17 ± 12.54)	4 (5.63 ± 4.50)	0.32	–	0.35	–	0.57	–
<i>rs316008</i>	A>C	438 (9.70 ± 13.65)	149 (7.80 ± 11.64)	5 (11.00 ± 18.51)	0.17	–	0.15	–	0.97	–
<i>rs3798156</i>	G>A	504 (9.45 ± 13.27)	86 (8.06 ± 13.04)	2 (5.50 ± 7.78)	0.33	–	0.36	–	0.62	–
<i>rs316012</i>	G>A	372 (10.12 ± 14.16)	197 (7.74 ± 11.59)	23 (7.67 ± 9.03)	0.04	0.56	0.03	0.42	0.58	–
<i>rs316013</i>	T>C	371 (10.13 ± 14.18)	197 (7.74 ± 11.59)	23 (7.67 ± 9.03)	0.04	0.55	0.03	0.41	0.58	–
<i>rs316018</i>	C>T	372 (10.12 ± 14.16)	197 (7.74 ± 11.59)	23 (7.67 ± 9.03)	0.04	0.56	0.03	0.42	0.58	–
<i>rs316019</i>	G>T	451 (9.73 ± 13.97)	129 (7.71 ± 10.74)	12 (7.01 ± 4.89)	0.10	–	0.09	–	0.68	–
<i>rs3912161</i>	A>G	499 (8.62 ± 12.24)	90 (12.02 ± 17.00)	2 (32.00 ± 29.70)	0.004	0.05	0.01	0.13	0.01	0.15
<i>rs2279463</i>	T>C	503 (9.42 ± 13.27)	87 (8.26 ± 13.09)	2 (5.50 ± 7.78)	0.40	–	0.43	–	0.62	–
<i>rs316021</i>	A>G	352 (10.21 ± 14.13)	214 (7.84 ± 11.98)	26 (7.48 ± 8.59)	0.03	0.42	0.02	0.30	0.55	–
<i>rs9364550</i>	G>T	520 (9.66 ± 13.74)	68 (6.23 ± 8.22)	4 (5.28 ± 1.81)	0.05	0.66	0.05	0.60	0.62	–
<i>rs315983</i>	C>T	395 (10.12 ± 13.91)	184 (7.31 ± 11.58)	13 (9.41 ± 11.36)	0.03	0.37	0.02	0.21	0.94	–
<i>rs10945657</i>	T>G	518 (9.27 ± 12.97)	74 (9.00 ± 14.92)	–	0.74	–	0.74	–	–	–
<i>rs3798158</i>	A>C	337 (9.53 ± 13.22)	217 (8.78 ± 13.04)	38 (9.19 ± 14.44)	0.64	–	0.58	–	0.95	–
<i>rs10755577</i>	C>T	487 (9.82 ± 13.75)	99 (6.59 ± 10.33)	5 (5.80 ± 3.64)	0.03	0.35	0.03	0.33	0.55	–
<i>rs13195185</i>	T>G	465 (9.90 ± 14.03)	120 (6.71 ± 9.52)	7 (7.94 ± 5.93)	0.02	0.32	0.02	0.21	0.86	–
<i>rs3127594</i>	T>A	558 (9.35 ± 13.21)	34 (7.36 ± 13.46)	–	0.35	–	0.35	–	–	–
<i>rs694812</i>	A>G	465 (9.90 ± 14.03)	120 (6.71 ± 9.52)	6 (8.27 ± 6.43)	0.02	0.32	0.02	0.22	0.90	–
		–/–	+/–	+/+						
<i>SLC22A2-ht1</i>		182 (9.49 ± 13.99)	285 (8.58 ± 12.58)	125 (10.36 ± 13.52)	0.71	–	0.66	–	0.25	–
<i>SLC22A2-ht2</i>		362 (9.50 ± 13.30)	205 (8.49 ± 12.67)	25 (11.52 ± 16.37)	0.88	–	0.59	–	0.38	–
<i>SLC22A2-ht3</i>		507 (8.68 ± 12.25)	83 (12.06 ± 17.33)	2 (32.00 ± 29.70)	0.004	0.05	0.01	0.14	0.01	0.15
<i>SLC22A2-ht4</i>		528 (9.60 ± 13.67)	61 (6.23 ± 8.32)	3 (5.20 ± 2.21)	0.05	0.71	0.05	0.65	0.67	–

The genotype distribution of each SNP is presented as the number of subjects. Data in parentheses indicate the percentage of FEV₁ fall by aspirin provocation (mean ± SE). Bold values indicate the case of p < 0.05.

* p values for linear regression analysis controlling age, sex, smoking status, and atopy as covariates.

** p values after correction for multiple testing.

sion. A previous study described the effect of introns on the rate of transcription of genes through experiments using transgenic mice [17], whereas another report showed that splicing affecting genomic variants can lead to splicing abnormalities causing human disease phenotypes, e.g. exon skipping, activation of cryptic splice sites, or alteration of the fine balance of the alternatively spliced isoforms that are produced [18]. It has also been reported that an intronic polymorphism (*rs3733072*) in the interleukin-17 receptor B (*IL-17RB*) gene is associated with the risk of asthma [19]. Thus, it is possible that *rs316021* is involved in the onset of AIA by affecting the transcription process of the *SLC22A2* gene. However, when comparing the lower minor allele frequency of *rs316021* in AIA patients (MAF = 0.172) to that in ATA controls (MAF = 0.245), we do not rule out a possible protective effect of the variant since haplotype *SLC22A2-ht3* with the major A allele is more frequent in AIA patients than in ATA controls and has a more significant association with AIA compared to *SLC22A2-ht4* with the minor G allele.

SLC22A2 is a polyspecific solute carrier that mediates the luminal release of ACh, which has recently been recognized as a novel regulator of airway remodeling and serves as a neurotransmitter of the central and peripheral neurons in human airways [8, 9]. Although ACh has been known as a typical neurotransmitter in the central and peripheral nervous systems and is synthesized from acetylcoenzyme A and choline by acetyltransferase, it is also one of the strongest bronchoconstrictors that plays an important role in the regulation of airway function, specifically during airway wall remodeling in disease and immunomodulation [20, 21]. The release of acetylcholine from bronchial epithelial cells mediates bronchoconstriction following the release of serotonin from mast cells. In addition, airway epithelial cells possess various muscarinic receptors that serve as potential targets of locally released ACh, and dysregulation of these receptors in airway epithelial cells is a major cause of asthma [20, 21]. Despite no direct evidence correlating the genetic variations of *SLC22A2* to an altered responsiveness to

cholinergic stimulation, the modest significant associations of *SLC22A3* polymorphisms with methamphetamine dependence, which is also related to cholinergic transmission, and polysubstance use in methamphetamine dependence have been recently reported [22]. Aspirin-induced bronchospasm following the inhalation of NSAIDs in aspirin-intolerant asthmatics with genetic polymorphisms of *SLC22A2*, therefore, could be related to the regulation of acetylcholine release from bronchial cells via altered *SLC22A2* functions.

The transporter activities of Madin-Darby canine kidney cells that are overexpressed in the human *SLC22A2* variants (*Thr199Ileu*, *Thr201Met*, and *Ala270Ser*) showed a significantly decreased uptake of [³H]methyl-4-phenylpyridinium acetate (MPP⁺) or [¹⁴C]tetraethylammonium (TEA) compared with cells that are overexpressed in the wild-type *SLC22A2* [23]. It has been shown that TEA plays a role in the increase of vital capacity among bronchial asthma patients [24] and significantly decreases the relaxant effects of propofol on ovalbumin-induced asthma in guinea pigs [25]. From these results, we suspect that the 3 *SLC22A2* SNPs (*Thr199Ileu*, *Thr201Met*, and *Ala270Ser*) mentioned above are involved in the development of bronchial asthma which results from a reduced vital capacity caused by a decrease in the transport of TEA. A previous study revealed an association between the *SLC22A2* polymorphism and other human diseases. It has been shown that the *Ala270Ser* polymorphism in the *SLC22A2* gene is significantly associated with systemic arterial hypertension in the Caucasian population. Patients who possessed the *Ser270* allele were less frequent-

ly affected by the clinical diagnosis of hypertension than were homozygote carriers of the wild-type allele *Ala270* [26]. In this study, the *Ala270Ser* polymorphism (*rs316019*) was also identified and analyzed for its association with AIA; however, we were not able to establish a significant association between this polymorphism and AIA.

In conclusion, we identified 18 genetic variants in the human *SLC22A2* gene. All of the polymorphisms were selected for genotyping in our asthma cohort association study, and statistical analyses revealed that *rs316021* was significantly associated with the risk of AIA and that *rs3912161* and *SLC22A2-ht3* were significantly associated with the maximum fall of FEV₁. Further studies on this gene including functional and replication studies elsewhere are needed to identify the roles of polymorphisms. These findings on the *SLC22A2* gene may provide useful information in the development of new methods for the diagnosis of aspirin intolerance, as well as new strategies for the control of this asthma phenotype.

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