

Association of thromboxane A2 receptor gene polymorphism with the phenotype of acetyl salicylic acid-intolerant asthma

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Summary

Background and objective The thromboxane A2 receptor (TBXA2R) is a receptor for a potent bronchoconstrictor, TBXA2 which is known to be related to bronchial asthma and myocardial infarction. TBXA2R antagonist and TBX synthase inhibitors have been found to be effective in the management of asthmatic patients. This study was aimed to evaluate whether genetic variants of TBXA2R may be related with development of acetyl salicylic acid (ASA)-intolerant asthma (AIA). **Methods** TBXA2R gene polymorphisms (TBXA2R+795T>C, TBXA2R+924T>C) were determined using a single-base extension method in 93 AIA patients compared with 172 patients with ASA-tolerant asthma (ATA) and 118 normal controls (NCs) recruited from the Korean population. HLA DPB1*0301 genotype was performed using a direct sequencing method.

Results The rare C allele frequency of TBXA2R+795T>C was significantly higher in AIA than in ATA ($P=0.03$) and the TBXA2R+795T>C polymorphism was also associated with extent of percent fall in forced expiratory volume in 1 s (FEV₁) after the inhalation of lysine–acetyl salicylic acid in AIA patients ($P=0.009$); AIA patients homozygous for the +795 C allele had a greater percent fall of FEV₁ compared with individuals with TBXA2R+795 CT or TT genotypes. The frequency of patients carrying both the TBXA2R+795T>C rare allele and HLA DPB1*0301 was significantly higher in AIA patients (29.4%) than in ATA patients (7.3%) ($P=0.008$, odds ratio = 5.3).

Conclusion These results suggest that the polymorphism of TBXA2R+795T>C may increase bronchoconstrictive response to ASA, which could contribute to the development of the AIA phenotype.

Keywords ASA-intolerant asthma, bronchoconstrictive response, thromboxane A2 receptor gene polymorphisms

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Introduction

Acetyl salicylic acid (ASA)-intolerant asthma (AIA) is a distinct clinical syndrome affecting 10–20% of adult asthmatic patients in whom ingestion of ASA/non-steroidal anti-inflammatory drugs causes bronchoconstriction [1] and is highly associated with rhinosinusitis and nasal polyp [2].

It is known that the pathogenesis of AIA is caused by arachidonic acid metabolites such as leukotrienes (LTs), prostaglandins (PGs) and thromboxane (TBX) [3–5]. Most reports related to AIA emphasize the role of cysteinyl LTs in AIA [6, 7]. Baseline LT E₄ concentrations and LT C₄ (LTC₄) synthase expression levels have been reported to be signifi-

cantly higher in AIA patients compared with ASA-tolerant asthma (ATA) patients [8–10]. TBXA2, an eicosanoid product induced by cyclooxygenase may also be an important mediator in AIA, as it can induce bronchoconstriction and bronchial hyper-responsiveness [11–13] and stimulate proliferation of human airway smooth muscle cells [14]. TBXA2 exerts its actions by interacting with the G protein-coupled TBXA2 receptor (TBXA2R). In this context, TBX receptor antagonists and TBX synthase inhibitors have been proven to be effective in the management of asthmatic patients [15–17] by lowering airway hyper-responsiveness (AHR) [18, 19]. This strongly suggests that TBXA2 and TBXA2R may play a crucial role in the pathogenesis of AIA.

Recently, genetic association studies have identified positive association between genetic polymorphism in the TBXA2R and risk of asthma, atopy and atopic dermatitis [20–23]. However, there has been no study of the role of TBXA2R polymorphisms in the pathogenesis of AIA. In this study, we have investigated whether genetic polymorphisms of the TBXA2R gene are associated with an AIA phenotype

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through a case-study of three groups classified as AIA, ATA and normal healthy control (NC).

Materials and methods

Subjects

Three subject groups (93 patients with AIA, 181 patients with ATA and 123 normal controls (NC)) were enrolled from Ajou University Hospital and Soonchunhyang University Hospital in Korea. Diagnosis of AIA was based on positive response to lysine—acetyl salicylic acid (L-ASA) bronchoprovocation test. The L-ASA bronchoprovocation test was performed with increasing doses of ASA (75–300 mg/mL, Althargyl, Arthromedica, Switzerland) according to a modified method as previously described [24]. Change of forced expiratory volume in 1 s (FEV₁) was followed up to 5 h after last dose of aspirin challenge. ASA-induced change (%) in FEV₁ was calculated as percentage of post-challenge FEV₁ to pre-challenge FEV₁. Methacholine bronchial challenge tests were performed as previously described [25]. NCs were recruited from the general population who answered negatively to a screening questionnaire for respiratory symptoms, had no past history of ASA hypersensitivity, and had an FEV₁ greater than 80% predicted, PC₂₀ methacholine greater than 25 mg/mL, and normal findings on simple chest radiograms. Atopy was defined as one or more positive reactions on skin prick test, using 12 common aeroallergens (Bencard, Brentford, UK) including *Dermatophagoides pteronyssinus*, *Dermatophagoides farine*, cat, dog, cockroach, tree pollen mixture, grass pollen mixture, mugwort, ragweed, Hop Japanese, *Aspergillus*, *Alternaria*, with histamine and saline controls. Total IgE was measured using the UniCAP system (Pharmacia Diagnostics, Välinge, Sweden). All subjects gave their informed consent and the study was approved by the institutional review board of Ajou University Hospital, Suwon, Korea.

Genotyping

Sequences of amplifying and extension primers for TBXA2R+795T>C and TBXA2R+924T>C were forward-5'-gagtgaccctggatctca-3', reverse 5'-gcgctctgccactctc-3', extension-5'-cgagcactgtctgggc-3' (TBXA2R+795T>C) and extension-5'-tcctggaccctgggtga-3' (TBXA2R+924T>C). A single-base extension method was used for genotyping of single nucleotide polymorphisms (SNPs) according to previously described methodology [22]. Primer extension reactions were performed with the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, CA, USA). To clean up the primer extension reaction, 1 U of shrimp alkaline phosphatase (SAP) was added to the reaction mixture, and the mixture was incubated at 37 °C for 1 h, followed by 15 min at 72 °C for enzyme inactivation. The DNA samples, containing extension products, and Genescan 120 Liz size standard solution was added to Hi-Di formamide (Applied Biosystems) according to the manufacturer's recommendations. The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice, and then electrophoresis was performed using the ABI Prism 3100 Genetic Analyzer. The results were analysed using the ABI Prism GeneScan and

Genotyper software (Applied Biosystems, Foster City, CA, USA). In addition, genetic polymorphisms of LT-related genes such as 5-lipoxygenase [ALOX5-1708G>A, ALOX5+21C>T, ALOX5+270G>A, ALOX5+1728A>G], 5-lipoxygenase-activating protein [ALOX5AP+218A>G], LTC4S [LTC4S-444A>C], cyclooxygenase 2 [PTGS2-162C>G, PTGS2+10T>G, PTGS2R228H, PTGS2V511A], and cysteinyl LT receptor 1 [CYSLTR1+927T>C] were analysed by single-base extension method according to previously described methodology [25]. HLA DPB1*0301 genotype was obtained from amplified gene products with the use of locus-specific primers and direct sequencing with the ABI 3100 genetic analyzer (Applied Biosystems) according to a previously described method [26].

Statistical analysis

Significant departures of genotype frequency from the Hardy–Weinberg equilibrium at each SNP were tested by χ^2 analysis. Differences in genotype frequency between case and control were tested by χ^2 test and calculation of odds ratios (ORs) with 95% confidence intervals (CIs). Logistic regression models were used for analyses of SNPs and haplotypes controlling age and sex as covariates with three alternative models (co-dominant, dominant and recessive models). Haplotypes of the TBXA2R gene were analysed using Haploview version 2.05 based on an EM algorithm [27]. Linkage disequilibrium between loci was measured using the absolute value of Lewontin's $|D'|$ [28]. Differences in the mean value of the phenotypic characteristics within AIA patients were compared using ANOVA test and *t*-test. Statistical analyses were undertaken using SPSS v11 (SPSS Inc., Chicago, IL, USA). *P*-values of <0.05 were considered to be significant.

Results

Subjects

The clinical characteristics of the study subjects are summarized in Table 1. There were significant differences in mean age, prevalence of sex, and atopic status between AIA and NC groups ($P<0.001$, $P=0.03$, $P<0.001$, respectively). Between the AIA and ATA groups, there were significant

Table 1. Clinical characteristics of the study subjects

	AIA (N = 93)	ATA (N = 172)	NC (N = 118)
Age (years)*	44.1 ± 14.0	44.8 ± 14.2	35.9 ± 14.5
Sex (M)*	34 (36.6%)	52 (30.2%)	61 (51.7%)
Atopy*	39 (45.3%)	52 (45.6%)	17 (15.5%)
Asthma duration (years)	6.9 ± 6.1	5.6 ± 6.2	NA
FEV ₁ (%)	81.0 ± 22.5	87.9 ± 24.6	NA
% Fall (FEV ₁)**	22.7 ± 11.3	5.6 ± 1.5	NA
PC ₂₀ methacholine (mg/mL)	3.9 ± 6.8	8.6 ± 13.3	NA
Serum total IgE (IU/mL)	333.4 ± 366.7	346.6 ± 460.0	NA
Rhinitis*	63(70.0%)	53 (39.3%)	NA

ASA, acetyl salicylic acid; AIA, ASA-intolerant asthma; ATA, ASA-tolerant asthma; NC, normal controls; FEV₁, forced expiratory volume in 1 s; N, number of patients; NA, not applicable.

* $P<0.05$, ** $P<0.01$.

Table 2. Allele, genotype and haplotype frequencies of the TBXA2R gene

Loci	Genotype/haplotype	AIA (N = 93)	ATA (N = 172)	NC (N = 118)	P-value*	
					AIA vs. ATA	AIA vs. NC
+795T>C (Exon3)	TT	28 (30.1%)	74 (43.3%)	40 (33.9%)	0.21	0.70
	CT	53 (57.0%)	71 (41.5%)	56 (47.5%)	0.60	0.18
	CC	12 (12.9%)	26 (15.2%)	22 (18.6%)	0.03	0.61
	Allele frequency (q)	0.41	0.36	0.42	0.21	0.71
+924T>C (Exon3)	TT	55 (58.5%)	110 (64.3%)	75 (63.6%)	0.56	0.86
	CT	36 (38.3%)	53 (31.0%)	36 (30.5%)	0.55	0.25
	CC	3 (3.2%)	8 (4.7%)	7 (5.9%)	0.35	0.47
	Allele frequency (q)	0.22	0.20	0.21	0.57	0.86
	Ht1 (T-T)	0.59	0.66	0.60	0.12	0.98
	Ht2 (C-T)	0.23	0.22	0.24	0.77	0.84
	Ht3 (C-C)	0.16	0.11	0.15	0.05	0.77

TBXA2R, thromboxane A2 receptor; ASA, acetyl salicylic acid; AIA, ASA-intolerant asthma; ATA, ASA-tolerant asthma; NC, normal controls; n, number of patients; q, minor allele frequency; NS, not significant.

*Each P-value was calculated with co-dominant, dominant and recessive models. Logistic regression analysis was used to control for age and sex as covariables.

Table 3. Mean ($\pm$ standard deviation) of the clinical characteristics within AIA patients stratified by TBXA2R genotype

Loci	Genotype	Asthma duration (years)	FEV ₁ (%)	% fall (FEV ₁) (%)	PC ₂₀ methacholine (mg/mL)
+795T>C	TT or CT	7.0 $\pm$ 6.5	82.4 $\pm$ 21.8	21.3 $\pm$ 9.6	3.8 $\pm$ 6.7
	CC	6.3 $\pm$ 4.1	72.0 $\pm$ 26.5	33.8 $\pm$ 17.8	2.2 $\pm$ 1.7
	P-value	0.71	0.16	0.009	0.47
+924T>C	TT or CT	6.8 $\pm$ 6.2	81.7 $\pm$ 22.5	22.8 $\pm$ 11.5	4.0 $\pm$ 6.9
	CC	10.5 $\pm$ 6.3	56.7 $\pm$ 1.6	20.5 $\pm$ 0.7	1.4 $\pm$ 1.6
	P-value	0.23	0.92	0.37	0.25

ASA, acetyl salicylic acid; AIA, ASA-intolerant asthma; TBXA2R, thromboxane A2 receptor; FEV₁, forced expiratory volume in 1 s. Bold indicates significant P-value.

differences in percent fall of FEV₁ during L-ASA broncho-provocation test ($P = 0.004$); with AIA patients showing a higher percent fall of FEV₁.

Allele, genotype and haplotype frequencies of thromboxane A2 receptor gene

We performed a genetic association study of two genetic polymorphisms of TBXA2R (TBXA2R+795T>C, TBXA2R+924T>C) in three groups of study subjects classified as AIA, ATA and NC. Table 2 shows the allele, genotype and haplotype frequencies of two polymorphisms (TBXA2R+795T>C, TBXA2R+924T>C) of TBXA2R gene. AIA patients showed significantly higher frequency of the combined homozygous CC genotype and heterozygous CT genotype group of TBXA2R+795T>C when compared with ATA patients ($P = 0.03$, multiple logistic regression analysis controlling for age and sex, recessive model). In contrast, there were no significant differences in allele and genotype frequencies of TBXA2R+924T>C observed among the three study groups. The two polymorphisms in the TBXA2R gene were in linkage disequilibrium ($|D'| = 1$) and three common haplotypes were constructed utilizing the EM algorithm. Among the three haplotypes, haplotype 3 consisting of the rare alleles (C, C) of both TBXA2R+795T>C and TBXA2R+924T>C was shown to have a significant difference in observed haplotype frequency between AIA and ATA groups ($P = 0.05$, OR = 1.663, 95% CI = 0.993–2.985).

Association of TBXA2R+795T>C with bronchoconstrictive response to ASA

We examined the association of both polymorphisms of the TBXA2R gene with asthma-related phenotypes (Table 3). There was a significant difference in percent fall of FEV₁ after L-ASA inhalation between subjects homozygous CC for the TBXA2R+795T>C compared with those who were either heterozygous CT or homozygous TT; AIA patients with the homozygous CC genotype had a greater decrease of FEV₁ (33.8 $\pm$ 17.8%, mean value $\pm$ SD) after inhalation of L-ASA than those carrying the common T allele, (21.3 $\pm$ 9.6%) ($P = 0.009$). In addition, AIA patients who were homozygous CC tended to have lower PC₂₀ methacholine (2.2 $\pm$ 1.7 mg/mL) and lower baseline FEV₁-value (72.0 $\pm$ 26.5%) than those carrying wild genotype (3.8 $\pm$ 6.7 mg/mL for PC₂₀ methacholine, 82.4 $\pm$ 21.8% for baseline FEV₁-value). Figure 1 shows the degree of bronchoconstrictive response to L-ASA according to the polymorphisms of TBXA2R gene.

Association of TBXA2R+795T>C with HLA DPB1*0301

We have previously reported a significant association between the HLA DPB1*0301 allele and AIA susceptibility in the Korean population [26]. Therefore, we examined whether there was any interaction between HLA DPB1*0301 and TBXA2R polymorphism in determining susceptibility to AIA. There was a highly significant interaction between the genes with carriers of the TBXA2R+795T>C allele who were also HLA DPB1*0301 positive being more frequent in the AIA

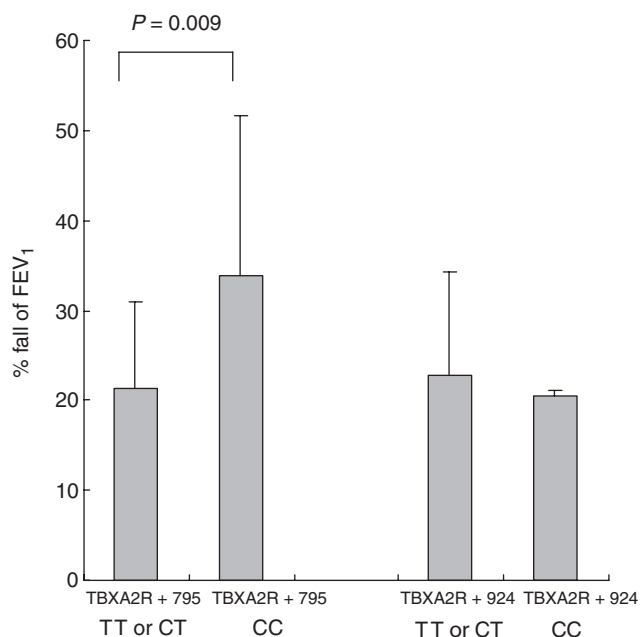


Fig. 1. Percent fall of forced expiratory volume in 1 s (FEV₁) after lysine-acetyl salicylic acid inhalation according to thromboxane A2 receptor (TBXA2R) genotype.

population (29.4%) than in the ATA population (7.3%) ($P = 0.008$, OR = 5.3, 95% CI = 1.409–19.772, Table 4a). However, no significant association was noted between TBXA2R+924T>C and HLA DPB1*0301 (data not shown).

Gene-gene interaction of TBXA2R+924T>C with ALOX5+21C>T

Another previously identified genetic risk factor for AIA in the Korean population is a haplotype of the ALOX5 gene [25]. Accordingly, we analysed possible gene-gene interaction between TBXA2R gene [TBXA2R+795T>C, TBXA2R+924T>C] and LT-related genes such as 5-lipoxygenase [ALOX5-1708G>A, ALOX5+21C>T, ALOX5+270G>A, ALOX5+1728A>G], 5-lipoxygenase-activating protein [ALOX5AP+218A>G], LTC4S [LTC4S-444A>C], cyclooxygenase 2 [PTGS2-162C>G, PTGS2+10T>G, PTGS2R228H, PTGS2V511A], and cysteinyl LT receptor 1 [CYSLTR1+927T>C]. This analysis revealed there was a significant interaction between the TBXA2R+924 C allele and ALOX5+21 T allele. The number of the subjects carrying the rare allele of both TBXA2R+924T>C and ALOX5+21C>T was significantly higher in the AIA group than in either of the other control groups ($P = 0.02$ AIA vs. ATA, $P = 0.007$ AIA vs. NC, Table 4b). No significant associations were noted with other LT-related genes for either the TBXA2R+924T>C or +795T>C polymorphisms ($P > 0.05$, respectively, data not shown).

Discussion

AIA is characterized by ASA hypersensitivity, bronchial asthma and chronic rhinosinusitis with nasal polyposis [29]. Arachidonic acid metabolites such as LTs, PGs and TBX have been considered as important mediators in the

pathogenesis of AIA. The role of TBXA2 in asthma can be evident through its induction of bronchoconstriction and AHR [11–13] and its receptor antagonist can be used for asthma management [15–17]. However, until now there has been no published data to demonstrate any association with AIA pathogenesis.

In the present study, we have firstly demonstrated a possible association of TBXA2R+795T>C in the development of AIA, as AIA patients had a higher frequency of the TBXA2R+795 CC genotype and AIA patients with this genotype exhibited greater bronchoconstrictive response to inhaled L-ASA. These results suggest that a genetic polymorphism of TBXA2R can augment bronchoconstrictive response to inhaled L-ASA and increase susceptibility to AIA. The key mechanism underlying bronchoconstrictive response to L-ASA in AIA patients has been explained by overproduction of LTs [6, 7, 30], that may be as a result of suppression of protective PG E₂ and elevation of LTC₄ synthase expression [9, 10]. In addition, Tornhamre et al. [31] reported that cyclooxygenase inhibition by ASA leads to attenuated TBX biosynthesis, which then causes overproduction of LT via uncoupling of LTC₄ synthase which is down-regulated by TBXA2. Based on these findings, one possible mechanism for how the TBXA2R gene polymorphism might contribute to AIA phenotype would be through the negative regulation of LTC₄S by TBXA2 being modulated by the TBXA2R+795T>C polymorphism. Furthermore, this genetic polymorphism of the TBXA2R showed a significant association with HLA DPB1*0301, which has previously been reported to be a strong genetic susceptibility factor for AIA [26]. Through combined association analysis of TBXA2R+795T>C and HLA DPB1*0301, a synergistic effect of TBXA2R+795T>C and HLA DPB1*0301 genotypes was found on the development of AIA ($P = 0.008$, OR = 5.3, 95% CI = 1.409–19.772). Based on these findings, we conclude that a gene-gene interaction of TBXA2R+795T>C and HLA DPB1*0301 can increase susceptibility to AIA.

Another genetic polymorphism of the TBXA2R (TBXA2R+924T>C) has previously been reported to be associated with atopy and asthma [20–22]. However, we found no significant association of this polymorphism with AIA in this study. However, when analysing potential gene-gene interactions between polymorphisms in the LT biosynthetic pathway and the TBXA2R polymorphisms, we found a significant interaction between TBXA2R+924T>C and ALOX5+21C>T, with the frequency of combined mutant genotypes of TBXA2R+924T>C and ALOX5+21C>T was being significantly higher in the AIA, the ATA and NC groups. This result suggested that a gene-gene interaction of TBXA2R and ALOX5 may be synergistically associated with AIA; however, further investigations will be needed to pursue how this gene-gene interaction is involved in the pathogenesis of AIA.

Previously, the levels of 11-dehydro TBXB₂, a stable metabolite of TBXA₂, have been shown to be significantly higher in atopic AIA patients than non-atopic AIA patients [32], suggesting a possible interaction between TBXA₂ and atopy. However, we did not find any significant association of polymorphisms in the TBXA2R gene with atopic status in the AIA patient group (data not shown).

Table 4a. Distribution of combined genetic variants of TBXA2R+795 T>C and HLA DPB1*0301

TBXA2R795 T>C	HLA DPB1*0301	AIA	ATA	AIA vs. ATA
TT	(Positive)	6 (26.1%)	2 (8.3%)	0.105
	(Negative)	17 (73.9%)	22 (91.7%)	
	N	23	24	
CT or CC	(Positive)	15 (29.4%)	3 (7.3%)	0.008 (OR = 5.3)
	(Negative)	36 (70.6%)	38 (92.7%)	
	N	51	41	

ASA, acetyl salicylic acid; TBXA2R, thromboxane A2 receptor; HLA, human leucocyte antigen; AIA, ASA-intolerant asthma; ATA, ASA-tolerant asthma; NC, normal controls; OR, odds ratio. Bold indicates significant *P*-value.

Table 4b. Distribution of combined genetic variants of TBXA2R+924 T>C and ALOX5+21C>T

TBXA2R+924 T>C	ALOX5+21 C>T	AIA	ATA	NC	AIA vs. ATA	AIA vs. NC
TT	CC	31 (66.0%)	64 (62.7%)	49 (66.2%)	0.705	0.977
	CT or TT	16 (34.0%)	38 (37.3%)	25 (33.8%)		
	N	47	102	149		
CT or CC	CC	13 (39.4%)	38 (65.5%)	29 (70.7%)	0.016 (OR = 2.9)	0.007 (OR = 3.7)
	CT or TT	20 (60.6%)	20 (34.5%)	12 (29.3%)		
	N	33	58	91		

ASA, acetyl salicylic acid; TBXA2R, thromboxane A2 receptor; AIA, ASA-intolerant asthma; ATA, ASA-tolerant asthma; NC, normal controls; OR, odds ratio. Bold indicates significant *P*-values.

In conclusion, the TBXA2R+795T>C polymorphism may increase bronchoconstrictive response to L-ASA, which could contribute to development of the AIA phenotype in the Korean population. Further studies will be needed to evaluate the function of these polymorphisms on TBXA2 responsiveness and how they might influence response to TBXA2R antagonist treatment in AIA patients.

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