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ORIGINAL ARTICLE

Association Analysis of *TEC* Polymorphisms with Aspirin-Exacerbated Respiratory Disease in a Korean Population

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The tyrosine-protein kinase Tec (TEC) is a member of non-receptor tyrosine kinases and has critical roles in cell signaling transmission, calcium mobilization, gene expression, and transformation. TEC is also involved in various immune responses, such as mast cell activation. Therefore, we hypothesized that *TEC* polymorphisms might be involved in aspirin-exacerbated respiratory disease (AERD) pathogenesis. We genotyped 38 *TEC* single nucleotide polymorphisms in a total of 592 subjects, which comprised 163 AERD cases and 429 aspirin-tolerant asthma controls. Logistic regression analysis was performed to examine the associations between *TEC* polymorphisms and the risk of AERD in a Korean population. The results revealed that *TEC* polymorphisms and major haplotypes were not associated with the risk of AERD. In another regression analysis for the fall rate of forced expiratory volume in 1 second (FEV_1) by aspirin provocation, two variations (*rs7664091* and *rs12500534*) and one haplotype (*TEC_BL2_ht4*) showed nominal associations with FEV_1 decline ($p = 0.03-0.04$). However, the association signals were not retained after performing corrections for multiple testing. Despite *TEC* playing an important role in immune responses, the results from the present study suggest that *TEC* polymorphisms do not affect AERD susceptibility. Findings from the present study might contribute to the genetic etiology of AERD pathogenesis.

Keywords: aspirin-exacerbated respiratory disease, aspirin-tolerant asthma, Tec protein tyrosine kinase, genetic polymorphisms, haplotypes

Introduction

Asthma is a disease that is caused by inflammation in the lung and bronchus and is affected by genetic and environmental influences. Aspirin-exacerbated respiratory disease (AERD), which was first reported in 1922, is a type of asthma. AERD is characterized by the following three symptoms: bronchial asthma, aspirin sensitivity, and nasal polyposis [1-3]. It has been reported that 10−20% of asthma patients have aspirin sensitivity, whereas 1−2% of the non-asthma population shows aspirin sensitivity [4, 5]. Although the mechanisms of AERD pathogenesis are still not fully understood, inflammatory responses by overproduction of leukotrienes are regarded as the main pathogenesis of AERD.

Aspirin is a commonly used medication, which belongs to the non-steroidal anti-inflammatory drugs. Despite being a widely used medication, aspirin intake also causes various side effects, including manifested gastrointestinal ulcer, stomach bleeding, and tinnitus, especially in higher doses. Although the side effects of aspirin are not common, these

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effects have been reported in about 10% of adult asthmatics. In AERD pathogenesis, aspirin inhibits the activation of cyclooxygenase-1 enzyme, leading to block of production of prostaglandin and thromboxane. This mechanism causes overproduction of leukotrienes, such as leukotriene B4, leukotriene C4, leukotriene D4, and leukotriene E4 [6, 7].

The tyrosine-protein kinase Tec (TEC) is a member of the non-receptor tyrosine kinases and has critical roles in cell signaling transmission, calcium mobilization, gene expression, and transformation [8]. It has been known that TEC family kinases are associated with various intracellular signaling mechanisms, such as cytokine receptors and lymphocyte surface antigens [9, 10]. In particular, it was demonstrated that the TEC family proteins are involved in regulation of leukotriene secretion via the mast cell signaling pathway [11]. Therefore, we hypothesized that *TEC* polymorphisms might be involved in AERD pathogenesis. In the present study, 38 *TEC* single nucleotide polymorphisms (SNPs) were genotyped in a total of 592 subjects, which comprised 163 AERD cases and 429 aspirin-tolerant asthma (ATA) controls, to examine the associations between *TEC* polymorphisms and AERD susceptibility.

Methods

Study subjects

Subjects in this study were recruited from the Asthma Genome Research Center, comprising the hospitals of Soonchunhyang, Chunnam, Chungbuk, Seoul National, and Chung-Ang Universities in Korea. All subjects provided written informed consents, and the study protocols were approved by the institutional review board of each hospital. Diagnosis of AERD was performed according to a modified method as previously described [12]. We also performed aspirin challenge in subjects with a history of aspirin hypersensitivity, presence of urticaria, nasal polyp, and sinusitis. The AERD case group included patients with 20% or greater decreases in forced expiratory volume in 1 second (FEV_1) or 15% to 19% decreases in FEV_1 with naso-ocular or cutaneous reactions, whereas subjects showing a rate of FEV_1 decline less than 15% without extrabronchial nasal or skin symptoms were included in the ATA group. The clinical diagnostic factors for the present study are summarized in Table 1.

SNP selection and genotyping

To investigate the associations between *TEC* polymorphisms and the risk of AERD, we selected candidate SNPs based on allele frequencies in the Asian population, linkage disequilibrium (LD) status, and National Center for Biotechnology information. The data for selection were obtained from the International HapMap database (http://hapmap.ncbi.nlm.nih.gov/). Genotyping of 38 *TEC* polymorphisms was performed in a total of 592 subjects, including 163 AERD cases and 429 ATA controls. Genotyping was carried out with 20 ng of genomic DNA by TaqMan assay using ABI prism 7900HT sequence detection system software version 2.3 (Applied Biosystems, Foster City, CA, USA) in all subjects. Assay IDs of all SNPs used in TaqMan assay are listed in Supplementary Table 1.

Table 1. Clinical profile of subjects in study with *TEC* polymorphisms

Clinical profile	Total subjects	AERD	ATA	p-value
No. of subjects	592	163	429	
Mean age of first medical examination (range, y)	46.15 (15.40–77.88)	43.13 (17.22–72.73)	47.30 (15.40–77.88)	**0.001**
Sex (male/female)	206/386	59/104	147/282	0.66
Height (cm)	160.78 ± 8.63	161.72 (143.00–196.00)	160.42 (140.00–199.00)	0.10
Weight (kg)	62.81 ± 10.84	61.25 ± 10.38	63.40 ± 10.97	0.03
BMI (kg/m^2)	24.24 ± 3.39	23.39 ± 3.25	24.58 ± 3.39	**0.0001**
Fall rate (%)	9.27 ± 13.24	24.63 ± 16.11	3.54 ± 4.85	**0.0001**
FVC %, predicted	88.54 ± 14.08	90.35 ± 14.04	87.85 ± 14.05	0.05
FEV_1 %, predicted	90.54 ± 16.97	87.58 ± 16.94	91.66 ± 16.87	**0.009**
PC20, methacholine (mg/mL)	6.43 ± 8.67	5.02 ± 7.83	6.91 ± 8.90	0.02
Current smoker (%)	27.70	21.47	30.07	0.02
Blood eosinophil (%)	6.01 ± 5.73	5.96 ± 5.21	6.03 ± 5.92	0.88
Total IgE (IU/mL)	357.65 ± 604.09	348.60 ± 596.44	361.00 ± 607.56	0.83
Positive rate of skin test (%)	56.42	52.76	57.81	0.27

Age indicates first medical examination.
AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second.

Statistics

We applied a widely used measure of linkage disequilibrium to all pairs of biallelic loci: Lewontin's D' (|D'|) [13] and r^2. Haplotypes of each individual were inferred using the PHASE algorithm (ver. 2.0), developed by Stephens *et al*. [14]. Linear regression analysis was performed to examine the differences in the rates of decline in FEV_1 following aspirin challenge among the genotypes and major haplotypes. The data were managed and analyzed using Statistical Analysis System, version 9.2 (SAS Inc., Cary, NC, USA). Associations for AERD under the logistic model were adjusted by smoking status, atopy, body mass index (BMI), age, and sex (male = 0, female = 1). Significant associations are shown in boldface ($p < 0.05$).

Results and Discussion

We recruited a total of 592 subjects, which consisted of 163 AERD cases and 429 ATA controls, for the present study. According to the results, four clinical characteristics showed significant differences between the case and control groups (Table 1). The fall rate by aspirin provocation was significantly higher in AERD subjects than ATA controls (24.63 ± 16.11 vs. 3.54 ± 4.85; p = 0.0001). Percentage of predicted FEV_1 in the AERD subjects showed decreased lung function than in ATA subjects (87.58 ± 16.94 vs. 91.66 ± 16.87; p = 0.009). Also, age of first medical examination and BMI were lower in AERD cases than ATA controls. The other diagnostic factors showed no significant differences between the case and control group.

In the present study, 38 *TEC* polymorphisms and 12 major haplotypes were used for the association analyses. Locations of the polymorphisms are shown in a genetic map of *TEC* with their LD status (Fig. 1A). Minor allele frequencies (MAFs) of the SNPs in Korean subjects are displayed in Supplementary Table 2 with their allele change, position, and p-value for Hardy-Weinberg equilibrium. All SNPs were in Hardy-Weinberg equilibrium. The LD blocks were obtained by using HaploView software, and the haplotypes of each LD block were calculated by PHASE software (Fig. 1B and 1C). We used major haplotypes that had frequencies higher than 0.05 for further analyses. The minor haplotypes that had frequencies lower than 0.05 were merged and presented as 'Others.' To compare genetic differences among ethnicities, we obtained MAFs of Caucasians, Han Chinese,

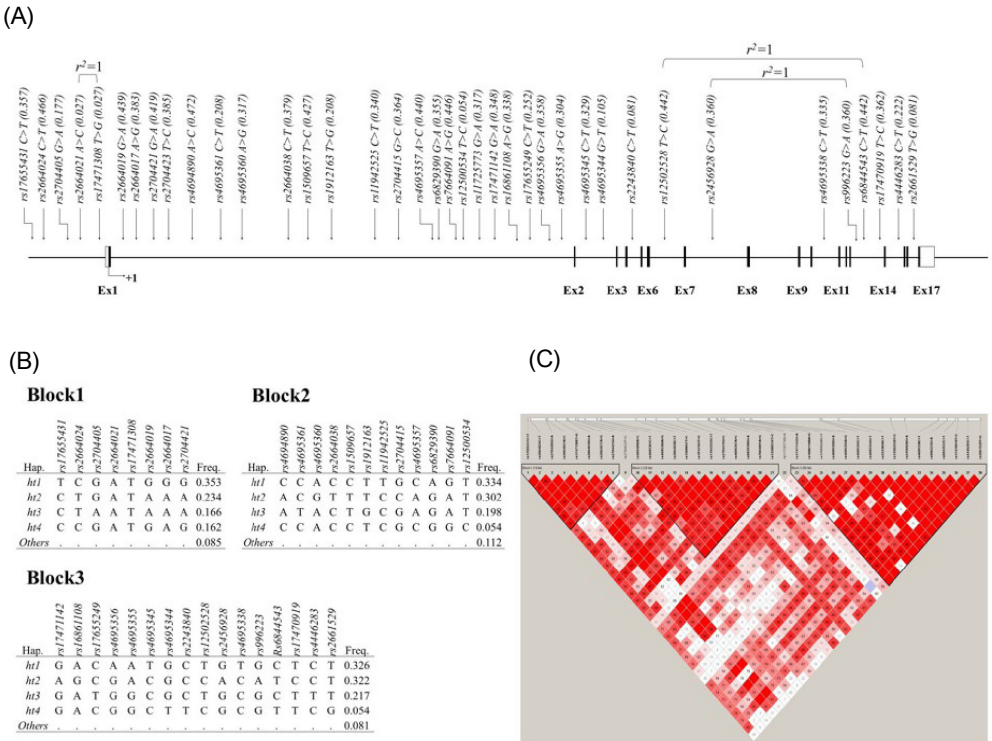

Fig. 1. Schematic physical map, haplotypes and linkage disequilibrium (LD) plot of *TEC*. (A) Polymorphisms identified in *TEC*. Coding exons are marked by shaded blocks and untranslated region by white blocks. The LD coefficients (r^2) are based on the genotypes of Korean samples. (B) Haplotypes are calculated using genotypes of *TEC* polymorphisms in a Korean population. Only those with frequencies over 0.05 are shown in tables. Haplotypes with frequencies lower than 0.05 are merged into "*Others.*" (C) LD coefficients (|D'|) among the selected SNPs based on the genotypes of whole study subjects in this study.

Table 2. Association analysis of *TEC* polymorphisms and haplotypes with the risk of AERD in a Korean population

SNP/Haplotype	Allele change	Position	OR (95% CI)	p-value	Statistical power (%)
rs17655431	C>T	Promoter	1.11 (0.85–1.45)	0.43	80.33
rs2664024	C>T	Promoter	0.98 (0.75–1.27)	0.87	82.29
rs2704405	G>A	Promoter	1.03 (0.73–1.44)	0.87	56.53
rs2664021	A>C	Promoter	0.82 (0.34–1.98)	0.66	4.53
rs17471308	T>G	Promoter	0.82 (0.34–1.98)	0.66	4.53
rs2664019	G>A	Intron 1	0.99 (0.76–1.30)	0.97	82.32
rs2664017	A>G	Intron 1	1.10 (0.84–1.44)	0.48	81.33
rs2704421	G>A	Intron 1	1.03 (0.79–1.35)	0.81	82.14
rs2704423	T>C	Intron 1	1.02 (0.79–1.33)	0.86	81.39
TEC_BL1_ht1	-	-	1.11 (0.85–1.45)	0.46	80.09
TEC_BL1_ht2	-	-	1.05 (0.77–1.43)	0.74	68.14
TEC_BL1_ht3	-	-	0.98 (0.69–1.39)	0.91	54.38
TEC_BL1_ht4	-	-	0.84 (0.59–1.22)	0.36	52.40
rs4694890	A>C	Intron 1	1.06 (0.82–1.38)	0.64	82.24
rs4695361	C>T	Intron 1	0.91 (0.66–1.25)	0.56	63.71
rs4695360	A>G	Intron 1	1.01 (0.77–1.34)	0.93	77.96
rs2664038	C>T	Intron 1	1.06 (0.82–1.37)	0.68	81.20
rs1509657	T>C	Intron 1	0.97 (0.74–1.27)	0.83	82.23
rs1912163	T>G	Intron 1	0.88 (0.64–1.21)	0.43	63.71
rs11942525	C>T	Intron 1	1.09 (0.83–1.43)	0.55	79.46
rs2704415	G>C	Intron 1	1.13 (0.86–1.48)	0.37	80.64
rs4695357	A>C	Intron 1	0.92 (0.71–1.21)	0.55	82.32
rs6829390	G>A	Intron 1	1.05 (0.80–1.38)	0.74	80.24
rs7664091	A>G	Intron 1	0.94 (0.71–1.22)	0.62	82.34
rs12500534	T>C	Intron 1	0.63 (0.33–1.20)	0.16	12.90
rs11725773	G>A	Intron 1	1.16 (0.89–1.52)	0.27	77.96
TEC_BL2_ht1	-	-	1.04 (0.79–1.37)	0.79	79.10
TEC_BL2_ht2	-	-	1.03 (0.78–1.37)	0.83	76.58
TEC_BL2_ht3	-	-	0.82 (0.59–1.15)	0.25	61.80
TEC_BL2_ht4	-	-	0.63 (0.33–1.20)	0.16	12.90
rs17471142	G>A	Intron 1	1.18 (0.89–1.55)	0.25	79.89
rs16861108	A>G	Intron 1	1.23 (0.94–1.63)	0.14	79.34
rs17655249	C>T	Intron 1	0.73 (0.53–1.00)	0.05	71.20
rs4695356	G>A	Intron 1	1.00 (0.76–1.32)	1.00	80.38
rs4695355	A>G	Intron 1	0.80 (0.60–1.07)	0.13	76.93
rs4695345	C>T	Intron 2	1.05 (0.80–1.38)	0.73	78.79
rs4695344	G>T	Intron 2	0.80 (0.52–1.23)	0.31	32.83
rs2243840	C>T	Intron 4	0.91 (0.56–1.48)	0.70	23.31
rs12502528	T>C	Intron 7	1.13 (0.86–1.48)	0.39	82.33
rs2456928	G>A	Intron 8	1.17 (0.89–1.54)	0.26	80.47
rs4695338	C>T	Intron 10	1.05 (0.80–1.39)	0.71	79.16
rs996223	G>A	Intron 13	1.17 (0.89–1.54)	0.26	80.47
rs6844543	C>T	Intron 13	1.13 (0.86–1.48)	0.39	82.33
rs17470919	T>C	Intron 13	1.15 (0.88–1.52)	0.31	80.55
rs4446283	C>T	Intron 14	0.80 (0.58–1.11)	0.18	66.41
rs2661529	T>G	Intron 16	0.90 (0.55–1.46)	0.66	23.31
TEC_BL3_ht1	-	-	1.07 (0.81–1.42)	0.62	78.59
TEC_BL3_ht2	-	-	1.26 (0.95–1.66)	0.11	78.25
TEC_BL3_ht3	-	-	0.80 (0.58–1.12)	0.19	65.85
TEC_BL3_ht4	-	-	1.22 (0.71–2.12)	0.47	12.18

Logistic analyses controlling for age, sex, smoking status, atopy, and body mass index as covariates, performed using the Statistical Analysis System (SAS).
AERD, aspirin-exacerbated respiratory disease; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Japanese, and Africans from the NCBI database (dbSNP) (Supplementary Table 2) and calculated LD blocks using genotype data from the International HapMap database (Supplementary Fig. 1). As a result, Asian populations showed similar MAFs, whereas the other populations showed distinct differences. However, we did not find any correlation in LD status in any ethnicity.

Logistic analyses were performed to investigate the associations between *TEC* polymorphisms and the risk of AERD. The result from analyses revealed that all SNPs and major haplotypes were not associated with the risk of AERD (Table 2). On the other hand, two SNPs (*rs7664091*, p = 0.04 and *rs12500534*, p = 0.03) and one major haplotype in LD block 2 (*TEC_BL2_ht4*, p = 0.03) showed marginal associations in the regression analysis with the decline rate of FEV_1 (Supplementary Table 3). However, the association signals of these polymorphisms disappeared after performing corrections for multiple testing (data not shown). Taken together, these results indicate that the *TEC* polymorphisms are not associated with AERD susceptibility, at least in the Korean population. The lack of associations in this study suggests that although TEC plays an important role in immune responses, *TEC* variants do not directly affect decreased pulmonary function by aspirin uptake. However, considering the fact that the difference in frequency of polymorphisms showed different effects in various populations, replication studies in AERD subjects in other ethnicities are recommended.

The present study still had several limitations. First, an average statistical power of 66.41% indicated an insufficient sample size. However, the rare condition of AERD made it difficult to recruit subjects in the Korean asthma cohort. Second, SNPs in coding sequence were not selected, due to very low frequencies, although non-synonymous SNPs (nsSNP) in exonic regions could affect risk of the disease. Further studies are required to examine the molecular role of nsSNPs in *TEC*.

In conclusion, we hypothesized that *TEC* might impact on AERD susceptibility. However, our results showed that 38 *TEC* polymorphisms and 12 major haplotypes were not associated with the risk of AERD. Although further studies are required to investigate the exact role of the *TEC* SNPs in immune responses, the preliminary results of the present study may provide useful information for AERD pathogenesis.

Supplementary materials

Supplementary data including three tables and one figure can be found with this article online at http://www.genominfo.org/src/sm/gni-12-58-s001.pdf.

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