

Lack of association between *GTF2H4* genetic variants and AERD development and FEV₁ decline by aspirin provocationJ. Y. Kim^{1,*}, J.-H. Kim^{1,*}, J. S. Bae^{*}, B.-L. Park[†], S.-T. Uh[‡], M.-K. Kim[§], I. S. Choi[¶], S. H. Cho^{**}, C.-S. Park^{††} & H. D. Shin^{*†}

Summary

Aspirin-exacerbated respiratory disease (AERD) is prevalent in about 10% of asthma patients and is characterized by a severe decline in forced expiratory volume in 1-s (FEV₁), an important phenotype for total lung capacity, upon ingestion of aspirin. The *general transcription factor IIIH subunit 4* (*GTF2H4*) is positioned at 6p21.33, a part of the major histocompatibility complex (MHC) class II region that contains a number of genes that play an important role in the immune system. In addition, genetic variants in another general transcription factor IIIH gene have revealed significant association with lung disease. To investigate whether *GTF2H4* genetic variants could be a causative factor for AERD development and FEV₁ decline by aspirin provocation, five common single-nucleotide polymorphisms (SNPs) were genotyped in 93 patients with AERD and 96 aspirin-tolerant asthma (ATA) controls. As a result, when adjusted for age, gender, smoking status and atopy as covariates, the *rs1264307* variant and two haplotypes showed nominal signals in

the association with AERD ($P = 0.02$ – 0.04), but the significances disappeared after corrections for multiple testing (corrected $P > 0.05$). In further multiple regression analysis, no genetic variants of *GTF2H4* showed significant associations with FEV₁ decline by aspirin provocation in asthmatics ($P > 0.05$). Despite the need for replications in larger cohorts, our preliminary findings suggest that *GTF2H4* variants may not be associated with susceptibility to AERD and obstructive symptoms in asthmatics.

Introduction

Approximately 300 million patients over the world are known to be suffering from asthma currently (Fanta, 2009), and among them, about 20% of asthma patients are identified as patients with aspirin-exacerbated respiratory disease (AERD) (Hedman *et al.*, 1999; McDonald *et al.*, 1972). AERD is characterized by symptoms such as lung inflammation and difficulty of breathing upon ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin (Widal *et al.*, 1993; Babu & Salvi, 2000). The clinical characteristics of AERD include a combination of aspirin sensitivity, bronchial asthma and chronic rhinosinusitis with nasal polyposis (Lee & Stevenson, 2011). Consistent inflammation often results in airway remodelling and an irreversible structure change of airway epithelium, leading to clinical symptoms of asthma (Holgate, 2010).

The human *general transcription factor IIIH subunit 4* (*GTF2H4*), located on chromosome 6p21.33, is a gene that encodes for a subunit of transcription factor II H (TFIIH), which is known to be involved in nucleotide excision repair and RNA transcription (Giglia-Mari *et al.*, 2006). *GTF2H4* is located in the major histocompatibility complex (MHC) region, which contains numerous genes that are involved in immunological functions. Previously, genetic variants of MHC region genes such as *HLA-DRB1*, *DQA1*, *NOTCH4* and *TNXB* have shown significant associations with autoimmune diseases, namely multiple sclerosis (MS), rheumatoid arthritis and asthma (Rioux *et al.*, 2009; Shiina *et al.*, 2004). In addition, several genetic variants in *GTF2H1*, another general transcription factor

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IIH gene, were found to have significant associations with lung cancer (Wu *et al.*, 2009).

Human *GTF2H4* has also been observed to be associated with several other diseases. Recent studies have suggested that chronic inflammation may be related to cervical cancer (Yang *et al.*, 2001; Castle *et al.*, 2001), suggesting that *GTF2H4* genetic variation may lead to cervical cancer via affecting chronic inflammation. Also, a variant in *GTF2H4* has been shown to be significantly associated with MS, an autoimmune disease (Briggs *et al.*, 2010). Based on the important role of *GTF2H4* in immune responses, we hypothesized that the genetic polymorphisms of *GTF2H4* may be associated with obstructive symptoms in allergic diseases and AERD development.

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. A total of 189 subjects were recruited from the Asthma Genome Research Center comprising hospitals of Soonchunhyang, Chungbuk, Chonnam, Chung-Ang and Seoul National Universities. All subjects provided informed consent, and study protocols were approved by the Institutional Review Board of each hospital.

All patients showed a history of dyspnoea and wheezing in the past 12 months and also one of the following: (i) >15% increase in FEV₁ or >12% increase plus 200 mL following inhalation of a short-acting bronchodilator, (ii) <10 mg mL⁻¹ PC₂₀ methacholine and (iii) >20% increase in FEV₁ following 2 weeks of treatment with inhaled steroids and long-acting bronchodilators. Twenty-four common inhalant allergens were used for a skin prick test (Kim *et al.*, 2008). Total IgE was measured with the CAP system (Pharmacia Diagnostics, Uppsala, Sweden), and atopy was defined as having a wheal reaction equal to or greater than histamine or 3 mm in diameter. Oral aspirin challenge (OAC) was performed with increasing doses of aspirin, using methods slightly modified from those described previously (Kim *et al.*, 2010; Nizankowska-Mogilnicka *et al.*, 2007). Aspirin-induced bronchospasms, as reflected by rate (%) of FEV₁ decline, were calculated as the pre-challenge FEV₁ minus the post-challenge FEV₁ divided by the pre-challenge FEV₁. Based on the OAC reactions, subjects were categorized into two groups as follows: 15% or greater decreases in FEV₁ with naso-ocular or cutaneous reactions as AERD case group and <15% decreases in FEV₁ without naso-ocular or cutaneous reactions as aspirin-tolerant asthma (ATA) control group.

Single-nucleotide polymorphism (SNP) genotyping

Among all known SNPs of *GTF2H4* based on Asian population (Japanese and Chinese) database from International HapMap (<http://hapmap.ncbi.nlm.nih.gov/>), five common SNPs were selected based on allele frequency [minor allele frequency (MAF) >0.05] and LD status. In the case of completely tagged SNPs ($r^2 = 1$), we selected one SNP after considering the location (e.g. functionally important SNPs were preferred). Then, these five SNPs were genotyped in 93 AERD and 96 ATA subjects. Genotyping was performed using TaqMan assay (Applied Biosystems, Carlsbad, CA, USA) with assessment of data quality by duplicate DNAs ($n = 10$). SNPs that could not satisfy the following criteria were excluded: (i) a minimum call rate of 95%, (ii) no duplicate error and (iii) Hardy–Weinberg equilibrium of $P > 0.05$. As a result, a total of five SNPs in *GTF2H4* were successfully genotyped.

Statistics

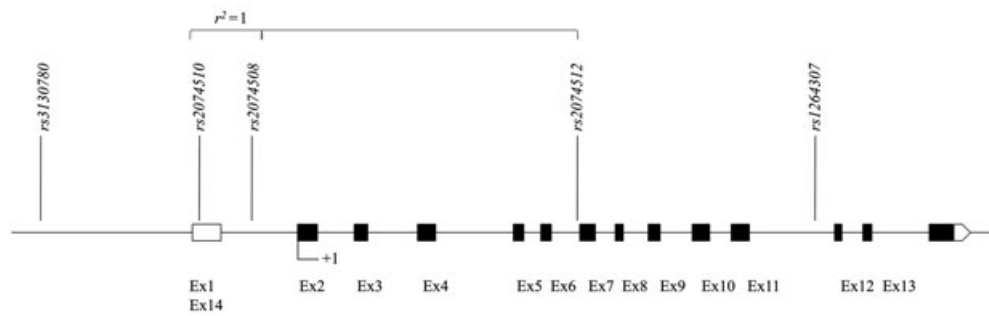
Lewontin's D' (|D'|) and the linkage disequilibrium (LD) coefficient r^2 between all pairs of biallelic loci were used to determine LD among the SNPs. LD was obtained using the HAPLOVIEW v4.2 (Broad Institute, Cambridge, MA, USA) program downloaded from the

Table 1. Clinical profiles of study subjects

Clinical profiles	Aspirin-exacerbated respiratory disease	Aspirin-tolerant asthma	P-value
Number of subjects (<i>n</i>)	93	96	
Sex (<i>n</i> , male/female)	32/61	24/72	0.156
Age [year, mean (range)]	44.39 (17–73)	45.79 (15–77)	0.497
Blood eosinophil (%)	6.29 ± 5.80	4.88 ± 4.19	0.060
PC ₂₀ , methacholine (mg mL ⁻¹)	4.23 ± 7.18	3.04 ± 4.27	0.193
Total IgE (IU mL ⁻¹)	321.65 ± 623.31	309.54 ± 426.04	0.878
FEV ₁ decline by aspirin provocation (%)	23.61 ± 14.48	0.94 ± 2.76	0.001
Ex-smoker/current smoker (%)	15.63/9.38	6.45/12.90	0.219
Positive rate of skin test (%)	61.46	56.99	0.532

Data of blood eosinophil, PC₂₀, total IgE, and FEV₁ decline by aspirin provocation are presented as mean ± SD.

Bold P-value indicates a significant difference between the values of Aspirin-exacerbated respiratory disease and Aspirin-tolerant asthma ($P < 0.05$).

(a) Map of *GTF2H4* (General transcription factor IIIH subunit 4) on chromosome 6p21.3**(b)** Haplotype information of *GTF2H4*

Hap.	rs3130780	rs2074510	rs2074508	rs2074512	rs1264307	Freq.
ht1	G	A	T	G	C	0.513
ht2	G	C	C	A	T	0.281
ht3	T	C	C	A	C	0.120
ht4	G	C	C	A	C	0.067
Others	.	.	.	.	.	0.019
Others are GATGT, TATGC and TCCAT						

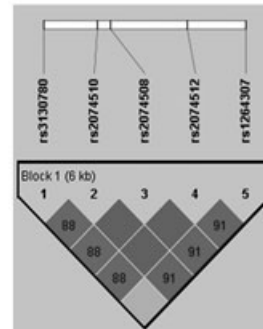
(c) LDs among *GTF2H4* polymorphisms

Figure 1. Physical map, haplotypes, and LD of the *GTF2H4* gene. (a) Schematic gene map and SNPs in the *GTF2H4* gene on chromosome 6p21.3. Black blocks represent coding exons, and white blocks represent 5'- and 3'-untranslated regions. The first base of translation site was denoted as nucleotide +1. (b) Haplotypes of *GTF2H4*. (c) LD coefficient (r^2) among *GTF2H4* SNPs in Korean asthmatics.

Broad Institute (Barrett *et al.*, 2005). Haplotypes were first estimated using PHASE software (Stephens *et al.*, 2001). Logistic analyses for associations were analysed using Statistical Analysis System (SAS) with age (continuous value), gender (male = 0, female = 1), smoking status (nonsmoker = 0, ex-smoker = 1, smoker = 2) and atopy (absence = 0, presence = 1) as covariates. To provide correction of the *P*-value, SNPSpD software (Queensland Institute of Medical Research, Queensland, Australia) that calculates the effective number of independent marker loci (<http://gump.qimr.edu.au/general/daledN/SNPSpD/>) was used. Also, power for genetic association analyses (PGA) software (Menashe *et al.*, 2008) was used to calculate statistical power for each polymorphism.

Results

Characteristics of study subject

A total of 189 asthma patients, including 93 patients with AERD as cases and 96 ATA patients as controls, were recruited for this study. From the clinical data as shown in Table 1, the numbers of female subjects were 2–3 times more than male subjects, which is consistent with previous reports on sex ratio of asthma according to the Global Allergy and Asthma European

Network (GA2LEN). Most importantly, a significant difference between patients with AERD and ATA controls for FEV₁ decline after aspirin challenge was found ($P < 0.001$). Other phenotype data showed that there were no significant differences between AERD and ATA subgroups ($P > 0.05$).

Genotyping, LD and haplotype of *GTF2H4* polymorphisms

For our study, a total of five common SNPs, with MAF over 0.05, were selected from the Asian population database of International HapMap Project. One SNP, *rs2074510*, is located on 5'-untranslated region (UTR) of *GTF2H4*, while other four SNPs are located in intronic regions (Fig. 1a). Furthermore, *rs2074510*, *rs2074508* and *rs2074512* showed complete linkage disequilibrium (LD) ($r^2 = 1$) in Korean asthmatics. From pairwise comparisons analysing LD block and haplotypes, the five SNPs formed one LD block (Fig. 1c) and yielded four major haplotypes with frequencies over 0.05 (Fig. 1b). As haplotype 3 (*GTF2H4*_ht3) was found to be equivalent with *rs3130780*, the haplotype was excluded from further association analysis. All SNPs were successfully genotyped and were in Hardy–Weinberg equilibrium ($P > 0.05$, Table 2).

Table 2. Genotype information of *GTF2H4* SNPs

SNP ID	Allele change	Position	Genotype (n)				MAF	HWE*
<i>rs3130780</i>	G > T	Promoter	GG (143)	GT (36)	TT (4)	Total (183)	0.120	0.344
<i>rs2074510</i>	A > C	5'-UTR	AA (55)	AC (90)	CC (44)	Total (189)	0.471	0.542
<i>rs2074508</i>	T > C	Intron	TT (55)	TC (88)	CC (44)	Total (187)	0.471	0.447
<i>rs2074512</i>	G > A	Intron	GG (55)	GA (89)	AA (45)	Total (189)	0.474	0.445
<i>rs1264307</i>	C > T	Intron	CC (92)	CT (83)	TT (14)	Total (189)	0.294	0.420

**P*-values of deviation from Hardy–Weinberg equilibrium (HWE). MAF, minor allele frequency and UTR, untranslated region. Genotype distribution of each SNP is presented as the number of subjects (FEV₁ decline %, mean ± SE).

Table 3. Association between *GTF2H4* SNPs and AERD

SNP/ haplotype	MAF		Statistical power (%)	Co-dominant			Dominant			Recessive		
	AERD (n = 93)	ATA (n = 96)		OR (95% CI)	<i>P</i>	<i>P</i> ^{cor}	OR (95% CI)	<i>P</i>	<i>P</i> ^{cor}	OR (95% CI)	<i>P</i>	<i>P</i> ^{cor}
<i>rs3130780</i>	0.139	0.102	37.1	1.37 (0.73–2.60)	0.33	0.86	1.35 (0.66–2.78)	0.42	1.00	2.74 (0.27–28.28)	0.4	1.00
<i>rs2074510</i>	0.446	0.495	53.1	0.76 (0.50–1.15)	0.20	0.52	0.91 (0.48–1.73)	0.76	1.00	0.51 (0.25–1.04)	0.07	0.17
<i>rs2074508</i>	0.445	0.495	53.1	0.76 (0.50–1.15)	0.19	0.50	0.87 (0.46–1.67)	0.68	1.00	0.52 (0.26–1.07)	0.08	0.20
<i>rs2074512</i>	0.452	0.495	45.6	0.78 (0.52–1.18)	0.24	0.63	0.91 (0.48–1.73)	0.76	1.00	0.55 (0.27–1.12)	0.1	0.26
<i>rs1264307</i>	0.280	0.307	29.0	0.81 (0.51–1.30)	0.39	1.00	1.04 (0.58–1.86)	0.89	1.00	0.23 (0.06–0.87)	0.03	0.08
<i>GTF2H4_ht1</i>	0.550	0.484	72.9	1.41 (0.92–2.16)	0.11	0.29	2.08 (1.01–4.26)	0.05	0.12	1.23 (0.63–2.41)	0.54	1.00
<i>GTF2H4_ht2</i>	0.272	0.292	17.1	0.86 (0.54–1.37)	0.53	1.00	1.12 (0.63–2.02)	0.70	1.00	0.24 (0.06–0.90)	0.04	0.09
<i>GTF2H4_ht4</i>	0.033	0.094	85.6	0.32 (0.12–0.84)	0.02	0.06	0.32 (0.12–0.87)	0.02	0.07	–	–	–

Haplotype *GTF2H4_ht3* is equivalent to *rs3130780*.

Effective number of corrections for multiple testing = 2.621.

MAF, minor allele frequency; AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; OR, odds ratio; CI, confidence interval.

Bold values indicate significant association between the genetic polymorphism and AERD (*P* < 0.05).

Association analyses of *GTF2H4* polymorphisms with AERD development and FEV₁ decline

To investigate whether genetic variations in *GTF2H4* could be associated with the development of AERD, this study employed logistic regression using three different genetic models (co-dominant, dominant and recessive) in the association analysis (Table 3). Initially, *rs1264307* (OR = 0.23, *P* = 0.03) and *GTF2H4_ht2* (OR = 0.24, *P* = 0.04) showed nominal associations with AERD under recessive model. In addition, haplotype *GTF2H4_ht4* was also marginally associated with the AERD under both co-dominant and dominant models (OR = 0.32, *P* = 0.02). However, significances of *rs1264307* and two haplotypes (*GTF2H4_ht2* and *GTF2H4_ht4*) disappeared after corrections for multiple testing (*P*^{co} > 0.05).

In further regression analyses to investigate possible associations of *GTF2H4* variants with FEV₁ decline by aspirin provocation in patients with AERD or ATA controls, no genetic variants of *GTF2H4* showed significant associations with FEV₁ decline by aspirin provocation in both AERD and ATA groups (*P* > 0.05, Table 4).

Discussion

Asthma is a complex disorder characterized by various phenotypes, with both genetic and environmental predisposition. Moreover, AERD is a rare and severe syndrome that can lead to death despite avoidance of aspirin. It has been generally known that women are 2.5-fold and 2.3-fold more prevalent to asthma and AERD than men respectively, possibly due to differences in gender physiques such as women's hormonal changes (Almqvist *et al.*, 2008; Boschetto *et al.*, 2003). In accordance with previous reports, the overall asthma subjects and patients with AERD in the present study were mostly women, which was about two-fold higher than men, suggesting that sex hormones affect immune responses and female airways.

Transcription factor II H is a part of the pre-initiation complex in the transcription process, and its primary function in the complex is thought to be the promoter opening over the start site (Frit *et al.*, 1999). The protein also forms a multi-protein complex to play an essential role in nucleotide excision repair, which is an important process for fixing DNA damage. Therefore, changes in TFIIH could debilitate DNA

Table 4. Association between *GTF2H4* SNPs and FEV₁ decline by aspirin provocation

	SNP/haplotype	C/C	C/R	R/R	<i>P_a</i>	<i>P_a^{cor.}</i>	<i>P_b</i>	<i>P_b^{cor.}</i>	<i>P_c</i>	<i>P_c^{cor.}</i>
In Aspirin-exacerbated respiratory disease	<i>rs3130780</i>	68 (22.15 ± 13.37)	19 (27.76 ± 18.26)	3 (25.67 ± 16.77)	0.20	0.46	0.14	0.33	0.88	1.00
	<i>rs2074510</i>	27 (21.12 ± 12.57)	49 (24.57 ± 14.93)	17 (24.80 ± 16.31)	0.50	1.00	0.42	0.94	0.82	1.00
	<i>rs2074508</i>	27 (21.12 ± 12.57)	47 (24.78 ± 15.20)	17 (24.80 ± 16.31)	0.49	1.00	0.39	0.89	0.84	1.00
	<i>rs2074512</i>	27 (21.12 ± 12.57)	48 (24.64 ± 15.07)	18 (24.58 ± 15.85)	0.53	1.00	0.42	0.94	0.87	1.00
	<i>rs1264307</i>	44 (24.09 ± 14.56)	46 (23.97 ± 14.52)	3 (11.10 ± 10.38)	0.41	0.93	0.70	1.00	0.13	0.29
	<i>GTF2H4_ht1</i>	16 (25.39 ± 16.66)	49 (24.64 ± 14.95)	25 (21.11 ± 12.98)	0.46	1.04	0.73	1.64	0.43	0.97
	<i>GTF2H4_ht2</i>	44 (24.07 ± 14.56)	43 (24.40 ± 14.92)	3 (11.10 ± 10.38)	0.43	0.97	0.74	1.68	0.12	0.27
	<i>GTF2H4_ht4</i>	84 (23.39 ± 14.90)	6 (29.43 ± 10.42)	–	0.50	1.13	0.50	1.13	–	–
In aspirin-tolerant asthma	<i>rs3130780</i>	75 (0.73 ± 2.75)	17 (1.54 ± 2.78)	1 (3.00)	0.17	0.39	0.19	0.43	0.53	1.00
	<i>rs2074510</i>	28 (0.84 ± 2.36)	41 (1.36 ± 2.75)	27 (0.40 ± 3.14)	0.61	1.00	0.84	1.00	0.28	0.64
	<i>rs2074508</i>	28 (0.84 ± 2.36)	41 (1.36 ± 2.75)	27 (0.40 ± 3.14)	0.61	1.00	0.84	1.00	0.28	0.64
	<i>rs2074512</i>	28 (0.84 ± 2.36)	41 (1.36 ± 2.75)	27 (0.40 ± 3.14)	0.61	1.00	0.84	1.00	0.28	0.64
	<i>rs1264307</i>	48 (1.13 ± 2.54)	37 (1.10 ± 2.63)	11 (–0.48 ± 3.84)	0.17	0.39	0.47	1.00	0.07	0.17
	<i>GTF2H4_ht1</i>	28 (0.53 ± 3.16)	43 (1.33 ± 2.71)	25 (0.71 ± 2.36)	0.79	1.00	0.40	0.90	0.66	1.49
	<i>GTF2H4_ht2</i>	51 (1.17 ± 2.52)	34 (1.04 ± 2.66)	11 (–0.48 ± 3.84)	0.13	0.31	0.36	0.81	0.07	0.17
	<i>GTF2H4_ht4</i>	79 (0.85 ± 2.87)	16 (1.70 ± 1.68)	1 (–4.40)	0.82	1.00	0.47	1.00	–	–

C/C, major homozygote; C/R, heterozygote; R/R, minor homozygote.

Genotype distribution of each SNP is presented as the number of subjects (FEV₁ decline %, mean ± SE).

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

Haplotype *GTF2H4_ht3* is equivalent to *rs3130780*.

Effective number of corrections for multiple testing = 2.621.

damage repair, as evidenced by the significant associations between genetic variants of *GTF2H1* and lung cancer (Wu *et al.*, 2009). Furthermore, it has been suggested that DNA damage in peripheral blood could cause chronic obstructive pulmonary disease (COPD) (Maluf *et al.*, 2007), which is characterized by major symptoms of chronic airway remodelling, lung parenchymal inflammation and expiratory airflow obstruction. These facts suggest that variants in *GTF2H4* could lead to a decreased efficiency of DNA damage repair, which in turn leads to higher prevalence of DNA damage, resulting in airway obstruction in asthma and/or allergic diseases.

Many genes located in the MHC region showed associations with autoimmune diseases. This region includes *HLA-A*, *-B* and *-C*, and *HLA-DP*, *-DQ* and *DR*, which are the most studied and known to carry the most immunological functions (Rioux *et al.*, 2009).

From the study by International MHC and Autoimmunity Genetics Network, SNPs that showed the most significant signals for seven studied diseases, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, rheumatoid arthritis, myasthenia gravis, selective IgA deficiency and MS were found to be located either in HLA genes or in other genes that were in LD with HLA genes. Several genes within MHC region also showed significant associations with asthma. In several papers, *HLA-DPB1*, a gene within MHC region, was found to be associated with AERD as well (Dekker *et al.*, 1997; Kim *et al.*, 2006; Shrestha Palikhe *et al.*, 2012). In a previous study, *HLA-DRB1* locus was found to be associated with two quantitative traits underlying asthma, IgE concentration and IgE responses to allergen (Moffatt *et al.*, 2001). In addi-

tion, a polymorphism in *TNF2* promoter was revealed to be associated with bronchial hyperreactivity in asthma (Li Kam Wa *et al.*, 1999). These recent results also have suggested a possible association of *GTF2H4* variations with asthma and its related phenotypes.

The present study is the first to investigate possible associations of *GTF2H4* variants within the MHC region with obstructive symptoms in asthmatics. From previous studies on *GTF2H4*, its genetic variants have been implicated as an important factor in inflammation mechanisms. The gene variants were previously associated with MS and cervical cancer, diseases that are also related to inflammation function (Castle *et al.*, 2001; Yang *et al.*, 2001; Compston & Coles, 2008). Furthermore, it has been reported that MS in Caucasians might be associated with the *GTF2H4 rs1264307* variant (Briggs *et al.*, 2010). However, results from this study showed that genetic variants in *GTF2H4* might not be associated with AERD development or FEV₁ decline after aspirin challenge in asthmatics.

Although the present study has several limitations, such as a small number of samples, low statistical powers, no inclusion of normal healthy controls and need of further replications in larger cohorts, our preliminary findings suggest that *GTF2H4* might have no association with obstructive diseases in asthma.

Ethical approval

All subjects provided informed consent, and study protocols were approved by the Institutional Review Board of Soonchunhyang, Chungbuk, Chonnam, Chung-Ang and Seoul National Universities.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgements

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