Association analysis of *DTD1* gene variations with aspirin-intolerance in asthmatics

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Abstract. Aspirin ingestion is a common precipitating factor of life-threatening asthma attacks, requiring some patients to undergo mechanical ventilation. The gene, D-tyrosyl-tRNA deacylase 1 (*DTD1*), may be a risk factor for aspirin-intolerant asthma (AIA) by catalyzing the hydrolysis of D-tryptophan and interacting with the tyrosyl-tRNA synthetase (tyrRS) enzyme, which promotes a pro-inflammatory phenotype. In order to investigate the association of *DTD1* variants with the risk of AIA in an asthma cohort, 38 single nucleotide polymorphisms (SNPs) were genotyped and 5 major haplotypes were obtained in 163 AIA cases and 429 aspirin-tolerant asthma (ATA) controls. Differences in *DTD1* SNP and haplotype distributions were analyzed using logistic and multiple regression models and were adjusted for age, gender, smoking status, atopy and body mass index (BMI) as covariates. Subsequent analyses revealed no association between *DTD1* variants and the risk of AIA. Although nominal evidence of an association was detected between several *DTD1* variants and the rate of decline of the forced expiratory volume in the first second (FEV_1) in AIA patients (*rs6136444, rs6136469, rs6081338* and *DTD1_ht5*; P=0.01-0.02), the signals reached the threshold of multiple testing corrections, suggesting that *DTD1* variants do not affect the abnormalities of the upper airways in AIA patients.

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Introduction

Aspirin-intolerant asthma (AIA) is the development of bronchoconstriction in asthmatic patients following the ingestion of aspirin, a non-steroidal anti-inflammatory drug (NSAID) (1,2), and is characterized by the triad of chronic rhinosinusitis, nasal polyps and exacerbated asthma akin to prolonged viral respiratory infection (2). The prevalence rate of aspirin intolerance has been reported to be around 10-20% among adult asthmatics (3,4), with an observed predominance among women (5,6). A previous study has reported that 25% of asthma patients who required emergency mechanical ventilation are aspirin intolerant (7), hence, ingestion of aspirin may be a precipitating factor in life-threatening asthma attacks (8). However, despite the well-defined clinical characteristics of the disease, genetic underpinnings of AIA pathogenesis are still unclear.

The etiology of AIA development is attributable to the combinatorial effects of environmental and genetic risk factors. We have previously identified several variants in the solute carrier family 6 (neurotransmitter transporter, betaine/GABA) member 12 (*SLC6A12*), the emilin/multimerin domain-containing protein 2 (*EMID2*) and the fibrous sheath interacting protein 1 (*FSIP1*) genes that are risk factors of AIA susceptibility (9-11), suggesting that complex genetic mechanisms underlie AIA pathogenesis. Although candidate gene-association studies and genome-wide association studies (GWAS) have provided unprecedented insights into the triggers and pathophysiology of asthma, the exact functional mechanisms of AIA development are still unclear and the roles of numerous candidate genes still need to be elucidated.

The human D-tyrosyl-tRNA deacylase 1 (*DTD1*) gene on chromosome 20p11.23 is a cellular component of the cytoplasm and is expressed mainly in the testis, ovary, spleen and in the adult and fetal brain (12). The protein encoded by this gene interacts with tyrosyl-tRNA synthetase (tyrRS) by catalyzing the hydrolysis of D-tyrosyl-tRNA, thereby preventing the misacylated accumulation of metabolically inactive tRNA molecules including several D-amino acids such as D-tyrosine, D-aspartic acid and D-tryptophan (13,14). During the activation of cellular immune responses, interferon (IFN)-γ induces the enzyme indoleamine 2,3-dioxygenase (IDO), which catalyzes tryptophan into kynurenine and serotonin (15), resulting in inhibition of T-cell proliferation (16) and increased severity of allergic asthma (17). Elevated levels of serum kynurenine and serotonin in individuals with various degrees of chronic airway obstruction compared to non-asthmatic controls provide evidence that tryptophan may play a role in asthma pathogenesis (18-20). In particular, inactivation or misregulation of the *DTD1* gene, may hamper the recycling of misacylated tRNA molecules and lead to an increase in the toxicity of D-amino acids including tryptophan (21).

With the crucial role of *DTD1* in inhibiting the harmful effects of D-tryptophan accumulation as well as its association with the pro-inflammatory promoting tyrRS, we hypothesized that genetic variations in *DTD1* may influence bronchial hypersensitivity in AIA patients. To elucidate the association between *DTD1* variants and the risk of AIA, a case-control analysis was carried out in a Korean population.

Materials and methods

Study subjects. Asthma patients were recruited from nine Korean hospitals belonging to the Asthma Genome Research Center, to serve as the primary subjects for our study. Each patient, as diagnosed by trained physicians, showed clinical symptoms that met the criteria for asthma according to the Global Initiative for Asthma (GINA) (22). Evaluation of the subjects included dyspnea and wheezing during the past year plus one of the following: i) airway reversibility measured by a positive bronchodilator response of a >15% increase in the forced expiratory volume in the first second (FEV_1) or a >12% increase in FEV_1 plus 200 ml following inhalation of a short-acting bronchodilator; ii) airway hyperreactivity to <10 mg/ml PC_{20} methacholine; or iii) >20% increase in FEV_1 following 2 weeks of treatment with inhaled steroids and long-acting bronchodilators (23). Twenty-four common inhalant allergens (e.g., dust mites, cat fur, dog fur, cockroaches, grasses, trees, ragweed pollen; Bencard Co. Ltd., Brentford, UK) were used in a skin-prick test. Total immunoglobulin E (IgE) was measured using the CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Atopy was defined as a wheal reaction ≥ to histamine or than 3 mm in diameter. Pulmonary function tests were performed using the Vmax Series 2130 Autobox Spirometer (SensorMedics, Yorba Linda, CA) with adherence to the American Thoracic Society (ATS) guidelines (24). The reference values of lung functions used were according to the Morris-Polgar standards (25,26). All asthmatics underwent oral aspirin challenge (OAC) that was performed with increasing doses of aspirin (10-450 mg) (27,28) with modifications. The subjects reported no increase in asthma symptoms or respiratory tract infections within 6 weeks prior to the test. Briefly, patients with a history of aspirin hypersensitivity were given 30 mg and those having no history of aspirin hypersensitivity were initiated with 100 mg of aspirin orally. Symptoms, external signs (urticaria and angioedema) and FEV_1 were documented every 30 min for a period of 2 h. In the absence of any symptoms or signs suggestive of an adverse reaction after 2 h, 60 or 100 mg of aspirin was administered and the same measurements were repeated every 1 h, increasing the doses up to 450 mg until the patient developed a reaction. If no reaction occurred 5 h after the final dose, the test was deemed negative. Changes in the FEV_1 were followed for 5 h after the final aspirin dose. Aspirin-induced bronchospasm, reflected by the rate (%) of decline in FEV_1, was calculated as the pre-challenge FEV_1 minus the post-challenge FEV_1 divided by the pre-challenge FEV_1. Categorization of patients was based on individual OAC reactions. Asthmatics exhibiting ≥20% decrease in FEV_1 or a 15-19% decrease in FEV_1 with naso-ocular or cutaneous reactions were diagnosed as AIA cases, whereas those demonstrating <15% decrease in FEV_1 without naso-ocular or cutaneous reactions were identified as aspirin-tolerant asthma (ATA) controls. This study was undertaken with the understanding and written consent of each subject, and the protocols were approved by the Institutional Review Board of each hospital.

SNP genotyping and haplotype construction. Candidate polymorphic SNPs of the *DTD1* gene were selected and screened from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) based on the minor allele frequencies (MAF) in the Asian population (Han Chinese and Japanese), the linkage disequilibrium (LD) status, and the importance of the position in the gene. Genomic DNA was extracted from peripheral blood lymphocytes using the Gentra Puregene kit (Gentra Systems, Minneapolis, MN) according to the manufacturer's protocol. SNP genotyping was performed using TaqMan assay (29) in the ABI PRISM 7900HT sequence detection system (Applied Biosystems, CA, USA). The genotyped data quality was assessed by duplicate DNA checking (n=10; rate of concordance in duplicates >99%). Using the Phase algorithm v.2.0 software (30), haplotypes were inferred from the successfully genotyped SNPs and those with a frequency >0.05 were included in the association analyses.

Statistical analyses. The LD between all pairs of biallelic loci were determined by Lewontin's D' (|*D*'|) and the LD coefficient r^2-values were examined using the Haploview algorithm (31). To determine the association between the *DTD1* genotype distributions in AIA cases and ATA controls, the odds ratios and 95% confidence intervals as well as the corresponding P-values were calculated using logistic regression analysis controlling for age (continuous variable), gender (male=0, female=1), smoking status (non-smoker=0, ex-smoker=1, smoker=2), atopy (absence=0, presence=1) and body mass index (BMI) as covariates to eliminate or reduce any confounding variables that might influence the findings. Data was managed and analyzed using the Statistical Analysis System (SAS) version 9.1 (SAS Inc., Cary, NC). In addition, the differences in the decline rate of FEV_1 following aspirin

Table I. Clinical profiles of the study subjects (n=592).

Clinical profile	Asthmatics (all subjects)	AIA	ATA
Number of subjects, n	592	163	429
Age, years, mean (range)	46.15 (15.40-77.88)	43.13 (17.22-72.73)[a]	47.30 (15.40-77.88)
Gender, n (male/female)	206/386	59/104	147/282
% Smokers, (current/ex-smokers)	27.70 (12.50/15.20)	21.47 (12.88/8.59)[a]	30.07 (12.35/17.72)
Height, cm	160.78±8.63	161.72±8.69	160.42±8.39
Weight, kg	62.81±10.84	61.25±10.38[a]	63.40±10.97
Body mass index, kg/m^2	24.24±3.39	23.39±3.25[a]	24.58±3.39
% FEV_1 decline by aspirin provocation	9.27±13.24	24.63±16.11[b]	3.54±4.85
% Blood eosinophils	6.01±5.73	5.96±5.21	6.03±5.92
FEV_1, % predicted	90.54 ±16.97	90.35±14.04[a]	91.66±16.87
PC_{20} methacholine, mg/ml	6.43±8.67	5.02±7.83[a]	6.91±8.90
Total IgE, IU/ml	357.65±604.09	348.60±596.44	361.00±607.56
Positive skin test, %	56.42	52.76	57.81
Positive nasal polyp, %	33.83	57.89[b]	26.06
Positive history of aspirin hypersensitivity, %	18.50	51.92[b]	6.00

Values are the means ± SE. BMI, body mass index; AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma. [a]P<0.05, [b]P<0.0001, statistically significant differences between AIA and ATA patients.

challenge among *DTD1* genotypes and haplotypes were examined using regression analysis.

To achieve optimal correction for multiple testing of markers representing SNPs in LD, the effective number of independent marker loci (29.1223) was calculated using the SNPSpD software (http://genepi.qimr.edu.au/general/daleN/SNPSpD/), a program that is based on the spectral decomposition (SpD) of matrices of pair-wise LD between markers (32).

Results

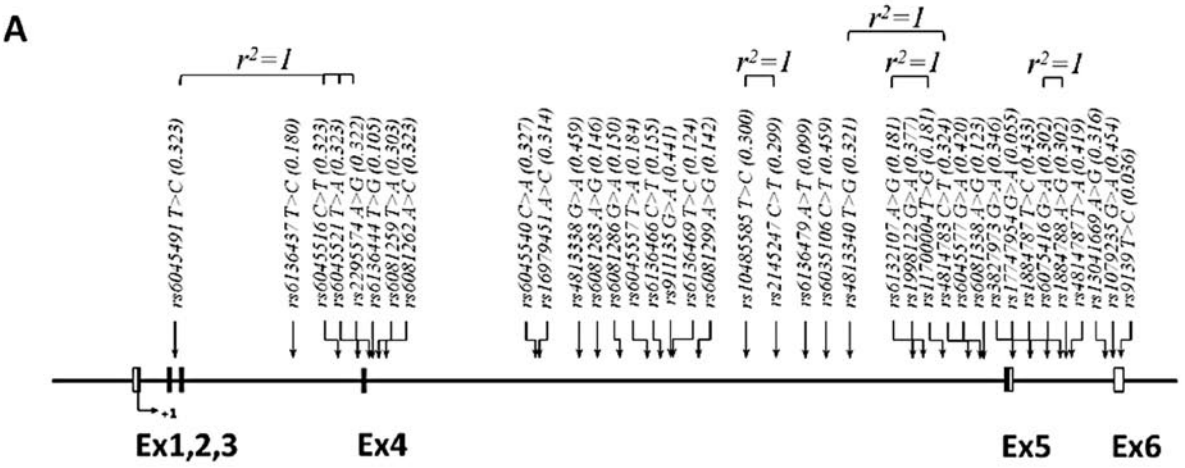
Among a total of 592 asthmatics recruited in our study, 163 subjects were identified as AIA cases and 429 subjects were categorized as ATA controls based on the results of the aspirin provocation test. Table I depicts the clinical characteristics of the study subjects. From the data obtained, it was observed that AIA patients had a lower BMI (23.39±3.25 kg/m^2) compared to the ATA controls (24.58±3.39 kg/m^2). Our findings also demonstrate a significant difference between the mean age of AIA patients (43.1 years) and that of the controls (47.3 years). In addition, results from the aspirin provocation test showed a significant increase in the aspirin-induced decline rate of FEV_1 in AIA patients compared to that of ATA controls (24.63% in AIA and 3.54% in ATA; P<0.0001). The predicted % FEV_1, smoking status and PC_{20} methacholine values were significantly lower in AIA patients than in the ATA controls (P<0.05). Furthermore, the rates of nasal polyps in AIA patients (57.89%) were significantly higher compared to ATA controls (26.06), suggesting that aspirin-intolerance can elicit meaningful deficits in the upper airways. A positive history of aspirin hypersensitivity was also found to be significantly prevalent in AIA patients (51.92%) compared to ATA subjects (6.00%).

With an average call rate of 99.9%, 38 *DTD1* SNPs were successfully genotyped in the asthma cohort of 592 Korean patients; 1 SNP was localized in intron 2 and 1 in exon 6, 3 in intron 3, 25 in intron 4 and 8 SNPs were positioned in intron 5 (Fig 1A and Table II). Results from the Hardy-Weinberg equilibrium (HWE) test showed no significant differences between the distribution of the observed genotypes and the expected distributions (P>0.05; Table II). The MAF of each SNP is shown in Table II. Using the genotyped SNPs, five major haplotypes with frequencies >0.05 (Fig. 1B) were obtained and included in the association analyses. The haplotypes were contained in one LD block (Fig. 1C) that was established from pair-wise comparisons of the 38 genotyped SNPs.

Results from logistic analyses showed no significant association between *DTD1* variants and the risk of AIA in a Korean population (Table III). Since the decline rate of FEV_1 induced by aspirin provocation is an important diagnostic marker of AIA, further association analysis with the *DTD1* genetic variants was performed using a regression model. Initial results revealed significant associations between three *DTD1* SNPs (*rs6136444, rs6136469* and *rs6081338*) and the decline rate of FEV_1 via a recessive model of genetic inheritance (P=0.01-0.02; Table IV), whereas one haplotype (*DTD1_ht5*) was also significantly associated with the decline rate of FEV_1 via co-dominant and dominant mechanisms (P=0.02; Table IV). However, with 29.1223 as the effective number of independent marker loci, the significant values were not retained after multiple testing corrections.

Discussion

The development of AIA can be attributed to combinatorial effects of genetic and environmental factors. In some patients,



B

Hap	rs6045491 T>C	rs6136437 T>C	rs6045516 C>T	rs6045521 T>A	rs2295574 A>G	rs6136444 T>G	rs6081259 T>A	rs6081262 A>C	rs6045540 C>A	rs16979451 A>C	rs4813338 G>A	rs6081283 A>G	rs6081286 G>A	rs6045557 T>A	rs6136466 C>T	rs911135 G>A	rs6136469 T>C	rs6081299 A>G	rs10485585 T>C	rs2145247 C>T	rs6136479 A>T	rs6035106 C>T	rs4813340 T>G	rs6132107 A>G	rs1998122 G>A	rs11700004 T>G	rs4814783 C>T	rs6045577 G>A	rs6081338 A>G	rs3827973 G>A	rs17747954 G>A	rs1884787 T>C	rs6075416 G>A	rs1884788 A>G	rs4814787 T>A	rs13041669 A>G	rs1079235 G>A	rs9139 T>C	Freq
DTD1_ht1	T	T	C	T	A	T	A	A	C	C	A	A	G	T	C	A	T	A	C	T	A	T	T	A	G	T	C	A	G	G	G	C	A	G	A	A	A	T	0.263
DTD1_ht2	T	T	C	T	A	T	T	A	C	A	G	A	G	T	C	G	T	A	T	C	A	C	G	A	A	T	T	G	A	A	G	T	G	A	T	G	G	T	0.260
DTD1_ht3	C	T	T	G	G	G	T	C	A	A	A	G	A	T	T	A	C	G	T	C	A	T	T	A	G	T	C	A	G	G	G	C	G	A	A	A	A	T	0.094
DTD1_ht4	C	C	T	T	G	T	T	C	A	A	G	A	G	A	C	G	T	A	T	C	T	C	T	G	G	G	C	G	G	G	G	T	G	A	T	A	G	T	0.094
DTD1_ht5	C	C	T	T	G	T	T	C	A	A	G	A	G	A	C	G	T	A	T	C	A	C	T	G	G	G	C	G	G	G	G	T	G	A	T	A	G	T	0.079
Others	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.063

C

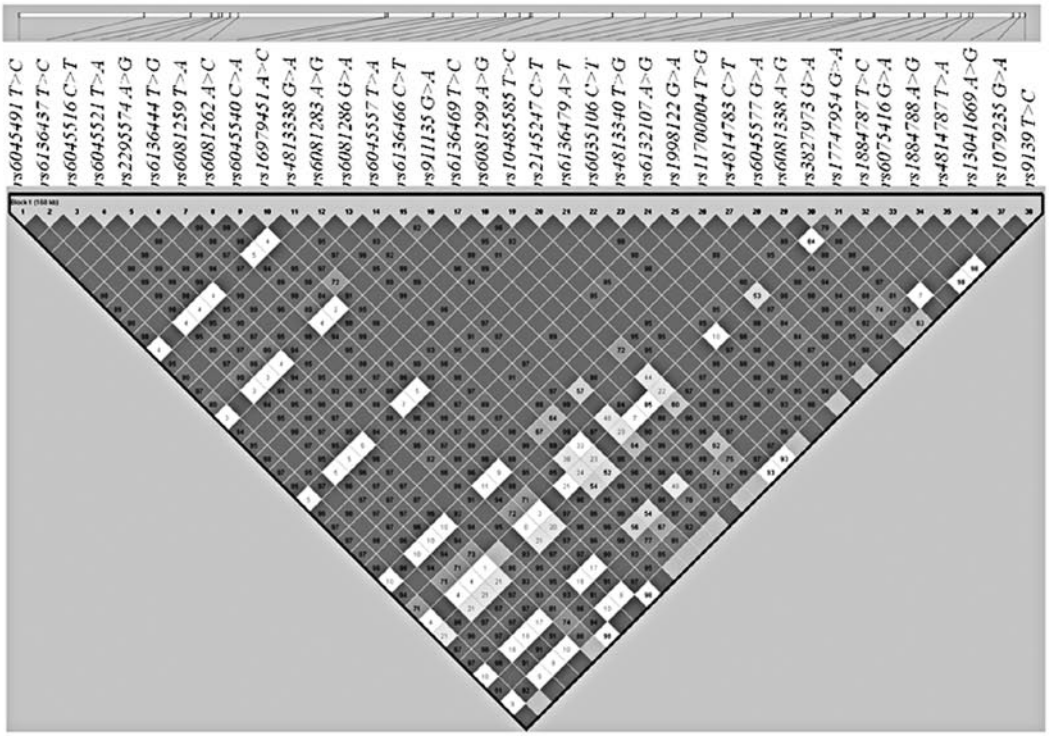


Figure 1. Physical map, haplotypes, and linkage disequilibrium of the *DTD1* gene. (A) Schematic gene map and SNPs in the *DTD1* gene on chromosome 20p11.23 (176 kb). Black blocks represent coding exons and white blocks represent 5' and 3' UTRs. The first base of translation site was denoted as nucleotide +1. SNPs in absolute linkage are indicated by brackets (r^2=1). (B) Haplotypes of *DTD1*. (C) LD coefficient (|D'|) among *DTD1* SNPs in a Korean population. UTR, untranslated region.

Table II. Genotype and allele frequency distribution of *DTD1* variants.

Loci	Position	Allele	MAF	Heterozygosity	HWE
rs6045491	Intron 2	T>C	0.323	0.437	0.258
rs6136437	Intron 3	T>C	0.180	0.295	0.253
rs6045516	Intron 3	C>T	0.323	0.437	0.258
rs6045521	Intron 3	T>A	0.323	0.437	0.258
rs2295574	Intron 4	A>G	0.322	0.436	0.223
rs6136444	Intron 4	T>G	0.105	0.189	0.407
rs6081259	Intron 4	T>A	0.303	0.422	0.945
rs6081262	Intron 4	A>C	0.323	0.438	0.170
rs6045540	Intron 4	C>A	0.327	0.440	0.196
rs16979451	Intron 4	A>C	0.314	0.431	0.904
rs4813338	Intron 4	G>A	0.459	0.497	0.464
rs6081283	Intron 4	A>G	0.146	0.250	0.905
rs6081286	Intron 4	G>A	0.150	0.255	0.782
rs6045557	Intron 4	T>A	0.184	0.300	0.271
rs6136466	Intron 4	C>T	0.155	0.262	0.773
rs911135	Intron 4	G>A	0.441	0.493	0.550
rs6136469	Intron 4	T>C	0.124	0.217	0.185
rs6081299	Intron 4	A>G	0.142	0.243	0.861
rs10485585	Intron 4	T>C	0.300	0.420	0.726
rs2145247	Intron 4	C>T	0.299	0.419	0.696
rs6136479	Intron 4	A>T	0.099	0.178	0.338
rs6035106	Intron 4	C>T	0.459	0.497	0.417
rs4813340	Intron 4	T>G	0.321	0.436	0.590
rs6132107	Intron 4	A>G	0.181	0.296	0.235
rs1998122	Intron 4	G>A	0.377	0.470	0.420
rs11700004	Intron 4	T>G	0.181	0.296	0.235
rs4814783	Intron 4	C>T	0.324	0.438	0.943
rs6045577	Intron 4	G>A	0.420	0.487	0.869
rs6081338	Intron 4	A>G	0.123	0.216	0.198
rs3827973	Intron 5	G>A	0.346	0.452	0.877
rs17747954	Intron 5	G>A	0.055	0.104	0.940
rs1884787	Intron 5	T>C	0.433	0.491	0.589
rs6075416	Intron 5	G>A	0.302	0.422	0.859
rs1884788	Intron 5	A>G	0.302	0.422	0.859
rs4814787	Intron 5	T>A	0.419	0.487	0.614
rs13041669	Intron 5	A>G	0.316	0.432	0.769
rs1079235	Intron 5	G>A	0.454	0.496	0.270
rs9139	Exon 6	T>C	0.036	0.069	0.351
DTD1_ht1			0.266	0.390	0.578
DTD1_ht2			0.265	0.390	0.307
DTD1_ht3			0.093	0.168	0.259
DTD1_ht4			0.093	0.169	0.489
DTD1_ht5			0.076	0.140	0.361

MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium.

aspirin-intolerance is manifested through severe acute asthma attacks requiring hospital admission and mechanical ventilation to support the failing lungs (7,8). Once developed, AIA cases are considered fatal despite treatment with fast-acting medicines and avoidance of aspirin. As a precipitating factor in life-threatening asthma attacks, researchers are

Table III. Association analysis of *DTD1* polymorphisms and haplotypes with AIA.

Loci	Position	MAF		Co-dominant		Dominant		Recessive	
		AIA (n=163)	ATA (n=429)	OR (95% CI)	P-value[a]	OR (95% CI)	P-value[a]	OR (95% CI)	P-value[a]
rs6045491	Intron 2	0.313	0.321	1.00 (0.75-1.33)	0.98	1.04 (0.72-1.51)	0.82	0.85 (0.43-1.68)	0.64
rs6136437	Intron 3	0.181	0.179	1.04 (0.74-1.47)	0.82	1.14 (0.77-1.68)	0.52	0.42 (0.09-1.89)	0.26
rs6045516	Intron 3	0.313	0.321	1.00 (0.75-1.33)	0.98	1.04 (0.72-1.51)	0.82	0.85 (0.43-1.68)	0.64
rs6045521	Intron 3	0.313	0.321	1.00 (0.75-1.33)	0.98	1.04 (0.72-1.51)	0.82	0.85 (0.43-1.68)	0.64
rs2295574	Intron 4	0.313	0.319	1.00 (0.75-1.34)	1.00	1.05 (0.72-1.52)	0.81	0.86 (0.43-1.71)	0.67
rs6136444	Intron 4	0.107	0.105	1.07 (0.70-1.63)	0.77	0.98 (0.62-1.56)	0.95	3.58 (0.69-18.51)	0.13
rs6081259	Intron 4	0.282	0.308	0.88 (0.66-1.18)	0.40	0.93 (0.64-1.34)	0.68	0.65 (0.32-1.32)	0.24
rs6081262	Intron 4	0.313	0.322	0.99 (0.74-1.32)	0.93	1.02 (0.71-1.48)	0.91	0.86 (0.44-1.71)	0.67
rs6045540	Intron 4	0.316	0.326	0.98 (0.73-1.31)	0.88	1.03 (0.71-1.49)	0.90	0.82 (0.42-1.63)	0.58
rs16979451	Intron 4	0.293	0.317	0.90 (0.68-1.20)	0.47	0.95 (0.66-1.38)	0.80	0.67 (0.34-1.32)	0.24
rs4813338	Intron 4	0.426	0.461	0.88 (0.67-1.14)	0.33	0.91 (0.61-1.36)	0.63	0.75 (0.46-1.22)	0.25
rs6081283	Intron 4	0.135	0.145	0.94 (0.64-1.38)	0.75	0.91 (0.60-1.39)	0.67	1.15 (0.30-4.38)	0.84
rs6081286	Intron 4	0.138	0.149	0.93 (0.64-1.35)	0.71	0.89 (0.58-1.35)	0.58	1.29 (0.39-4.25)	0.68
rs6045557	Intron 4	0.184	0.184	1.03 (0.73-1.46)	0.86	1.13 (0.77-1.66)	0.54	0.40 (0.09-1.81)	0.23
rs6136466	Intron 4	0.138	0.155	0.89 (0.61-1.29)	0.53	0.84 (0.55-1.27)	0.40	1.29 (0.40-4.18)	0.67
rs911135	Intron 4	0.399	0.446	0.81 (0.62-1.06)	0.13	0.83 (0.56-1.23)	0.35	0.66 (0.39-1.10)	0.11
rs6136469	Intron 4	0.123	0.121	1.00 (0.67-1.50)	1.00	0.94 (0.60-1.45)	0.77	2.53 (0.55-11.74)	0.23
rs6081299	Intron 4	0.126	0.141	0.86 (0.58-1.27)	0.45	0.82 (0.53-1.26)	0.36	1.24 (0.32-4.80)	0.76
rs10485585	Intron 4	0.276	0.304	0.86 (0.65-1.15)	0.32	0.89 (0.62-1.29)	0.55	0.65 (0.32-1.31)	0.23
rs2145247	Intron 4	0.276	0.304	0.86 (0.65-1.15)	0.32	0.90 (0.62-1.30)	0.56	0.64 (0.32-1.30)	0.22
rs6136479	Intron 4	0.110	0.094	1.21 (0.79-1.88)	0.38	1.25 (0.79-1.98)	0.34	0.86 (0.09-8.51)	0.89
rs6035106	Intron 4	0.414	0.465	0.82 (0.62-1.07)	0.14	0.87 (0.58-1.30)	0.48	0.64 (0.39-1.05)	0.08
rs4813340	Intron 4	0.367	0.311	1.24 (0.94-1.64)	0.12	1.21 (0.83-1.76)	0.32	1.64 (0.94-2.86)	0.08
rs6132107	Intron 4	0.184	0.179	1.06 (0.75-1.49)	0.76	1.16 (0.78-1.70)	0.47	0.42 (0.09-1.89)	0.26
rs1998122	Intron 4	0.420	0.371	1.20 (0.91-1.58)	0.19	1.17 (0.79-1.73)	0.43	1.45 (0.87-2.42)	0.15
rs11700004	Intron 4	0.184	0.179	1.06 (0.75-1.49)	0.76	1.16 (0.78-1.70)	0.47	0.42 (0.09-1.89)	0.26
rs4814783	Intron 4	0.367	0.315	1.22 (0.93-1.61)	0.15	1.16 (0.79-1.68)	0.45	1.69 (0.97-2.95)	0.07
rs6045577	Intron 4	0.380	0.426	0.82 (0.63-1.07)	0.15	0.84 (0.57-1.23)	0.37	0.66 (0.39-1.12)	0.12
rs6081338	Intron 4	0.123	0.120	1.01 (0.67-1.52)	0.96	0.95 (0.61-1.47)	0.81	2.53 (0.55-11.74)	0.23
rs3827973	Intron 5	0.368	0.340	1.12 (0.85-1.47)	0.43	1.24 (0.85-1.80)	0.27	0.99 (0.55-1.75)	0.96
rs17747954	Intron 5	0.034	0.063	0.54 (0.28-1.07)	0.08	0.55 (0.27-1.09)	0.08	-	0.98
rs1884787	Intron 5	0.408	0.436	0.91 (0.69-1.19)	0.48	0.99 (0.67-1.47)	0.95	0.73 (0.44-1.21)	0.22
rs6075416	Intron 5	0.279	0.308	0.88 (0.66-1.17)	0.38	0.91 (0.63-1.32)	0.61	0.67 (0.33-1.35)	0.26
rs1884788	Intron 5	0.279	0.308	0.88 (0.66-1.17)	0.38	0.91 (0.63-1.32)	0.61	0.67 (0.33-1.35)	0.26
rs4814787	Intron 5	0.396	0.422	0.90 (0.69-1.17)	0.43	0.95 (0.64-1.40)	0.78	0.75 (0.45-1.25)	0.27
rs13041669	Intron 5	0.359	0.305	1.23 (0.93-1.62)	0.14	1.20 (0.82-1.74)	0.35	1.60 (0.91-2.82)	0.10
rs1079235	Intron 5	0.414	0.459	0.85 (0.64-1.11)	0.22	0.89 (0.60-1.34)	0.58	0.68 (0.41-1.13)	0.13
rs9139	Exon 6	0.028	0.042	0.68 (0.32-1.46)	0.32	0.68 (0.32-1.46)	0.32	-	-
DTD1_ht1		0.224	0.277	0.76 (0.56-1.03)	0.08	0.74 (0.51-1.08)	0.12	0.58 (0.26-1.30)	0.18
DTD1_ht2		0.294	0.256	1.16 (0.87-1.54)	0.32	1.10 (0.76-1.60)	0.61	1.57 (0.82-2.97)	0.17
DTD1_ht3		0.083	0.094	0.87 (0.54-1.41)	0.58	0.84 (0.51-1.38)	0.49	2.20 (0.19-24.96)	0.52
DTD1_ht4		0.101	0.090	1.17 (0.75-1.83)	0.49	1.20 (0.75-1.93)	0.45	0.86 (0.09-8.51)	0.89
DTD1_ht5		0.067	0.080	0.83 (0.49-1.40)	0.48	0.84 (0.50-1.44)	0.53	-	0.98

AIA, aspirin-intolerant asthma; ATA, aspirin tolerant asthma; OR, odds ratio; CI, confidence interval. [a]P<0.05. Co-dominant, dominant and recessive models of logistic regression analyses were used to calculate ORs and the 95% CIs controlling for age, gender, smoking status, atopy and BMI as covariates.

Table IV. Regression analysis of *DTD1* polymorphisms and haplotypes with fall rate of FEV_1 by aspirin provocation.

Loci	C/C	C/R	R/R	Pa	Pa^{corr}	Pb	Pb^{corr}	Pc	Pc^{corr}
rs6045491	270 (9.37±12.93)	265 (9.32±13.59)	57 (8.19±13.01)	0.77	-	0.86	-	0.74	-
rs6136437	395 (9.21±13.02)	181 (9.42±13.90)	16 (7.59±10.43)	0.96	-	0.94	-	0.7	-
rs6045516	270 (9.37±12.93)	265 (9.32±13.59)	57 (8.19±13.01)	0.77	-	0.86	-	0.74	-
rs6045521	270 (9.37±12.93)	265 (9.32±13.59)	57 (8.19±13.01)	0.77	-	0.86	-	0.74	-
rs2295574	270 (9.37±12.93)	265 (9.32±13.59)	56 (8.32±13.09)	0.8	-	0.87	-	0.77	-
rs6136444	471 (9.39±13.23)	115 (7.91±12.09)	6 (22.25±25.23)	0.85	-	0.66	-	**0.01**	NS
rs6081259	289 (9.11±13.01)	251 (9.76±13.85)	52 (7.37±11.15)	0.79	-	0.76	-	0.24	-
rs6081262	268 (9.38±12.97)	268 (9.28±13.53)	56 (8.32±13.09)	0.78	-	0.85	-	0.78	-
rs6045540	265 (9.33±12.98)	269 (9.37±13.56)	58 (8.16±12.90)	0.78	-	0.89	-	0.7	-
rs16979451	280 (8.99±12.91)	253 (9.93±14.06)	56 (7.28±10.89)	0.9	-	0.57	-	0.22	-
rs4813338	173 (9.15±13.19)	300 (9.72±13.31)	117 (8.18±13.16)	0.74	-	0.73	-	0.33	-
rs6081283	434 (9.49±13.38)	146 (8.18±12.07)	12 (12.84±19.79)	0.76	-	0.49	-	0.26	-
rs6081286	431 (9.52±13.42)	147 (8.08±12.01)	14 (12.49±18.37)	0.73	-	0.45	-	0.29	-
rs6045557	391 (9.22±13.07)	184 (9.41±13.82)	17 (7.62±10.10)	0.94	-	0.98	-	0.74	-
rs6136466	426 (9.59±13.48)	150 (8.01±11.94)	15 (11.79±17.77)	0.59	-	0.34	-	0.34	-
rs911135	186 (9.50±13.31)	298 (9.53±13.32)	108 (7.95±12.81)	0.44	-	0.86	-	0.24	-
rs6136469	453 (9.46±13.46)	132 (7.88±11.36)	7 (19.97±23.81)	0.86	-	0.44	-	**0.02**	NS
rs6081299	438 (9.60±13.49)	142 (7.79±11.57)	11 (13.92±20.38)	0.52	-	0.27	-	0.2	-
rs10485585	294 (9.11±12.92)	246 (9.71±13.99)	52 (7.68±11.08)	0.85	-	0.77	-	0.34	-
rs2145247	294 (9.11±12.92)	245 (9.73±14.01)	52 (7.60±11.09)	0.84	-	0.77	-	0.32	-
rs6136479	477 (8.72±12.62)	111 (11.59±15.50)	4 (5.58±9.14)	0.09	-	0.06	-	0.62	-
rs6035106	173 (9.22±13.21)	302 (9.84±13.52)	117 (7.68±12.42)	0.52	-	0.8	-	0.16	-
rs4813340	267 (8.60±12.78)	248 (9.60±13.61)	65 (10.75±13.67)	0.26	-	0.34	-	0.36	-
rs6132107	394 (9.21±13.02)	182 (9.42±13.90)	16 (7.59±10.43)	0.92	-	0.98	-	0.7	-
rs1998122	220 (8.58±13.26)	288 (9.48±13.17)	83 (10.18±13.41)	0.39	-	0.48	-	0.49	-
rs11700004	394 (9.21±13.02)	182 (9.42±13.90)	16 (7.59±10.43)	0.92	-	0.98	-	0.7	-
rs4814783	268 (8.60±12.75)	258 (9.46±13.60)	65 (10.75±13.67)	0.28	-	0.39	-	0.34	-
rs6045577	202 (9.12±13.06)	287 (9.70±13.42)	101 (8.22±13.12)	0.78	-	0.73	-	0.34	-
rs6081338	454 (9.45±13.45)	131 (7.91±11.40)	7 (19.97±23.81)	0.87	-	0.46	-	**0.02**	NS
rs3827973	252 (8.32±12.52)	269 (10.27±13.95)	71 (8.54±12.67)	0.45	-	0.18	-	0.65	-
rs17747954	529 (9.51±13.48)	61 (7.05±10.67)	2 (1.70±0.99)	0.12	-	0.13	-	0.55	-
rs1884787	190 (9.06±13.10)	296 (9.83±13.72)	106 (7.86±11.95)	0.73	-	0.64	-	0.23	-
rs6075416	290 (9.17±13.03)	250 (9.62±13.86)	52 (7.69±11.07)	0.82	-	0.84	-	0.38	-
rs1884788	290 (9.17±13.03)	250 (9.62±13.86)	52 (7.69±11.07)	0.82	-	0.84	-	0.38	-
rs4814787	199 (9.22±13.21)	294 (9.62±13.56)	99 (8.12±12.23)	0.69	-	0.84	-	0.33	-
rs13041669	276 (8.48±12.48)	252 (9.69±13.90)	63 (10.72±13.69)	0.24	-	0.3	-	0.4	-
rs1079235	174 (9.07±13.04)	306 (9.94±13.81)	112 (7.56±11.71)	0.61	-	0.63	-	0.15	-
rs9139	546 (9.39±13.43)	45 (7.34±10.43)	-	0.3	-	0.3	-	-	-
DTD1_ht1	324 (9.31±12.93)	226 (9.59±13.92)	42 (6.72±11.46)	0.56	-	0.95	-	0.2	-
DTD1_ht2	324 (8.58±12.39)	220 (10.09±14.33)	48 (9.71±13.37)	0.34	-	0.28	-	0.79	-
DTD1_ht3	485 (9.57±13.42)	104 (7.41±11.79)	3 (17.83±23.69)	0.33	-	0.21	-	0.2	-
DTD1_ht4	484 (8.78±12.59)	104 (11.50±15.80)	4 (5.58±9.14)	0.1	-	0.07	-	0.62	-
DTD1_ht5	506 (9.73±13.63)	84 (6.48±10.14)	2 (-0.10±6.93)	**0.02**	NS	**0.02**	NS	0.32	-

Co-dominant, dominant and recessive models of multiple linear regression analyses were performed controlling for age, gender, smoking status, atopy and BMI as covariates. C/C, C/R and R/R indicate the homozygotes of the common allele, and the heterozygotes and homozygotes of the rare allele, respectively. Bold values indicate P<0.05. Pa, Pb and Pc refer to P-values of the co-dominant, dominant and recessive models, respectively. Pa^{corr}, Pb^{corr} and Pc^{corr} refer to P-values after multiple testing corrections (29.1223). NS, not significant.

now actively engaged in identifying mechanisms involved in the development of AIA that may lead to novel targets for treatment of the disease. Despite previous reports on genetic markers of bronchial hypersensitivity as a result of aspirin intake, the exact mechanisms of the disease are still unclear and susceptibility genes still need to be identified. Findings from this study may contribute to the current knowledge of AIA pathogenesis.

Although D-amino acids are thought to not be incorporated in proteins, several aminoacyl-tRNA synthetases (aaRSs) such as the tyrRS are capable of transferring the D-isomer of their amino acid onto their cognate tRNA, resulting in accumulation of the metabolically inactive D-aminoacyl-tRNAs (21). The *DTD1* gene counters this process and interacts with tyrRS by possessing a D-tyrosyl-tRNA deacylase that cleaves the ester bond between a tRNA molecule and a D-amino acid (21). Misregulation of *DTD1* gene expression may increase the toxicity of D-amino acids such as D-tryptophan, an enzyme that has been implicated in AIA etiology by producing kynurenine and serotonin (18-20). Furthermore, Wakasugi and Schimmel have revealed that cytoplasmic tyrRS has proinflammatory cytokine functions similar to interleukin-8 (IL-8) and to the endothelial monocyte-activating polypeptide II (EMAP II) (33). Previous studies have demonstrated the association between an IL-8 polymorphism and respiratory syncytial virus (RSV) bronchiolitis, a condition that may progress to bronchial asthma (34,35). In addition, IL-8 has been implicated in neutrophilic inflammation associated with severe asthma (36,37), whereas EMAP II has been known to stimulate the production of tumor necrosis factor-α (TNFα) (38), an AIA-susceptibility gene (39). Given the indirect role of *DTD1* in AIA susceptibility by interacting with tyrRS and catalyzing the formation of D-amino acids, we investigated the association of *DTD1* genetic variants with the risk of AIA in a Korean population.

In this study, differences in the distributions of 38 genotyped SNPs and 5 major haplotypes in *DTD1* were analyzed for a possible association with the risk of AIA and decline rate of FEV_1. Results from the logistic regression analysis revealed a lack of association between *DTD1* variants and AIA susceptibility. Furthermore, since the decline rate of FEV_1 is a crucial diagnostic marker for AIA, further analysis was performed. Our initial findings revealed associations of several *DTD1* SNPs (*rs6136444*, *rs6136469* and *rs6081338*) and of one haplotype (*DTD1_ht5*) with the decline rate of FEV_1 induced by aspirin provocation. Patients with the rare allele (R/R genotype) of the polymorphisms were in greater risk of developing AIA compared to subjects having other genotypes (C/C and C/R). However, the significant signals were not retained after multiple testing corrections, suggesting that *DTD1* polymorphisms do not affect pulmonary function abnormalities in AIA patients.

Although the exact molecular mechanisms are not clear, the *DTD1* gene can counteract the accumulation of inactive tRNA molecules (14) and promote defense mechanisms against the harmful effects of D-amino acids including tryptophan, an enzyme that is down-regulated by aspirin (40) and has been implicated in AIA susceptibility in several studies (18-20). The small sample size (n=592) may serve as a potential limitation to this preliminary study, and therefore, in order to clarify the relationship between *DTD1* variants and the risk of AIA with high statistical power, further replication studies should use larger scale samples in various ethnic groups (n>1,000).

To our knowledge, this study is the first to explore the relationship between *DTD1* gene variations and AIA pathogenesis. To conclude, our findings provide evidence that *DTD1* variants do not influence AIA and the decline rate of FEV_1 in a Korean population. However, considering the important function of *DTD1* in AIA pathogenesis, future replication studies in a larger cohort are recommended. These studies may be useful in the current genetic etiology of AIA susceptibility.

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