

***KIF3A*, a Cilia Structural Gene on Chromosome 5q31, and Its Polymorphisms Show an Association with Aspirin Hypersensitivity in Asthma**

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Received: 18 July 2010 / Accepted: 7 September 2010
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Abstract

Introduction The *kinesin family number 3A (KIF3A)* gene on the human chromosomal 5q31–33 region, which is known as a susceptibility locus for immune diseases including asthma, plays a crucial role in generation of cilia.

Results A treatment with aspirin in the human bronchial epithelial cells increased the mRNA expression level of *KIF3A* compared to that of the untreated control ($P \leq 0.01$), and nasal polyp epithelia from aspirin-intolerant asthma (AIA) patients also showed a higher expression of *KIF3A* protein than aspirin-tolerant asthma controls. Further

logistic analyses revealed that most polymorphisms of *KIF3A* were significantly associated with AIA ($P = 0.0004\text{--}0.02$; $P^{\text{corr}} = 0.004\text{--}0.04$) and the decline of forced expiratory volume at 1 s (FEV₁)% by aspirin provocation ($P = 0.004\text{--}0.04$; $P^{\text{corr}} = 0.03$).

Discussion Our findings suggest that the *KIF3A* gene and/or its polymorphisms might have a susceptibility effect on AIA, providing a new step toward controlling aspirin intolerance in asthmatics.

Keywords Aspirin-intolerant asthma · *KIF3A* · single nucleotide polymorphism (SNP) · haplotype

Jeong-Hyun Kim and Ji-Yeon Cha contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s10875-010-9462-x) contains supplementary material, which is available to authorized users.

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Introduction

Aspirin, also known as acetyl salicylic acid (ASA), is a common medicine used to relieve pain and inflammation. Prevalence of aspirin hypersensitivity is about 0.6–2.5% in the general populations, whereas up to 20% of asthmatics are sensitive to aspirin [1, 2]. Aspirin-intolerant asthma (AIA) is a distinct clinical syndrome associated with the upper and lower airway eosinophil infiltration. AIA was first described in 1922 [3, 4], and its clinical characteristics are popularized as the aspirin triad: aspirin hypersensitivity, bronchial asthma, and chronic rhinosinusitis with nasal polyposis. Generally, AIA begins at about 30 years of age and occurs more frequently in women, approximately two third of the total cases, than in men [4]. Furthermore, the inhibition of leukotriene pathways by leukotriene receptor antagonists has been known as a first-line medication for the long-term AIA management [5, 6]. Although knowledge on bronchoconstriction among aspirin-intolerant asthmatics has been attributed to sensitivity to aspirin and other nonsteroidal anti-inflammatory drugs, the pathogenesis of AIA has not been elucidated completely.

Much attention has been focused on the relation of motile cilia in the airway epithelia and respiratory disease [7, 8]. In the case of Bardet–Biedl syndrome, which has been correlated with an increased incidence of asthma (25–28%), abnormal functions of cilia have been discovered [8]. The abnormal ciliary structure and/or function has also been found in primary ciliary dyskinesia, a condition characterized by disease of the respiratory tract [7]. In addition, leukotriene, especially leukotriene D₄, has been shown to stimulate ciliary activity in human [9]. The *kinesin family number 3A* (*KIF3A*) gene on the chromosomal region 5q31 encodes a motor subunit of kinesin-2, an important component for cilia formation, and plays a crucial role in generation and mechanism of cilia [10, 11]. Moreover, a different expression of *KIF3A* in nasal epithelial cells between exacerbated asthmatics and normal controls and a significant association of *KIF3A* polymorphisms with asthma have been suggested by a previous report [12].

A large number of studies have implicated that the human chromosomal region 5q31–33, which contains cytokine gene cluster and immune-related genes, is important in the development of inflammatory diseases including asthma in several populations [13–15]. Furthermore, functional analyses of the *interleukin (IL)-12B* and *DCNP1* genes on chromosome 5q31–33 have elucidated the associations between polymorphisms and haplotypes of the genes and asthma [16–18]. However, the exact and comprehensive mechanisms underlying the pathology of aspirin hypersensitivity in asthma remain unclear.

To investigate the genetic role of the *KIF3A* gene, which is positioned on chromosome 5q31, in the development of

aspirin hypersensitivity in asthma, we utilized in vitro assays functional experiments and performed association analyses of the genetic polymorphisms with the risk of AIA.

Methods

Cell Culture and Aspirin Treatment

Human bronchial epithelial cell line (BEAS2B) was obtained from ATCC (Rockville, MD, USA). The cells were maintained with Dulbecco's modified Eagle's medium/F-12 nutrient mixture Han (WelGENE Inc., Korea) containing 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) at 37°C in 5% CO₂ incubator. Cells were treated with 0.2 mM aspirin for 4 and 12 h incubation. The cells were washed with serum-free media, added with 0.25% trypsin and 0.03% EDTA solution, and were allowed to sit at room temperature until the cells began to be detached. After dispersing with fresh medium, the cells were obtained by centrifugation at 1,200 rpm for 5 min at 4°C and were washed twice in fresh medium.

Reverse Transcriptase Polymerase Chain Reaction

Total RNA was extracted using TRI reagent (Molecular Research Center, Inc., USA) and chloroform according to the manufacturer's instructions and treated with RNase-free DNase-I (Stratagene, USA). First-strand cDNA synthesis was performed using SuperScript® II Reverse Transcriptase (Invitrogen, USA) according to the manufacturer's instructions. The cDNA amplification for human *KIF3A* and *beta-actin* genes was performed using specific primer pairs (5'-ATAGTTCCCGTTCCCATG-3' and 5'-CTGACCCACTGATATCAGAG-3' for *KIF3A*; 5'-GGACTTCGAGCAAGAGATGG-3' and 5'-AGCACTGTGTTGGCGTACAG-3' for *β-actin*) under the following PCR condition: 95°C for 5 min; 28 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min; and 72°C for 10 min and electrophoresed on 1% agarose gel with visualization by ethidium bromide stain.

Immunohistochemistry

For immunostaining, the VectaStain mouse ABC Elite kit (Vector Laboratories, Burlingame, CA, USA) was used. Serial 4-µm tissue sections were obtained, and deparaffinization was performed. Endogenous peroxides were blocked with 1.4% H₂O₂ in methanol for 30 min, and non-specific binding was blocked by adding 1.5% normal goat serum for 30 min. The sections were incubated with the *KIF3A* antibody (1:100, BD Transduction Laboratories™) at 4°C. After washing with Tris-buffered saline, the sections were

incubated sequentially with biotinylated goat anti-rabbit immunoglobulin G (H + L; 1:200; Vector Laboratories) for 30 min. The color reaction was developed using 3,3'-diaminobenzidine tetrachloride (Zymed Laboratories Inc., South San Francisco, CA, USA), and Harris hematoxylin was applied as the counter stain. The sections were then rinsed with distilled water, treated with 1% acetic acid for 2 min, dehydrated through alcohols, cleared in xylene, and mounted using Synthetic Mountant. Photomicrographs were made using a nikon microscope (Nikon, Japan).

Study Subjects

A total of 371 asthma patients were recruited from the Asthma Genome Research Center comprising hospitals of Soonchunhyang, Chungbuk, Chunnam, Yonsei, Hanlym, and Seoul National Universities in Korea. Ethical approvals were obtained from the institutional review board of each hospital. All of the subjects were Korean whose physical examination results exhibited clinical symptoms compatible with asthma, based on the Global Initiative for Asthma guidelines [13]. All patients showed a history of dyspnea and wheezing during the previous 12 months, plus one of the followings: (1) >15% increase in FEV₁ or >12% increase plus 200 ml following inhalation of a short-acting bronchodilator, (2) <10 mg/ml PC20 methacholine, and (3) >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, and total IgE was measured using the CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Oral aspirin challenge (OAC) was performed in all asthmatics with increasing doses of aspirin using methods that were slightly modified from those described previously [19, 20]. Aspirin-induced bronchospasms, as presented by rate (%) of FEV₁ decline, were calculated as scores of the pre-challenge FEV₁ minus the post-challenge FEV₁ divided by the pre-challenge FEV₁. The AIA and aspirin-tolerant asthma (ATA) groups were classified depending on the OAC reactions as follows: Subjects with 15% or greater decreases in FEV₁ or appearance of naso-ocular or cutaneous reactions were designated as the AIA group, and those with less than 15% decreases in FEV₁ without naso-ocular or cutaneous reactions were the ATA group. The clinical parameters are summarized in Table 1.

Single Nucleotide Polymorphism Selection and Genotyping

Genomic DNA was isolated from the peripheral blood of patients using the Wizard genomic DNA purification kit (Promega, USA). A total of 22 common single nucleotide polymorphisms (SNPs) based on the frequencies in Asian population from International HapMap Project ([\[map.ncbi.nlm.nih.gov/index.html.en\]\(http://map.ncbi.nlm.nih.gov/index.html.en\)\) were selected for genotyping. The SNPs were genotyped in 103 AIA patients and 268 ATA controls using TaqMan assay in the ABI-PRISM 7900HT sequence detection system \(Applied Biosystems, USA\) with the assessment of data quality by duplicate DNAs \(\$n=10\$ \). Genotyped data were obtained using the ABI-PRISM sequence detection system software version 2.3.](http://hap</p>
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Statistics

The association of each SNP and haplotypes in the *KIF3A* gene with AIA was determined by logistic analyses adjusted for age, gender, smoking status, atopy, and body mass index as covariates using the Statistical Analysis System (SAS). The *Haploview* v4.1 software downloaded from the Broad Institute (<http://www.broadinstitute.org/mpg/haploview>) was used to determine linkage disequilibrium (LD) of SNPs in the *KIF3A* gene [21]. Lewontin's D' ($|D'|$) and LD coefficient r^2 between all pairs of biallelic loci were examined to measure linkage disequilibrium [22]. After haplotypes were estimated using PHASE algorithm (ver. 2.0) [23], association analyses were carried out using SAS for the associations with AIA. Regression analyses for associations of SNPs and haplotypes with the rate (percent) of FEV₁ decline by aspirin provocation were using SAS. Significant associations were indicated by a P value < 0.05.

Results

Increased Expression of *KIF3A* in the Aspirin-Induced Bronchial Epithelial Cells

To investigate whether the expression of *KIF3A* is modulated in the bronchial epithelia, the mRNA and protein levels of *KIF3A* were measured. Interestingly, the mRNA expression of the *KIF3A* gene following treatment with aspirin (0.2 mM) in the transformed human bronchial epithelial cells (BEAS-2B) showed a significant increase in treatment with aspirin at both of 4 and 12 h (Fig. 1a; $P \leq 0.05$, two-tailed t test). Furthermore, immunohistochemical assay in nasal polyp epithelia from patients also exhibited an increase in the expression of *KIF3A* protein of AIA patients compared to that of ATA controls (Fig. 1b), indicating that expressions of *KIF3A* might be increased in aspirin-induced bronchial epithelial cells.

Characteristics of Study Subject

We further investigated a possible association between genetic polymorphisms of *KIF3A* and aspirin hypersensitivity in asthma. Clinical and demographic characteristics of 103 AIA patients and 268 ATA controls in this study are

Table 1 Clinical profiles of study subjects ($n=371$)

Clinical profile	AIA	ATA	<i>P</i>
Subjects (<i>n</i>)	103	268	
Age of onset [year, mean (range)]	43.2 (19.0–73.0)	47.8 (15.4–79.1)	0.004
Sex (male/female)	36:67	87:181	–
BMI (kg/m^2)	23.68 ± 3.38	24.56 ± 3.52	–
Decline of FEV_1 by aspirin provocation (%)	34.04 ± 13.71	3.48 ± 4.52	<0.0001
Log [blood eosinophil (%)]	0.77 ± 0.31	0.74 ± 0.33	–
FEV_1 , predicted (%)	84.67 ± 16.95	89.75 ± 18.46	0.01
FVC, predicted (%)	89.76 ± 12.41	87.83 ± 14.90	–
Log [PC20, methacholine (mg/ml)]	0.43 ± 0.42	0.46 ± 0.31	–
Log [total IgE (IU/ml)]	2.27 ± 0.56	2.17 ± 0.62	–
Current smoker (%)	19.42	33.21	0.005
Positive rate of skin test (%)	47.57	56.34	–

AIA aspirin-intolerant asthma,
ATA aspirin-tolerant asthma,
BMI body mass index, FEV_1
forced expiratory volume at 1 s,
FVC forced vital capacity

summarized in Table 1. AIA cases included 36 males (35.0%) and 67 females (65.0%) with a mean age of 43.2, and ATA controls included 87 males (32.5%) and 181 females (67.5%) with a mean age of 47.8. Most importantly, AIA patients showed about 10-fold increase in the rate of FEV_1 decline by aspirin provocation compared to that of ATA controls ($P<0.0001$; Table 1), showing that AIA phenotype appears well supported and distinct from ATA. In addition, the predicted FEV_1 , age of onset, and smoking status in AIA patients were statistically lower than those in ATA controls ($P\leq 0.01$).

Association of *KIF3A* Polymorphisms with the Risk of AIA

Due to infrequent SNPs in the coding regions of *KIF3A* depending on public database, a total of 22 common polymorphisms in introns were genotyped in the study subjects (Fig. 2a and Supplementary Table 1). The genotype distributions of all loci were in Hardy–Weinberg equilibrium ($P>0.05$, Supplementary Table 1). Pair-wise comparisons among 22 SNPs showed one tight LD block, and haplotypes were inferred using *PHASE* software (Fig. 2b, c). All of the SNPs and the five haplotypes with

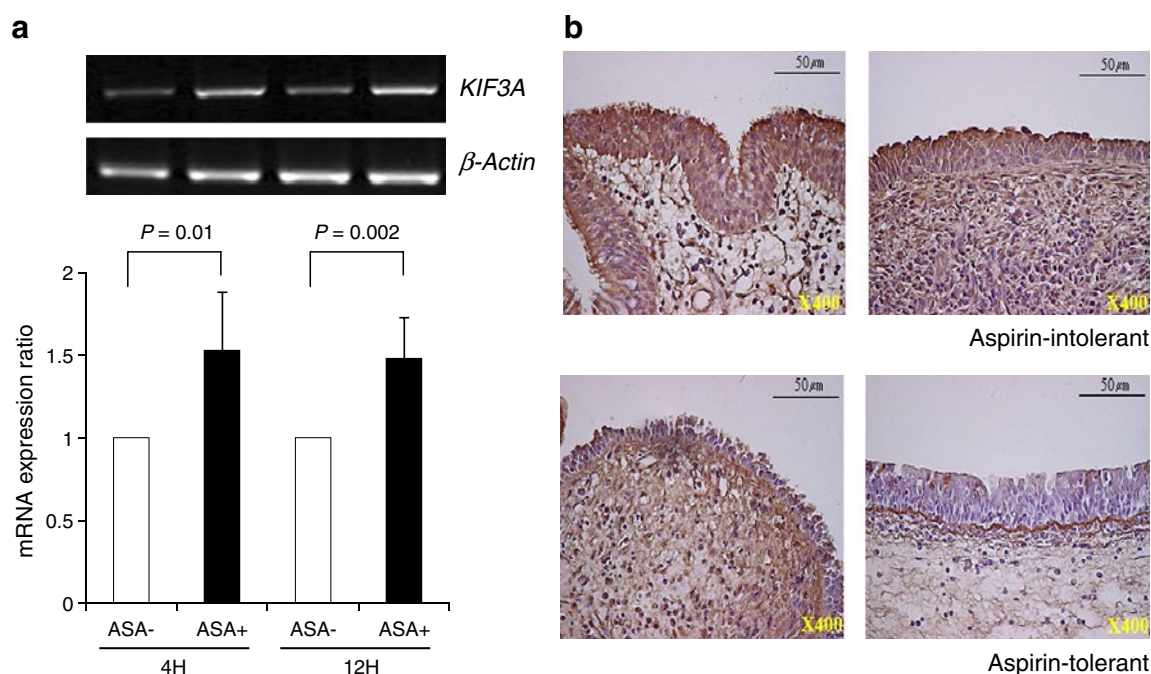


Fig. 1 Expressions of *KIF3A*. **a** RT-PCR. Significant difference of mRNA expression of *KIF3A* is found between aspirin untreated (ASA–) and treated (ASA+) human bronchial epithelial cells (BEAS-2B; $n=5$, $P<0.05$). **b** Immunohistochemistry. Higher protein

expression of *KIF3A* in nasal polyp epithelia from aspirin-intolerant asthmatics was shown compared to aspirin-tolerant asthmatics ($n=4$). Statistical significance was calculated by two-tailed *t* tests. All data are expressed as mean $\pm$ SEM. ASA acetylsalicylic acid

Table 2 Logistic analysis of *KIF3A* polymorphisms with the risk of AIA

SNP/haplotype	SNP ID	Position	MAF		Co-dominant		Dominant		Recessive	
			AIA	ATA	OR (95% CI)	P	P ^{corr}	OR (95% CI)	P	P ^{corr}
+2779C>G	rs7732667	Intron 1	0.309	0.228	1.65 (1.15–2.38)	0.007	0.06	1.52 (0.94–2.47)	0.09	0.78
+9552A>T	rs3798129	Intron 2	0.296	0.222	1.64 (1.13–2.38)	0.009	0.08	1.58 (0.97–2.56)	0.07	0.58
+16965G>T	rs2074529	Intron 5	0.189	0.140	1.53 (1.00–2.33)	0.05	0.46	1.36 (0.81–2.28)	0.25	–
+22420A>G	rs17166215	Intron 8	0.306	0.221	1.71 (1.18–2.47)	0.005	0.04	1.55 (0.96–2.51)	0.07	0.66
+23857C>T	rs4266392	Intron 8	0.189	0.146	1.48 (0.97–2.26)	0.07	0.63	1.30 (0.77–2.18)	0.33	–
+23996G>A	rs1080001	Intron 8	0.301	0.216	1.68 (1.17–2.43)	0.006	0.05	1.52 (0.94–2.47)	0.09	0.77
+26323C>G	rs11740584	Intron 8	0.186	0.145	1.43 (0.93–2.19)	0.10	0.88	1.23 (0.73–2.07)	0.44	–
+26522A>G	rs7737031	Intron 9	0.301	0.219	1.67 (1.16–2.41)	0.006	0.06	1.50 (0.93–2.43)	0.10	0.88
+28374C>G	rs2299006	Intron 9	0.304	0.223	1.66 (1.15–2.41)	0.007	0.06	1.48 (0.91–2.40)	0.12	–
+29464T>C	rs12517135	Intron 9	0.306	0.223	1.69 (1.17–2.44)	0.005	0.05	1.52 (0.94–2.46)	0.09	0.79
+30080C>T	rs2299007	Intron 9	0.476	0.375	1.65 (1.17–2.32)	0.005	0.04	1.44 (0.87–2.38)	0.16	–
+30372G>A	rs2299010	Intron 9	0.471	0.369	1.64 (1.16–2.31)	0.005	0.05	1.40 (0.85–2.32)	0.19	–
+30570C>G	rs2299011	Intron 9	0.301	0.220	1.66 (1.15–2.40)	0.007	0.06	1.49 (0.92–2.41)	0.11	0.95
+30966A>G	rs3798130	Intron 9	0.304	0.226	1.62 (1.12–2.34)	0.01	0.09	1.46 (0.90–2.38)	0.12	–
+32117G>C	rs17166203	Intron 9	0.301	0.222	1.65 (1.14–2.38)	0.008	0.07	1.46 (0.90–2.37)	0.12	–
+33043T>C	rs12186803	Intron 9	0.301	0.216	1.68 (1.16–2.43)	0.006	0.05	1.51 (0.93–2.45)	0.09	0.82
+33980G>T	rs2285700	Intron 10	0.184	0.142	1.46 (0.96–2.24)	0.08	0.71	1.28 (0.76–2.15)	0.36	–
+34913G>A	rs3213639	Intron 12	0.189	0.144	1.49 (0.97–2.28)	0.07	0.59	1.31 (0.78–2.20)	0.31	–
+36545G>T	rs3756755	Intron 13	0.121	0.075	2.10 (1.17–3.76)	0.01	0.12	2.17 (1.19–3.93)	0.01	0.10
+36860G>A	rs1468216	Intron 14	0.121	0.076	2.02 (1.13–3.61)	0.02	0.16	2.08 (1.15–3.76)	0.02	0.13
+39500A>G	rs2237059	Intron 16	0.307	0.219	1.74 (1.20–2.53)	0.004	0.03	1.57 (0.97–2.57)	0.07	0.61
+40614G>A	rs17623617	Intron 16	0.102	0.107	0.89 (0.51–1.58)	0.70	–	0.85 (0.47–1.55)	0.60	–
<i>KIF3A_h1l</i>			0.417	0.491	0.70 (0.51–0.98)	0.04	0.34	0.52 (0.32–0.87)	0.01	0.11
<i>KIF3A_h12</i>			0.155	0.144	1.03 (0.65–1.63)	0.90	–	0.93 (0.55–1.58)	0.79	–
<i>KIF3A_h13</i>			0.160	0.123	1.39 (0.89–2.16)	0.15	–	1.13 (0.65–1.96)	0.66	–
<i>KIF3A_h14</i>			0.092	0.103	0.84 (0.47–1.50)	0.55	–	0.79 (0.43–1.47)	0.46	–
<i>KIF3A_h15</i>			0.083	0.045	2.11 (1.06–4.17)	0.03	0.29	2.22 (1.10–4.50)	0.03	0.24

P values were adjusted for age of onset, sex, atopy, smoking status, and body mass index

AIA aspirin-intolerant asthma, ATA aspirin-tolerant asthma, MAF minor allele frequency, OR odds ratio, CI confidence interval

Table III Regression analysis of *KIF3A* polymorphisms with the decline of FEV₁% after injection of aspirin

SNP/ Haplotype	SNP ID	C/C		C/R		R/R		Co-dominant		Dominant		Recessive	
		<i>n</i>	Decline of FEV ₁ (%)	<i>n</i>	Decline of FEV ₁ (%)	<i>n</i>	Decline of FEV ₁ (%)	<i>P</i>	<i>P</i> ^{corr}	<i>P</i>	<i>P</i> ^{corr}	<i>P</i>	<i>P</i> ^{corr}
+2779C>G	rs7732667	213	10.65±15.12	124	12.57±16.95	30	16.27±15.81	0.02	0.20	0.05	0.48	0.06	0.55
+9552A>T	rs3798129	217	10.56±15.05	123	13.05±16.96	28	15.72±16.20	0.02	0.15	0.03	0.28	0.09	0.78
+16965G>T	rs2074529	268	11.48±15.78	87	11.56±15.51	13	19.59±18.37	0.18	–	0.39	–	0.07	0.60
+22420A>G	rs17166215	215	10.58±15.26	124	12.69±16.67	28	17.27±15.84	0.01	0.10	0.03	0.29	0.03	0.31
+23857C>T	rs4266392	264	11.57±15.88	90	11.34±15.30	13	19.59±18.37	0.21	–	0.45	–	0.07	0.61
+23996G>A	rs1080001	218	10.55±15.19	120	12.82±16.87	28	17.27±15.84	0.01	0.09	0.03	0.28	0.03	0.31
+26323C>G	rs11740584	264	11.57±15.76	88	11.22±15.49	13	19.59±18.37	0.25	–	0.54	–	0.06	0.57
+26522A>G	rs7737031	216	10.67±15.21	121	12.72±16.84	28	17.27±15.84	0.01	0.11	0.04	0.33	0.04	0.32
+28374C>G	rs2299006	214	10.64±15.26	124	12.48±16.72	28	17.27±15.84	0.01	0.13	0.05	0.41	0.03	0.30
+29464T>C	rs12517135	214	10.63±15.27	125	12.55±16.67	28	17.27±15.84	0.01	0.12	0.04	0.37	0.03	0.31
+30080C>T	rs2299007	131	10.85±15.82	178	11.66±16.03	59	14.24±15.32	0.14	–	0.30	–	0.15	–
+30372G>A	rs2299010	134	10.78±15.72	175	11.69±16.12	58	14.48±15.33	0.12	–	0.29	–	0.13	–
+30570C>G	rs2299011	215	10.69±15.24	121	12.64±16.90	28	17.27±15.84	0.01	0.13	0.04	0.40	0.04	0.32
+30966A>G	rs3798130	212	10.64±15.19	123	12.62±16.72	29	16.52±16.07	0.02	0.16	0.04	0.38	0.06	0.50
+32117G>C	rs17166203	216	10.67±15.21	124	12.49±16.72	28	17.27±15.84	0.02	0.14	0.05	0.43	0.03	0.30
+33043T>C	rs12186803	219	10.54±15.15	121	12.76±16.81	28	17.27±15.84	0.01	0.10	0.03	0.30	0.03	0.30
+33980G>T	rs2285700	268	11.55±15.78	87	11.33±15.52	13	19.59±18.37	0.21	–	0.47	–	0.07	0.60
+34913G>A	rs3213639	265	11.55±15.85	89	11.39±15.38	13	19.59±18.37	0.21	–	0.45	–	0.07	0.61
+36545G>T	rs3756755	304	10.80±15.31	63	16.58±17.64	1	10.00	0.004	0.03	0.004	0.03	0.79	–
+36860G>A	rs1468216	303	10.92±15.52	64	15.91±16.89	1	10.00	0.01	0.11	0.01	0.10	0.79	–
+39500A>G	rs2237059	214	10.37±15.00	121	12.76±16.82	28	17.27±15.84	0.006	0.05	0.02	0.17	0.03	0.24
+40614G>A	rs17623617	291	11.69±15.85	74	12.15±15.70	2	19.50±28.99	0.79	–	0.86	–	0.57	–
<i>KIF3A</i> _{ht1}		108	13.36±15.39	174	11.19±16.18	86	11.01±15.74	0.19	–	0.16	–	0.45	–
<i>KIF3A</i> _{ht2}		270	12.32±16.44	89	10.32±14.15	9	10.27±13.36	0.20	–	0.17	–	0.76	–
<i>KIF3A</i> _{ht3}		282	11.61±15.66	74	10.85±15.95	12	21.70±17.46	0.23	–	0.62	–	0.02	0.22
<i>KIF3A</i> _{ht4}		296	11.73±15.83	70	11.81±15.77	2	19.50±28.99	0.92	–	1.00	–	0.56	–
<i>KIF3A</i> _{ht5}		328	11.07±15.44	39	17.82±18.16	1	10.00	0.01	0.13	0.01	0.11	0.79	–

P values were adjusted for age of onset, sex, atopy, smoking status, and body mass index. C/C, C/R, and R/R indicate the homozygote of the common allele and the heterozygote and homozygote of the minor allele, respectively. Decline of FEV₁ represents mean ± SE value

AIA aspirin-intolerant asthma, ATA aspirin-tolerant asthma

predicted as a putative branch point adenosine with a score of 3.66 and 3.61, respectively (Supplementary Fig. 1).

Discussion

To our knowledge, this is the first study to investigate the association between *KIF3A*, as a ciliary structure gene, and AIA. In this study, results from logistic and regression analyses revealed significant associations of *KIF3A* polymorphisms that have a tight LD with AIA ($P=0.0004$ – 0.02 ; $P^{\text{corr}}=0.004$ – 0.04) and associations with the decline of FEV₁ by aspirin provocation ($P=0.004$ – 0.04 ; $P^{\text{corr}}=0.03$). Furthermore, in vitro analysis showed higher mRNA expression of *KIF3A* in the aspirin-induced bronchial

epithelial cells and protein expression in nasal polyp epithelia in AIA patients. Therefore, our findings suggest that *KIF3A* might play an important role in the development of aspirin hypersensitivity in asthmatics.

Motor cilia, which cover the respiratory epithelium, play a critical role in efficient clearance of the lower respiratory tract. Abnormalities of cilia predispose to recurrent respiratory tract and pulmonary infections, leading to respiratory diseases such bronchiectasis [24, 25]. Moreover, primary ciliary dyskinesia, also known as immotile cilia syndrome, and Bardet–Biedl syndrome, which is caused by mutations in ciliary functional genes and provides predisposition for asthma, have been implicated to be relevant in dysmotile and/or immotile cilia [8, 26]. On the other hand, leukotriene D₄, a potent bronchoconstrictor in asthma, has been

clarified to affect ciliary activity and orientation of cilia even in humans [9]. The regulation in production of critical mediators, such as leukotrienes, has been known as an important factor for the susceptibility to AIA [4, 27]. These facts provide a possibility that *KIF3A*, as an important subunit for cilia formation, affect susceptibility to AIA.

According to many studies, chromosome region 5q31–33, which contains a cluster of cytokine genes, is a susceptibility locus for allergy and asthma-related traits [14, 28, 29]. Inspired by the relation between ciliary activity and respiratory epithelium, we identified a new association between aspirin hypersensitivity in asthma and the *KIF3A* gene, which is located on chromosome 5q31. More recently, the *protocadherin-1* (*PCDH1*), as a structural gene for cell adhesion, on chromosome 5q31–33 has been identified as a novel susceptibility gene for bronchial hyperresponsiveness [30]. In addition, it has been recently reported that *KIF3A* polymorphisms potentially contribute to tuberculosis susceptibility, supporting the role of this gene in immune disorders [31]. Therefore, our findings that common SNPs of *KIF3A* are significantly associated with AIA and increase of the aspirin-induced bronchospasms, with in vitro evidential effects of *KIF3A* on respiratory epithelia, provide a new insight to the role of *KIF3A* in asthma-related traits.

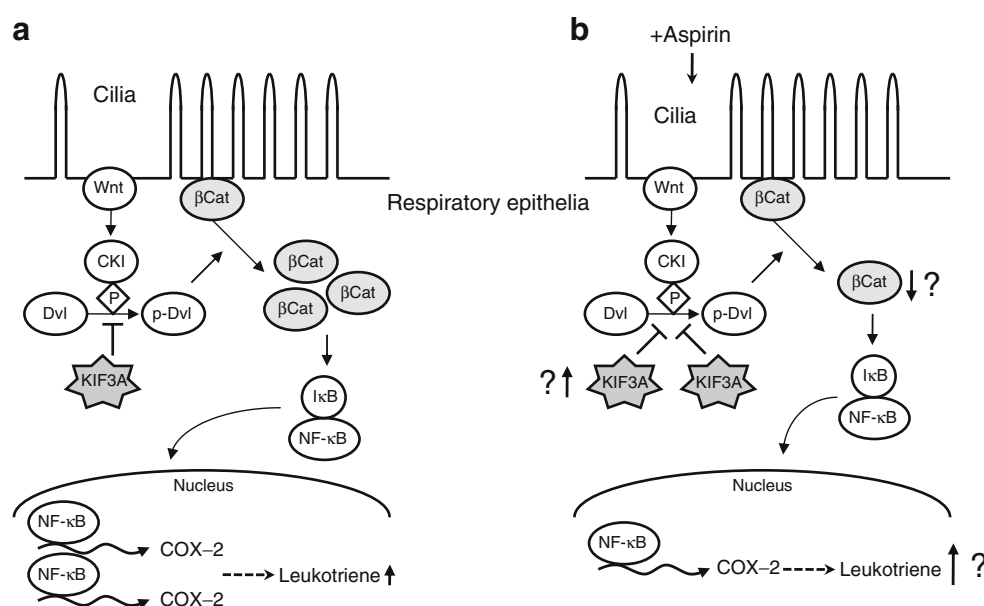
Linkage disequilibrium, as a nonrandom association, provides a profound evidence for association with quantitative characters and complex diseases [32]. To find other potential genes in nearby regions of *KIF3A*, additional analysis of LD near *KIF3A* in Asian populations (Chinese and Japanese) from the International HapMap Project was performed. Interestingly, the *IL-4* gene emerged to have a strong LD with *KIF3A* in the same LD block (Supplementary Fig. 2). Many studies have implicated that *IL-4* and its

polymorphisms are associated with total serum IgE levels and asthma [33, 34]. Furthermore, basophils, which are involved in aspirin-exacerbated respiratory disease, have been implicated to respond to IgE-dependent stimulation by secreting IL-4 [35, 36]. Recently, IL-4-induced overexpression of cysteinyl leukotriene (CysLT) has been shown to be blocked through signal transducer and activator of transcription 6 (STAT6)-mediated transcriptional inhibition [37]. Considering these results, variations in the *KIF3A* gene, which is in LD with *IL-4*, may affect the inhibition of IL-4-STAT6, resulting to failed aspirin desensitization.

Given that *KIF3A* polymorphisms have no direct effects on ciliary structure or activity, another explanation for association between *KIF3A* and aspirin hypersensitivity in asthma is suggested to be related with the over-production of CysLTs by cyclooxygenase (COX) pathway down-regulation, which consequently diverts arachidonic acid into prostaglandins, through the constraint of β -catenin-dependent Wnt signaling derived from abnormality of *KIF3A* (Fig. 3) [4, 11, 38]. Based on in vitro studies, the stabilization of β -catenin in the cilia cells from *Kif3a*^{−/−} mice have been suggested [11]. This stabilization of β -catenin may lead to NF- κ B activation, which also plays a crucial role in immune responses, resulting in the activation of target effectors, such as COX-2 [39]. In addition, significant decreases of NF- κ B and COX-2 in nasal polyps from AIA patients compared to ATA controls have been observed previously [38]. Based on these evidences, therefore, the blockage of β -catenin stabilization by the increase of *KIF3A*, which was shown in this study, may inhibit COX pathway and consequently increase CysLTs productions.

In the present study, regression analysis adjusted for age, gender, atopy, and smoking status as covariates identified

Fig. 3 A possible model for association between *KIF3A* and aspirin-intolerant asthma. **a** In normal conditions, a moderate amount of β -catenin is stabilized. **b** In aspirin-induced or aspirin-intolerant conditions, the increased *KIF3A* represses the phosphorylation of Dvl by CKI, leading to a break to the stabilization of β -catenin, which inhibits the activation of NF- κ B. This inactivation of NF- κ B results in decreases of inflammatory factors, such as COX-2. The decrease of COX-2 diverts to increase of CysLTs productions. CKI casein kinase I, Dvl disheveled, p-Dvl phosphorylated Dvl, β Cat β -catenin



that most of the common SNPs discovered in this study showed more significant associations with aspirin-intolerant asthma in the co-dominant and recessive models, with the highest significant signal detected in the recessive model, compared to the dominant model (Table II). This finding suggests that the heterozygote might have a similar functional activity of the KIF3A phenotype to common homozygote. In other words, only minor homozygotes of each SNP may have an effect on the mean value of aspirin hypersensitivity, as supported by the increased decline of FEV₁% in minor homozygote (R/R; Table III). However, heterozygotes of rs3756755 and rs1468216 appear to have its specific phenotypic mean value, with a small number of minor homozygote.

Alternative splicing is an important process for the regulation of gene expression, leading to different protein isoforms. Through the use of alternative splicing database, it has been possible to predict alternative splice events on genome-wide scale [40]. In this study, rs3798129 and rs3798130 were predicted as putative branch point sites for alternative splicing using an EMBL-EBI splice site prediction tool (<http://www.ebi.ac.uk/asd-srv/wb.cgi?method=2>), which calculates the position and score of branch point. About ten alternative splice variants of *KIF3A* have been detected from the Ensembl Genome Browser (<http://www.ensembl.org/index.html>). Moreover, given that several KIF3A isoforms derived from alternative splicing events in intron 9 have been observed, it is possible that the rs3798130 in intron 9 may produce dysfunctional products in respiratory epithelia, resulting in increased expression of KIF3A, as shown in the present study. Further functional studies are needed to determine whether these putative alternative splice variants may be related to aspirin hypersensitivity in asthmatics. In addition, insufficient sample size of AIA patients serves as the limitation of this study. However, recruitment of AIA patients was completed at about 6 years due to the rareness of the condition.

Conclusions

Considering the in vitro results and the significant association between *KIF3A* polymorphisms and AIA, our findings could provide supporting information for aspirin-induced hypersensitivity and/or asthma. Although further functional evaluations and future replications in independent study cohorts are needed, our preliminary results suggest that *KIF3A* might be a susceptible genetic factor to AIA, with a possibility that *KIF3A* polymorphisms could produce splicing variants, resulting in a higher gene expression.

Acknowledgments This work was supported by a grant from the Korea Health 21 R&D Project (A010249) and by grant number M1-

0302-00-0073 from the Korea Science and Engineering Foundation, funded by the Korean government (Ministry of Education, Science and Technology; No. 2009-0080157), and a Priority Research Centers Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2009-0093822). The DNA and nasal polyp samples were generously provided by the 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.

Conflicts of Interest No conflict of interest declared.

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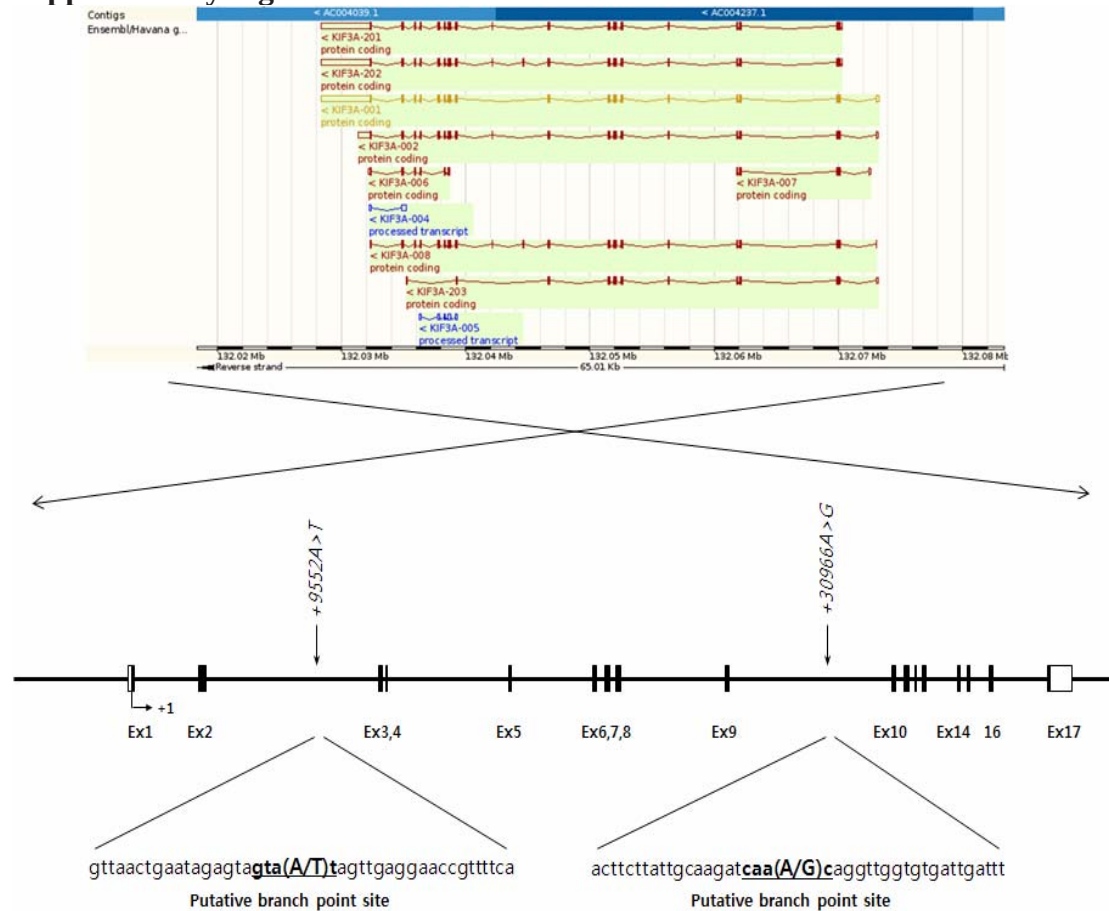
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Supplementary Table 1 Polymorphisms of *KIF3A* investigated in this study

SNP position	SNP ID	Position	Genotype (n)			MAF	Heterozygosity	HWE
+2779C>G	rs7732667	Intron 1	GG	CG	CC	0.250	0.375	0.057
			215	125	30			
+9552A>T	rs3798129	Intron 2	TT	AT	AA	0.243	0.367	0.081
			219	124	28			
+16965G>T	rs2074529	Intron 5	GG	GT	TT	0.154	0.260	0.090
			270	88	13			
+22420A>G	rs17166215	Intron 8	AA	AG	GG	0.245	0.370	0.099
			217	125	28			
+23857C>T	rs4266392	Intron 8	CC	CT	TT	0.158	0.266	0.143
			266	91	13			
+23996G>A	rs1080001	Intron 8	GG	AG	AA	0.240	0.365	0.053
			220	121	28			
+26323C>G	rs11740584	Intron 8	GG	CG	CC	0.156	0.264	0.112
			266	89	13			
+26522A>G	rs7737031	Intron 9	AA	AG	GG	0.242	0.367	0.066
			218	122	28			
+28374C>G	rs2299006	Intron 9	GG	CG	CC	0.245	0.370	0.103
			216	125	28			
+29464T>C	rs12517135	Intron 9	TT	CT	CC	0.246	0.371	0.115
			216	126	28			
+30080C>T	rs2299007	Intron 9	CC	CT	TT	0.403	0.481	0.958
			132	179	60			
+30372G>A	rs2299010	Intron 9	GG	AG	AA	0.397	0.479	0.897
			135	176	59			
+30570C>G	rs2299011	Intron 9	GG	CG	CC	0.243	0.367	0.068
			217	122	28			
+30966A>G	rs3798130	Intron 9	AA	AG	GG	0.248	0.373	0.072
			214	124	29			
+32117G>C	rs17166203	Intron 9	CC	CG	GG	0.244	0.369	0.095
			218	125	28			
+33043T>C	rs12186803	Intron 9	TT	CT	CC	0.240	0.365	0.058
			221	122	28			
+33980G>T	rs2285700	Intron 10	GG	GT	TT	0.154	0.260	0.090
			270	88	13			
+34913G>A	rs3213639	Intron 12	GG	AG	AA	0.157	0.264	0.124
			267	90	13			
+36545G>T	rs3756755	Intron 13	GG	GT	TT	0.088	0.160	0.230
			307	63	1			
+36860G>A	rs1468216	Intron 14	GG	AG	AA	0.089	0.162	0.215
			306	64	1			
+39500A>G	rs2237059	Intron 16	AA	AG	GG	0.243	0.368	0.071
			216	122	28			
+40614G>A	rs17623617	Intron 16	GG	AG	AA	0.105	0.189	0.245
			294	74	2			

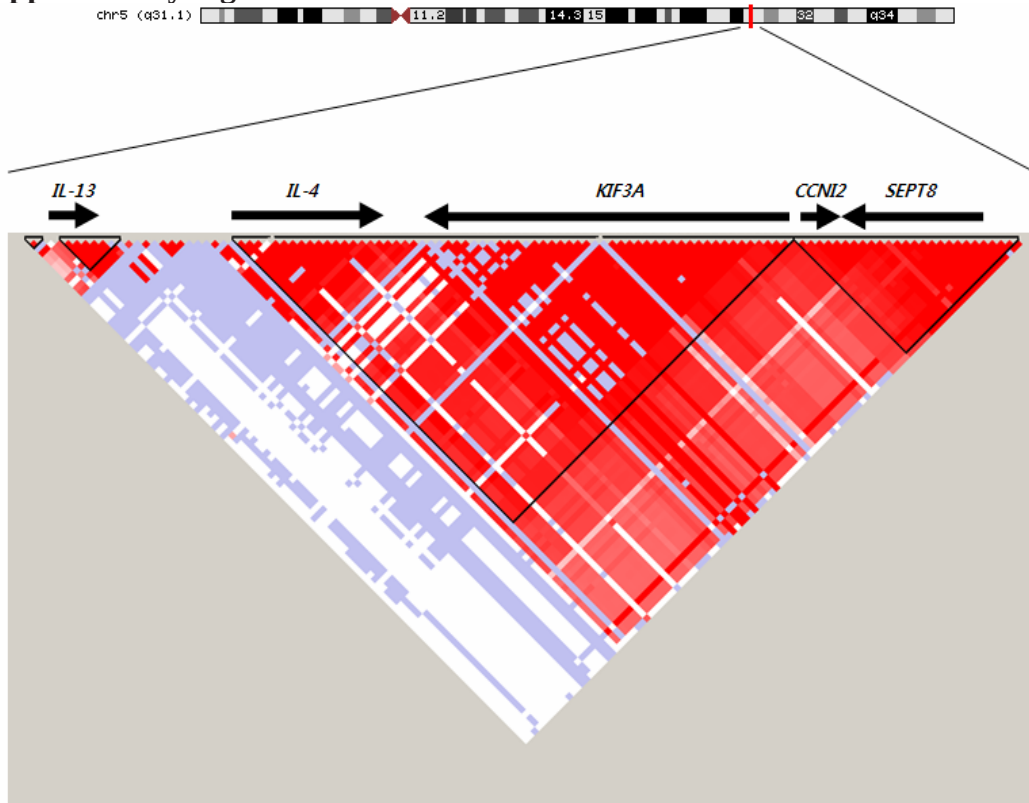
The first base of the translation start site (ATG) is denoted as nucleotide +1.
MAF, Minor allele frequency; HWE, Hardy-Weinberg equilibrium

Supplementary Figure 1



Supplementary Figure 1 Prediction of branch point. Two SNPs, +9552A/T (rs3798129) and +30966A/G (rs3798130), are predicted as a possible branch point adenosine with a score of 3.66 and 3.61, respectively, using an EMBL-EBI splice site prediction tool (<http://www.ebi.ac.uk/asd-srv/wb.cgi?method=2>).

Supplementary Figure 2



Supplementary Figure 2 LD plot nearby *KIF3A*. The LD near *KIF3A* in Asian populations (Japanese and Chinese) is analyzed from the International HapMap Project (<http://hapmap.ncbi.nlm.nih.gov/>). LD coefficient (D') among SNPs of *IL-13*, *IL-4*, *KIF3A*, *CCNI2*, and *SEPT8* genes in Asian populations. The *IL-4* gene has a LD with *KIF3A* in the same LD block.