

Genetic variations in *KIFC1* and the risk of aspirin exacerbated respiratory disease in a Korean population: an association analysis

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Received: 30 June 2011 / Accepted: 17 December 2011
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Abstract Modest effects of genes in various pathways are significant in the etiology of complex human diseases, including aspirin exacerbated respiratory disease (AERD). By functioning as a relevant component of respiratory processes, the human *kinesin family member C1* (*KIFC1*) is hypothesized to play a role in AERD pathogenesis. A case–control analysis was carried out by comparing the genotype distribution of six *KIFC1* single-nucleotide polymorphisms between 93 AERD cases and 96 aspirin-tolerant asthma controls in a Korean population. After controlling for confounds, logistic and regression models via various modes of genetic inheritance facilitated the association analysis. Initial results revealed significant association at 0.05 level of significance between several *KIFC1*

variations and AERD ($P = 0.01–0.05$, $OR = 1.81–1.90$) as well as fall rate of forced expiratory volume in the 1st second, an important diagnostic marker of airways constriction ($P = 0.04–0.05$). However, the signals were not deemed significant after multiple testing corrections ($P^{\text{corr}} > 0.05$). Although the results do not support a major role of *KIFC1* in AERD pathogenesis in a Korean asthma cohort, further replication and validation studies are required to clarify the current findings.

Keywords Aspirin exacerbated respiratory disease · FEV_1 · Haplotype · Single nucleotide polymorphism · *KIFC1*

Introduction

Despite the therapeutic effects of aspirin, ingestion of the drug may also induce adverse reactions including aspirin exacerbated respiratory disease (AERD), a condition that is characterized by asthma and nasal polyposis. Although over-production of cysteinyl-leukotrienes (cys-LTs) in the 5-lipoxygenase (5-LO) pathway has been implicated as the main determinant of bronchoconstrictive response to aspirin [1], the observed association between AERD and genes involved in other pathways [2–4] suggest that the exact genetic mechanisms underlying aspirin-induced airway inflammation still remain unclear.

Previous reports have shown association between genes on chromosome 6p21.3 and AERD [2, 3], indicating that the locus harbors protein products that may be relevant in disease pathogenesis. Located on the short arm of chromosome 6p21.3 at the centrometric side of the class II major histocompatibility complex (MHC) region [5] is the human *kinesin family member C1* (*KIFC1*), also referred to

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as *HSET*) gene comprising 11 exons (673 amino acid residues) over a full length of 2,421 base pair (bp) [6]. One of the genes flanking the centrometric end of *KIFC1* [6] downregulates the renin-angiotensin system (RAS), a hormone system that has been observed to be of elevated levels in acute asthma patients [7] and nasal polyps specimen [8].

By transporting membranous organelles and protein complexes in a microtubule- and ATP-dependent approach [9], the kinesin superfamily of proteins (KIFs) extends its function in regulating ciliogenesis. Previous separate studies have observed an abnormal orientation of cilia in patients with primary ciliary dyskinesia (PCD, also referred to as immotile cilia syndrome), an inflammation of the respiratory tract [10], and have implicated genes involved in ciliogenesis with Bardet–Biedl syndrome (BBS), a disease that has been associated with increased incidence of asthma [11]. Furthermore, in a recent study on Korean asthmatics, the current authors detected significant association of variations in *kinesin family member 3A* (*KIF3A*) with AERD ($P = 0.0004$ – 0.05 , $P^{\text{corr}} = 0.004$ – 0.01 depending on the genetic model) and decline rate of forced expiratory volume in the 1st second (FEV_1) [12], the most common parameter used in assessing bronchodilation. In vitro analysis revealed increased expression of *KIF3A* in the bronchial epithelial cell line and nasal polyps epithelia of AERD patients [12]. Although *KIF3A* and *KIFC1* do not have the same expression profiles, both kinesins are considered reverse-direction-oriented motor proteins. The association between *KIF3A* and AERD suggests a possible link between KIFs and aspirin-induced airway bronchospasm.

Unlike other members of the kinesin superfamily, the role of *KIFC1* in human diseases remains highly unknown. However, due to the relevance of the gene in respiratory processes, *KIFC1* is currently hypothesized to be involved in AERD pathophysiology, and a case–control analysis was performed in a Korean asthmatic cohort.

Materials and methods

Study patients

Asthma patients who provided written informed consent to participate in the study were recruited from nine hospitals comprising the Asthma Genome Research Center in Korea. The study protocol was approved by the Institutional Review Board of each participating hospital. Evaluation of asthma and tests measuring total immunoglobulin E (IgE), atopy, and individual reactions to inhalant allergens were carried out as described previously [13]. To identify patients who were aspirin-intolerant, all subjects underwent oral aspirin challenge that was performed with increasing

modifications in doses of aspirin (10–450 mg) according to previous methods [13]. Patients exhibiting either $\geq 20\%$ decrease in FEV_1 or 15–19% decrease in FEV_1 with naso-ocular or cutaneous reactions were categorized as AERD cases, whereas those demonstrating $< 15\%$ decrease in FEV_1 without naso-ocular or cutaneous reactions were classified as aspirin-tolerant asthma (ATA) controls.

SNP selection and genotyping, and haplotype construction

Tagging single-nucleotide polymorphisms (SNPs) in the *KIFC1* gene were screened from the International HapMap database (version: release #27; <http://www.hapmap.org>) based on linkage disequilibrium (LD) status in the Asian population (Chinese Hans and Japanese) and location in the gene. SNPs having $\text{MAF} > 0.05$ and tagging SNPs if several polymorphisms showed high LD (> 0.98) were selected for genotyping. Genotypes were derived using TaqMan assay [14] in the ABI prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA). LD relations between the screened SNPs were evaluated using the Haploview software (Cambridge, MA, USA; <http://www.broad.mit.edu/mpg/haploview>) [15]. Haplotypes were generated using PHASE algorithm ver. 2.0 and those with a frequency > 0.05 were deemed relevant in the study.

Statistical analyses

Lewontin's D' ($|D'|$) and LD coefficient r^2 were used to specify LD relations between all pairs of biallelic loci. To determine the association between *KIFC1* and the risk of AERD, logistic analysis was carried out controlling for age (continuous value), sex (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 confounding variables. Furthermore, differences in FEV_1 decline between AERD and ATA groups were examined using regression model with adjusted age, sex, smoking status, atopy, and BMI. Statistical analysis was performed using the Statistical Analysis System (SAS) version 9.1 (SAS Inc., Cary, NC), statistical power of single associations was determined using the Power for Genetic Association Analyses (PGA) software [16], and multiple testing corrections was calculated using the effective number of independent marker loci (M_{eff}) that accounts for the eigenvalue spectral decomposition of all the genotypes represented in the correlation matrix [17] and was extracted from the SNPSpD program. Furthermore, in silico analyses of the SNPs located in the 5' untranslated region (UTR) and introns were carried out using the UTRScan program and the European Molecular

Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) splice site prediction software (<http://www.ebi.ac.uk/asd-srv/wb.cgi?method=2>), respectively.

Results

Clinical characteristics of the study patients

Based on individual reaction to the oral aspirin challenge, the overall asthma patients ($n = 189$) were stratified into 93 AERD cases (males = 32, females = 61) and 96 ATA controls (males = 24, females = 72; Table 1). From the clinical profiles of the study subjects, fall rate of FEV₁ by aspirin provocation (AERD = 23.61 vs. ATA = 0.94), positive rate of aspirin intolerance (AERD = 26.67% vs. ATA = 8.42%), and positive rate of nasal polyps (AERD = 63.86% vs. ATA = 29.27%) are observed to be significantly prevalent in AERD patients ($P = 0.001$, Table 1). No significant differences between the cases and controls were observed for other clinical factors. The demographics and clinical characteristics of the study patients are summarized in Table 1.

Distribution of *KIFC1* variants

With high rates of concordance in duplicates (>99%), six *KIFC1* SNPs were successfully genotyped to facilitate comparison (Table 2; Fig. 1a). Except for *rs211462* and *rs211457* in intron 1, most of the analyzed variants were common in a Korean population (minor allele frequency (MAF) > 0.05; Table 2). A tight LD (Fig. 1b) was inferred from pairwise comparisons of the genotyped polymorphisms, with the exception of two rare variants, *rs211462* and *rs211457* due to its low MAF. The two relevant

haplotypes, *KIFC1_h1* and *KIFC1_h2*, with MAF > 0.05 were found to be equivalent to *rs465223* (which is in LD with *rs465506*) and *rs376006* (which is in LD with *rs456993*), respectively (Fig. 1c), and therefore, association analysis was not necessary.

Association analysis of *KIFC1* variants with aspirin-induced airways inflammation and in silico analysis

Table 3 evince that four *KIFC1* SNPs, *rs456993*, *rs465506*, *rs465223* and *rs376006*, initially showed significant associations with the risk of AERD using various modes of genetic inheritance ($P = 0.01$ – 0.05 , OR = 1.81–1.90). However, the signals were reduced to nominal evidence of association ($P^{\text{corr}} > 0.05$; Table 3) after multiple testing corrections was calculated with $M_{\text{eff}} = 4.9097$.

Differences in the distribution of *KIFC1* SNPs with respect to fall rate of FEV₁ between AERD patients and ATA controls were further assessed. Similarly, the significant association signals initially detected in three *KIFC1* polymorphisms, *rs456993*, *rs211457* and *rs376006* ($P = 0.04$ – 0.05 depending on the genetic model; Table 4) disappeared after multiple comparisons ($P^{\text{corr}} > 0.05$).

Discussion

Although the role of *KIFC1* in human diseases has not yet been elucidated, the gene is hypothesized to influence an individuals' susceptibility to AERD by exerting important influences on bronchial functions, including ciliogenesis. In the current study, initial analysis on the differences between the genotype frequency distribution of *KIFC1* polymorphisms revealed statistically significant association between

Table 1 Clinical profiles of the study patients ($n = 189$)

Clinical profile	AERD	ATA	<i>P</i> value
Number of subjects (<i>n</i>)	93	96	
Age [year, mean (range)]	44.39 (17–73)	45.79 (15–77)	0.497
Onset age [year, mean (range)]	38.01 (0–70)	37.99 (5–73)	0.995
Sex (male/female)	32/61	24/72	0.156
BMI (kg/m ²)	23.47 ± 3.18	24.41 ± 3.29	0.049
Ex-smoker/current smoker (%)	15.63/9.38	6.45/12.90	0.219
Blood eosinophil (%)	6.29 ± 5.80	4.88 ± 4.19	0.060
Predicted FVC (%)	89.90 ± 14.74	87.76 ± 12.80	0.293
Predicted FEV ₁ (%)	86.63 ± 16.74	88.26 ± 17.04	0.509
PC ₂₀ methacholine (mg/ml)	4.23 ± 7.18	3.04 ± 4.27	0.193
Total IgE (IU/ml)	321.65 ± 623.31	309.54 ± 426.04	0.878
Fall rate of FEV ₁ by aspirin provocation	23.61 ± 14.48	0.94 ± 2.76	0.001
Positive rate of skin test (%)	61.46	56.99	0.532
Positive rate of aspirin intolerance (%)	26.67	8.42	0.001
Positive rate of nasal polyp (%)	63.86	29.27	0.001

Values are mean ± SE. BMI body mass index, AERD aspirin exacerbated respiratory disease, ATA aspirin-tolerant asthma

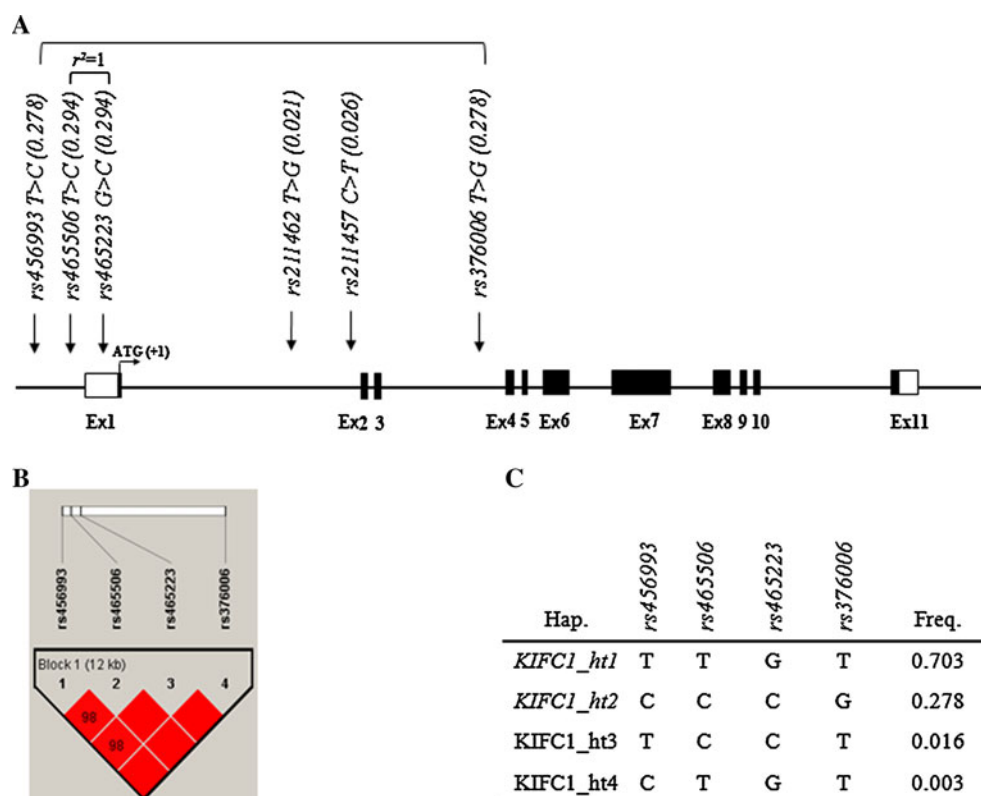
Table 2 Genotype and allele distribution of *KIFC1* variants

SNP	Allele	Position	Genotype				Heterozygosity	MAF
<i>rs456993</i>	T>C	5' near gene	T	CT	C	N	0.402	0.278
			98	71	16	185		
<i>rs465506</i>	T>C	5' near gene	T	CT	C	N	0.415	0.294
			92	80	15	187		
<i>rs465223</i>	G>C	5' UTR	G	CG	C	N	0.415	0.294
			93	81	15	189		
<i>rs211462</i>	T>G	Intron 1	T	GT	G	N	0.042	0.021
			180	8	0	188		
<i>rs211457</i>	C>T	Intron 1	C	CT	T	N	0.052	0.026
			180	8	1	189		
<i>rs376006</i>	T>G	Intron 3	T	GT	G	N	0.401	0.278
			99	75	15	189		

MAF minor allele frequency

Fig. 1 Physical map, LD, and haplotypes of the *KIFC1* gene.

a Schematic gene map and SNPs of *KIFC1* on chromosome 6p21.3 (18.04 kb). Black blocks represent coding exons and white blocks represent 5' and 3' UTR. The first base of translation site is denoted as nucleotide +1. SNPs in absolute linkage are indicated by brackets ($r^2 = 1$). **b** LD coefficient (D') among *KIFC1* SNPs in a Korean population. UTR untranslated region. **c** Haplotypes of *KIFC1*



several gene variants and the risk of AERD as well as fall rate of FEV₁ via co-dominant and dominant mechanisms. For most of the polymorphisms, patients who were homozygous for the rare allele (R/R genotype) were more prone to airways obstruction compared to subjects having other genotypes (C/C and C/R). However, the association signals did not withstand multiple testing corrections, suggesting that the tested *KIFC1* polymorphisms may not be correlated with aspirin-induced airways inflammation in a Korean population.

Despite the current findings, data from ATA patients in Koreans showed that *rs211457* in intron 1 deviated from Hardy–Weinberg equilibrium (HWE), suggesting error in genotyping or lack of ATA subjects having the genotype. This study is the first to investigate the relationship between *KIFC1* and aspirin-related lung function abnormalities.

From the genotyped polymorphisms, *rs465223* is located at the 5' UTR of the gene. Although the association signal was not deemed significant after corrections,

Table 3 Association analysis of *KIFC1* SNPs with AERD

SNP	MAF	HWE*		Co-dominant		Dominant		Recessive		Statistical power
		AERD	ATA	OR (95% CI)	P**	P ^{corr***}	OR (95% CI)	P**	P ^{corr***}	
<i>rs456993</i>	0.337	0.224	0.674	0.914	0.02	NS	1.90 (1.05–3.45)	0.04	NS	32.70
<i>rs465506</i>	0.352	0.240	0.565	0.775	0.01	NS	2.04 (1.13–3.70)	0.02	NS	33.71
<i>rs465223</i>	0.349	0.240	0.536	0.775	0.01	NS	2.01 (1.12–3.63)	0.02	NS	34.03
<i>rs211462</i>	0.016	0.026	0.874	0.793	0.42	–	0.54 (0.12–2.42)	0.42	–	7.60
<i>rs211457</i>	0.016	0.036	0.874	0.011	0.18	–	0.42 (0.10–1.79)	0.24	–	6.92
<i>rs376006</i>	0.333	0.224	0.876	0.914	0.02	NS	1.98 (1.10–3.56)	0.02	NS	33.10

Co-dominant, dominant and recessive models of logistic analysis controlling for age, sex, smoking status, atopy and BMI

OR odds ratios, CI confidence interval, NS not significant

* P values of Hardy–Weinberg equilibrium (HWE), ** P values at 0.05 levels of significance, *** P values after multiple testing corrections. Significant values are shown in bold

rs465223 was found to be marginally correlated with the risk of AERD at 0.05 level of significance. Variants in the UTRs have been implicated in gene expression by mediating translational control and stability [18], thus playing crucial roles in protein synthesis. Although little has been known about the 5' end of *KIFC1*, a previous study has reported a base substitution from G → C within the 5' UTR of the gene in a patient with PCD [6]. However, the effect of the substitution on gene expression has not been analyzed. In an attempt to predict the function of *rs465223*, in silico analysis was carried out using a program that employs a collection of sequences and regulatory motifs of the untranslated regions of eukaryotic mRNAs. Although findings from UTRScan analysis revealed that *rs465223* in the 5' UTR of *KIFC1* may not be within the sequence length where regulatory elements bind with the gene, further in vitro or ex vivo functional studies would provide more meaningful functional characterization of the variant.

Furthermore, increasing evidence suggest that variants in both coding and non-coding regions can affect splicing of the gene transcript. Refuting previous reports that intronic polymorphisms have no role in protein synthesis, recent research reveal that these variations function in exon skipping and altering the balance of the alternatively spliced isoforms that are produced, thereby causing disease phenotypes [19, 20]. In the current study, *KIFC1 rs376006* in intron 1 consistently showed weak association signals with the risk of AERD and FEV₁ decline using various modes of genetic inheritance. In silico analysis was performed to analyze the potential function of the SNP. Although results of analysis in silico revealed that none of the analyzed intronic variants are potential branch point sites for alternative splicing, validation of the functions of these intronic SNPs are warranted.

In the current study, common variations were used to determine the genetic factors of AERD. To date, researchers are considering the importance of rare variants in disease pathogenesis, giving rise to Next Generation Sequencing with one-base ultra-resolution and high-density chip microarrays. In addition, aside from investigating the genetic factors of AERD susceptibility, epigenetic analyses of methylation patterns between tissues of normal and asthmatic targets would be helpful in identifying the pathogenesis of AERD. Furthermore, the possibility that other polymorphisms within *KIFC1* or variations of flanking genes may be responsible for the marginal association effect cannot be ruled out. Indeed, the 5' of the *synaptic ras-GTPase-activating protein (SynGAP)* gene is localized in the centrometric end of *KIFC1* [6]. Although not related to the kinesin superfamily, *SynGAP* may play a role in diseases of the lower and upper airways by downregulating RAS. Also, since the effects of polymorphisms differ according to race and/or geographical location, the present results may not

Table 4 Regression analysis of *KIFC1* SNPs with FEV₁ decline by aspirin provocation

SNP	C/C	C/R	R/R	<i>P</i> ^{a,*}	<i>P</i> ^{COF a,**}	<i>P</i> ^{b,*}	<i>P</i> ^{COF b,**}	<i>P</i> ^{c,*}	<i>P</i> ^{COF c,**}
<i>rs456993</i>	98 (9.90 ± 13.76)	71 (13.62 ± 17.54)	16 (16.53 ± 14.32)	0.05	NS	0.07	–	0.17	–
<i>rs465506</i>	92 (9.85 ± 14.10)	80 (13.91 ± 16.91)	15 (15.17 ± 13.71)	0.06	–	0.06	–	0.39	–
<i>rs465223</i>	93 (9.97 ± 14.07)	81 (13.96 ± 16.81)	15 (15.17 ± 13.71)	0.06	–	0.06	–	0.40	–
<i>rs211462</i>	180 (12.39 ± 15.60)	8 (4.21 ± 4.97)	–	0.12	–	0.12	–	–	–
<i>rs211457</i>	180 (12.53 ± 15.56)	8 (4.21 ± 4.97)	1 (–4.40)	0.04	NS	0.05	NS	0.18	–
<i>rs376006</i>	99 (10.01 ± 13.73)	75 (14.23 ± 17.35)	15 (15.17 ± 13.71)	0.06	–	0.05	NS	0.40	–

^a Co-dominant, ^b dominant and ^c recessive models of regression analysis controlling for age, sex, smoking status, atopy and BMI. C/C, C/R, and R/R refer to major homozygote, heterozygote, and minor homozygote, respectively

NS not significant

* *P* values at 0.05 levels of significance. ** *P* values after multiple testing corrections. Significant values are shown in bold

hold true for other populations [21]. After performing power calculations of single associations, the average statistical power to detect effect sizes with the current sample is at 24.68% (Table 3), suggesting insufficient sample size. In order to address these limitations and identify the exact role of *KIFC1* in respiratory diseases, further replications using larger sample size are warranted.

To conclude, the current study failed to demonstrate convincing associations between *KIFC1* genetic variations and the risk of AERD as well as decrease in FEV₁ in a Korean population. However, due to the small sample size and observed deviation of an SNP from HWE in a Korean population, findings from this study need to be replicated in larger cohorts to determine the exact association between *KIFC1* and aspirin-induced airways inflammation.

Acknowledgments This work was supported by Korea Science and Engineering Foundation (KOSEF) funded by the Korea government (MEST) (No. 2009-0080157). This study was supported by a grant of the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A010249). The DNA samples were generously provided by Soonchunhyang University, Bucheon Hospital Biobank, a member of the National Biobank of Korea, supported by the Ministry of Health, Welfare and Family Affairs, Republic of Korea.

Conflict of interest The authors report no competing interests.

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