Human Immunology xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Association of single nucleotide polymorphisms on Interleukin 17 receptor A (*IL17RA*) gene with aspirin hypersensitivity in asthmatics

Jong Sook Park [a,1], Byung-Lae Park [b,1], Myung-ok Kim [a], Jeong-Seok Heo [a], Ji-Sun Jung [a], Da-Jeong Bae [a], Soo Taek Uh [c], Mi-Kyeong Kim [d], Inseon S. Choi [e], Sang Heon Cho [f], Chein-Soo Hong [g], Jae-Young Lee [h], Byoung Whui Choi [i], Hyoung Doo Shin [b,j,*], Choon-Sik Park [a,*]

[a] *Genome Research Center for Allergy and Respiratory Diseases, Soonchunhyang University Bucheon Hospital, 1174 Jung Dong, Wonmi Ku, Bucheon, Gyeonggi Do 420-767, Republic of Korea*
[b] *Department of Genetic Epidemiology, SNP Genetics, Inc., 14th Floor, Woorim Lions B/D, 371-28 Kasan Dong, Geumcheon Ku, Seoul 110-834, Republic of Korea*
[c] *Division of Allergy and Respiratory Medicine, Soonchunhyang University Seoul Hospital, 657-58, Hannam-dong, Yongsan-gu, Seoul 140-743, Republic of Korea*
[d] *Department of Internal Medicine, Chungbuk National University, College of Medicine, 62 Gaesin-dong, Heungduk-gu, Cheongju, Chungcheongbuk-do 361-711, Republic of Korea*
[e] *Department of Allergy, Chonnam National University Medical School and Research Institute of Medical Sciences, 8 Hakdong, Dong-gu, Gwangju 501-757, Republic of Korea*
[f] *Department of Internal Medicine and Institute of Allergy and Clinical Immunology, Seoul National University College of Medicine, 28 Yongon-Dong, Chongno-Ku, Seoul 110-744, Republic of Korea*
[g] *Division of Allergy and Immunology, Institute of Allergy, Department of Internal Medicine, Yonsei University College of Medicine, 250 Sungsan-ro, Seodaemun-gu, Seoul 120-752, Republic of Korea*
[h] *Pulmonary, Allergy & Critical Care Medicine, Hallym University College of Medicine, 39 Hallymdaehak-gil, Chuncheon, Gangwon-do 200-702, Republic of Korea*
[i] *Division of Pulmonology and Allergy, Department of Internal Medicine, Chung-Ang University Yongsan Hospital, 65-207 Hangang-Ro 3 Ga, Yongsan-Gu, Seoul 140-757, Republic of Korea*
[j] *Department of Life Science, Sogang University, 1 Shinsu-dong, Mapo-gu, Seoul 121-742, Republic of Korea*

ARTICLE INFO

Article history:
Received 21 March 2012
Accepted 15 November 2012
Available online xxxx

ABSTRACT

Aim: To investigate the association of single nucleotide polymorphisms (SNP) on *IL17RA* gene with Aspirin Exacerbated Respiratory Disease (AERD) and the functional effect of these variants on expression of *IL17RA* gene products.
Material & methods: 15 SNPs of *IL17RA* gene were analyzed in 825 normal controls and 143 subjects with AERD and 411 with aspirin-tolerant asthma (ATA) and functionally characterized using measurement of protein and m-RNA expression.
Result: Minor alleles frequencies of the three SNPs (*−1075 A > G, −947 A > G, −50 C > T)* and one haplotype (*BL1_ht1*) were significantly lower in AERD compared to those in ATA (p^{corr} = 0.002–0.03). IL17RA protein expression and mRNA amount in $CD14^+$ peripheral blood monocytes and mononuclear cells were significantly increased in subjects carrying the common alleles homozygote compared with those carrying the minor alleles.
Conclusions: The minor alleles of the three SNPs may decrease the risk of AERD via attenuation of *IL17RA* gene expression.
© 2012 American Society for Histocompatibility and Immunogenetics All rights reserved.

1. Introduction

Aspirin Exacerbated Respiratory Disease (AERD) refers to the development of nonallergic hypersensitivity reactions in asthmatics following the ingestion of aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) [1]. The syndrome is characterized by aspirin hypersensitivity, bronchial asthma, nasal polyposis, and hyperplastic eosinophilic sinusitis [2]. Although the pathogen-

Abbreviations: AERD, aspirin exacerbated respiratory disease; ATA, aspirin tolerant asthma; ASA, acetyl salicylic acid; LD, linkage disequilibrium; IL-17RA, interleukin 17 receptor A; IL-17, interleukin 17; COX, cyclooxygenase; LOX, lipooxygenase; Cys-LT, cysteinyl leukotriene; OAC, oral aspirin challenge; HWE, Hardy–Weinberg equilibrium; FEV_1, forced expiratory volume in one second; Ht, haplotype; LD, linkage disequilibrium; ONPG, ortho-nitrophenyl-D-galactopyranoside; PC20, provocative concentration of 20 total in FEV_1; SNP, single-nucleotide polymorphism.

* Corresponding authors. Addresses: Division of Allergy and Respiratory Medicine, Department of Internal Medicine Soonchunhyang University Bucheon Hospital, 1174, Jung Dong, Wonmi Ku, Bucheon, Gyeonggi Do 420-021, Republic of Korea (Choon-Sik Park), Department of Life Science, Sogang University, 1 Shinsu-dong, Mapo-gu, Seoul 121-742, Republic of Korea. Fax: +82 2 2026 4299 (H.D. Shin), fax: +82 32 621 5023 (C.-S. Park).
E-mail addresses: hdshin@sogang.ac.kr (H.D. Shin), mdcspark@unitel.co.kr (C.-S. Park).
[1] These authors equally contributed to this work as the coauthors.

0198-8859/$36.00 - see front matter © 2012 American Society for Histocompatibility and Immunogenetics All rights reserved.
http://dx.doi.org/10.1016/j.humimm.2012.11.002

Please cite this article in press as: Park JS et al. Association of single nucleotide polymorphisms on Interleukin 17 receptor A (*IL17RA*) gene with aspirin hypersensitivity in asthmatics. Hum Immunol (2012), http://dx.doi.org/10.1016/j.humimm.2012.11.002

UNCORRECTED PROOF

esis of AERD remains unknown, a two-compartment model has been proposed involving increased cysteinyl leukotriene (CysLT) production and CysLT receptor over-expression in inflammatory cells of the respiratory tract [3,4]. Several studies have investigated the genetic background of aspirin intolerance whereby polymorphisms for genes encoding LTC4S [5], ALOX5 [6], CYSLTR1 [7], CYSLTR2 [8] and TBXA2R [9] were found to be associated with an increased risk of AERD. The CysLT receptor is selectively antagonized by several leukotriene modifiers, including montelukast, pranlukast, and zafirlukast [10]. However, clinical studies have demonstrated that the response to these drugs is limited [11,12], suggesting the presence of an alternative pathway leading to AERD.

As in other asthmatics, the airways of AERD patients show extensive cellular inflammation, with marked eosinophilia, cytokine over-production, and up-regulation of inflammatory molecules [13]. Cytokines are signaling molecules with known pro-inflammatory functions and are important in the pathogenesis of asthma. However, there are few studies examining the genetic susceptibility of other inflammatory mediators and molecules in AERD [14,15]. Several members of the recently described *IL-17* cytokine family (*IL-17A, IL-17F, IL-17E*) have been shown to participate in inflammatory cell recruitment as well as in the amplification of an allergic response [16], and a specific type of T cell, capable of producing *IL-17A* and *IL-17F*, has been described [17]. These cytokines exert an effect via the interleukin 17A receptors A–C [18]. Interleukin 17 receptor A, IL-17RA, is a ubiquitous type I membrane glycoprotein that binds with low affinity to interleukin 17A. The IL-17RA gene is constitutively expressed in several human cell types, such as the lung epithelial cells, B cells, and myelomonocytic cells [18,19]. Activation of p38 mitogen-activated protein kinase (p38 MAPK) by IL-17A results in up-regulation of inducible nitric oxide synthase and COX-2 genes [20]. Activation of *IL-17RA* and *IL-17RB* induce the activation of JNK, p38 MAPK, and NF-κB [21], which regulate the expression of genes responsible for the inflammatory and remodeling processes in asthma.

Considering the biological effects of *IL-17RA* and *B*, genetic variants of these receptors may play a role in the development of asthma and its subphenotypes. We previously reported that a Single nucleotide polymorphisms in the *IL-17RB* gene was associated with the development of asthma [22]. However, to our knowledge, there is no previous report on analysis of any potential association between polymorphisms in *IL-17RA* and asthma or AERD. We, thus, screened a Korean population for SNPs in the *IL17RA* gene and performed statistical analysis to examine the relationship between the polymorphic loci of *IL-17RA* and asthma or AERD. Additionally, the functional effects of the SNPs were validated *in vitro*.

2. Material and methods

2.1. Subjects

The subjects, who were all Korean, were recruited from the Asthma Genome Research Center, which involves eight tertiary university hospitals in Korea. All patients were diagnosed with asthma according to the Global Initiative for asthma (GINA) guidelines [23]. All patients had a history of dyspnea and wheezing during the previous 12 months, plus one of the following: (1) >15% increase in FEV_1 or >12% increase plus 200 mL following inhalation of a short-acting bronchodilator, (2) <10 mg/mL PC20 methacholine, and (3) >20% increase in FEV_1 following 2 weeks of treatment with inhaled steroids and long-acting bronchodilators. Normal controls were recruited from among the spouses of the patients and from members of the general population who responded negatively to a screening questionnaire for respiratory symptoms and had an FEV1 >75% of the predicted value, a PC_{20} metacholine >10 mg/ml, and normal findings on a simple chest radiograph. Twenty-four common inhalant allergens were used for skin prick tests. Atopy was defined as a wheal reaction equal to or greater than histamine or at least 3 mm in diameter. Total IgE was measured using the CAP system (Pharmacia Diagnostics, Uppsala, Sweden). The patients with asthma had not experienced exacerbations of asthma or respiratory tract infections during the 6 weeks preceding the oral aspirin challenge (OAC). The OAC was carried out by using increasing doses of aspirin [24,25]. Briefly, patients with a history of aspirin hypersensitivity were given 30 mg orally. Respiratory and nasal symptoms, blood pressure, external signs (urticaria and angio-oedema) and FEV_1 were documented every 30 min for a period of 1.5 h. In the absence of any symptom or sign suggesting an adverse reaction after 1.5 h, increasing dosages of aspirin (60, 100, 300, and 400 mg) were administered until the patient developed the reaction and the same measurements were repeated every 1 h. Those having no history were started on 100 mg of aspirin and gradually increased the dosage of aspirin as 200, 350, and 450 mg until the patient developed the reaction. If no reaction occurred 4 h after the final dose, the test was deemed negative. Aspirin-induced bronchospasm, reflected by a decline (%) in FEV_1, were calculated as the pre-challenge FEV_1 minus the post-challenge FEV_1 divided by the pre-challenge FEV_1. OAC reactions were categorized into two groups as follows: (1) 15% or greater decrease in FEV_1 or nasal reactions such as rhinorrhea, nasal congestion (aspirin-intolerant asthma [AERD]) and (2) less than 15% decrease in FEV_1 without naso-ocular or cutaneous reactions (aspirin-tolerant asthma [ATA]). Peripheral venous blood was collected before aspirin challenge. Peripheral blood mononuclear cells were separated on Ficoll–Hypaque solution. All subjects gave informed consent to participate in the study. The protocols were approved by the local ethics committees of hospital.

2.2. Genotyping

Using data obtained from the National Center for Biotechnology Information (build 36), 15 among 31 SNPs of the IL-17RA gene were selected as candidate SNPs by taking into consideration those over 5% minor allele frequency and LDs. HapMap DB data based on the Han Chinese and Japanese populations was used for calculation of LDs and a SNP was selected one of SNPs with almost absolute LDs ($r^2 > 0.95$). The selected polymorphisms were then genotyped in the 143 AERD and 411 ATA subjects and 822 normal controls using the TaqMan assay [26]. Primer Express (Applied Biosystems) was used to design both the PCR primers and the MGB TaqMan probes. One allelic probe was labeled with the FAM dye and the other with the fluorescent VIC dye. Typically, PCR was run on the TaqMan Universal Master mix without UNG (Applied Biosystems) at a primer concentration of 900 nM and a TaqMan MGB–probe concentration of 200 nM. The reaction was performed using a 384-well format in a total reaction volume of 5 μL using 20 ng of genomic DNA. The plate was then placed in a thermal cycler (PE 9700, Applied Biosystems) and heated for 2 min at 50 °C and for 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The TaqMan assay plate was then transferred to a Prism 7900 HT instrument (Applied Biosystems) where the fluorescence intensity of each well was read. Fluorescence data files from each plate were analyzed by automated software (SDS 2.1). Genotyping quality control was performed in 10% of the samples by duplicate checking and the rate of concordance in duplicates was >99%. The sequences and detailed descriptions of the primers used are summarized in Supplementary Table 1.

2.3. Measurement of IL17RA protein expression on $CD14^+$ monocytes from asthmatics

Peripheral blood mononuclear cells (PBMC) were purified from the heparinized peripheral venous blood of the asthmatic patients

UNCORRECTED PROOF

Please cite this article in press as: Park JS et al. Association of single nucleotide polymorphisms on Interleukin 17 receptor A (*IL17RA*) gene with aspirin hypersensitivity in asthmatics. Hum Immunol (2012), http://dx.doi.org/10.1016/j.humimm.2012.11.002

Table 1
Clinical profiles of study subjects.

Clinical profiles	BA	NC	*P*	AERD	ATA	*P*
Number of subjects	554	825		143	411	
Age [mean (range)]	45.04 (15–77)	50.75 (4–87)	**<0.001**	42.69 (17–72)	46.27 (15–77)	**<0.001**
Sex (male/female)	195/359	340/485	NS	52/91	143/268	NS
BMI (kg/m2)	22.34±1.82	24.40±3.23	**<0.001**	23.54±2.98	24.65±3.40	**<0.001**
NS/ES (%)[a]	72.32/13.5	70.55/13.33	NS	74.65/10.56	70.15/17.23	NS
Positive rate of atopy (%)	60.34	22.42	NS	60.84	59.12	NS
FEV1 decrease (%) following OAC[b]	17.36±8.04	-	-	24.24±16.05	3.56±4.93	**<0.001**
FVC1%, predicted	88.34±13.97	95.68±15.60	NS	83.91±18.47	83.92±17.87	NS
FEV1%, predicted	90.83±17.01	103.14±15.78	NS	83.22±19.84	84.16±20.52	NS
Log[PC20, methacholine (mg/ml)]	0.55±0.44	1.37±0.15-	**<0.001**	0.52±0.45	0.57±0.41	NS
Log[Total IgE (IU/ml)]	2.28±0.74	1.71±0.60	**<0.001**	2.23±0.54	2.14±0.64	NS
Log[Peripheral blood eosinophil (%)]	0.74±0.42	0.48±0.21	**<0.001**	0.75±0.31	0.73±0.32	NS

AERD: Aspirin Exacerbated Respiratory Disease; ATA: aspirin Tolerant Asthma. *P*-values are obtained using T test or X2 test between AERD and ATA.[1]
[a] NS/ES: Non-smoker/Ex-smoker.
[b] OAC: oral aspirin challenge.

using a density gradient reagent (Histopaque-1077, Sigma, St. Louis, MO). Mouse phycoerythrin (PE)-conjugated monoclonal anti-human *IL17R* mAb (R&D Systems, Minneapolis, MN) and FITC-conjugated mouse anti-human CD14 (BD Pharmingen) was incubated with 5×10^5 PBMC, then washed twice with PBS. The stained cells were analyzed using a flow cytometer (FACScan; Becton-Dickinson, Mountain View, CA). For isotype-matched controls, PE-conjugated mouse IgG_1 (R&D Systems) and FITC-conjugated mouse IgG2a kappa (BD Biosciences, San Jose, CA) were used at the same concentration for each antibody tested.

2.4. RNA extraction, and real-time PCR of the IL17RA gene

Total RNA from PBMCs was isolated using the modified guanidinium thiocyanate-phenol-chloroform extraction method [27]. DNA contamination was eliminated by treatment with 1 μL of RNase-free DNase I for 15 min at 37 °C. We quantified RNA and reverse transcribed cDNA from 3 μg of total RNA. DNase I (10,000 U/mL; Stratagene, La Jolla, CA)-treated RNA was reverse-transcribed by incubating with 0.5 mM dNTP, 2.5 mM $MgCl_2$, 5 mM DTT, 1 μL of random hexamer (50 μg/μL) and SuperScript II RT (200 unit/μL; Life Technologies, Grand Island, NY) at 42 °C for 50 min, and heat-inactivated at 70 °C for 15 min. The cDNA fragment of *IL17RA* gene covered exon 1–4 (Fig. 4a). cDNA was aliquoted into tubes containing specific primer pairs for the human *PGK1* (phosphoglycerate kinase 1) and IL-17RA genes for amplification (317 bp fragments, respectively) by real-time PCR. The primers for *PGK1* and *IL17RA* were designed using the Primerbank and Primer3 software (http://pga.mgh.harvard.edu/primerbank/ and http://frodo.wi.mit.edu/primer3/). Nucleotide sequences for the primers consisted of the following; *PGK1* forward; 5′-CCTGGGCGGAGCTAAAGTTG-3′, *PGK1* reverse; 5′-AGACCTAATGTCCAAAGCTGAGA-3′, *IL17RA* forward; 5′-CTGCGACT CCTGGACCAC-3′, *IL17RA* reverse; 5′-CTGTCCAAACTGAGGCATCA-3. Real-time PCR was performed using the StepOne Real-Time PCR Sys-

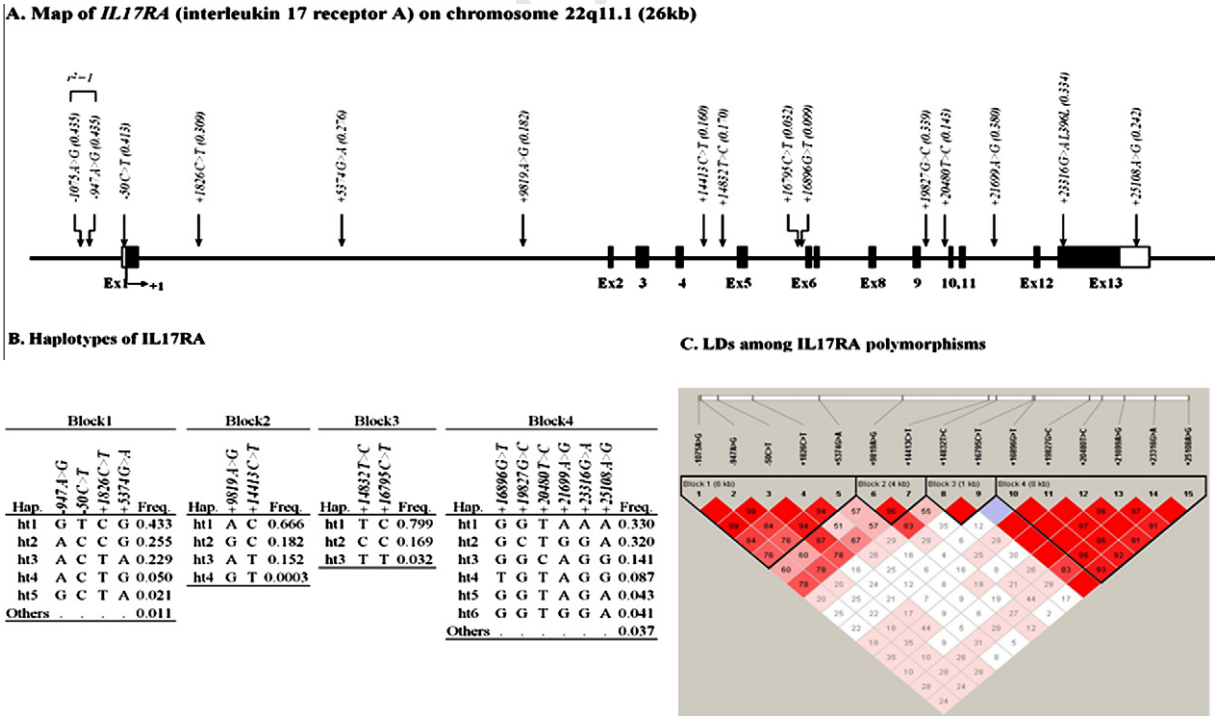


Fig. 1. Physical map, haplotype and LDs information of *IL17RA* gene. (**A**) Genetic map of *IL17RA* and the locations of the SNPs. The first base in the translation start site is denoted as nucleotide +1. Black and white blocks represent the coding and untranslated regions of *IL17RA*, respectively. (**B**) Haplotypes of *IL17RA* gene. (**C**) LDs among *IL17RA* polymorphisms.

Please cite this article in press as: Park JS et al. Association of single nucleotide polymorphisms on Interleukin 17 receptor A (*IL17RA*) gene with aspirin hypersensitivity in asthmatics. Hum Immunol (2012), http://dx.doi.org/10.1016/j.humimm.2012.11.002

UNCORRECTED PROOF

Table 2
Association analysis of *IL17RA* polymorphisms with the risk of AERD, controlling age, sex, smoking status, atopy and BMI in a Korean population.

Locus	Position	rs number	Amino acid change	Frequency			AERD – ATA			AERD – NC			ATA – NC		
				AERD (n = 143)	ATA (n = 411)	NC (n = 822)	OR (95%CI)	P	P^{corr}	OR (95%CI)	P	P^{corr}	OR (95%CI)	P	$\mathbf{Pc^{corr}}$
−1075 A > G	Promoter	rs4819553		0.332	0.471	0.469	0.60 (0.45–0.79)	**0.0002**	**0.003**	**0.56 (0.42–0.74)**	**0.00004**	**0.0006**	0.99 (0.83–1.18)	0.91	1
−947 A > G	Promoter	rs4819554		0.332	0.471	0.469	0.60 (0.45–0.79)	**0.0002**	**0.003**	**0.56 (0.42–0.74)**	**0.00004**	**0.0006**	0.99 (0.83–1.18)	0.91	1
−50 C > T	5'UTR	rs917864		0.31	0.449	0.449	0.57 (0.43–0.76)	**0.0001**	**0.002**	**0.55 (0.41–0.73)**	**0.00003**	**0.0005**	0.98 (0.82–1.17)	0.79	1
+1826 C > T	Intron1	rs2241043		0.367	0.288	0.305	1.45 (1.07–1.96)	**0.02**	0.3	1.21 (0.92–1.58)	0.18	1	0.89 (0.73–1.08)	0.23	1
+5374 G > A	Intron1	rs5748863		0.329	0.257	0.258	1.46 (1.07–2.00)	**0.02**	0.3	1.32 (0.99–1.75)	0.06	0.88	0.99 (0.80–1.23)	0.94	1
+9819 A > G	Intron1	rs6518660		0.234	0.164	0.183	1.63 (1.15–2.31)	**0.006**	0.09	**1.41 (1.01–1.97)**	**0.05**	0.7	0.89 (0.69–1.14)	0.35	1
+14413 C > T	Intron4	rs13053889		0.185	0.151	0.155	1.27 (0.87–1.84)	0.21	1	1.20 (0.85–1.71)	0.3	1	0.98 (0.76–1.26)	0.89	1
+14832 T > C	Intron4	rs9606615		0.162	0.173	0.169	0.92 (0.62–1.34)	0.65	1	0.92 (0.64–1.33)	0.66	1	1.08 (0.85–1.38)	0.52	1
BL1_ht1				0.259	0.36	0.24	0.61 (0.45–0.84)	**0.002**	**0.03**						
BL1_ht2				0.234	0.191	0.229	1.30 (0.93–1.80)	0.12	1	1.27 (1.06–1.53)	0.0116	0.174	1.18 (0.96–1.45)	0.12	1
BL1_ht3				0.115	0.088	0.055	1.42 (0.91–2.21)	0.12	1	1.07 (0.87–1.30)	0.5301	1	0.98 (0.79–1.22)	0.85	1
BL1_ht4				0.094	0.073	**0.065**	1.30 (0.79–2.13)	0.3	1	0.65 (0.44–0.96)	0.0315	0.4725	**0.58 (0.37–0.90)**	**0.02**	0.23
+16795 C > T	Intron5	rs17807076		0.045	0.028	0.797	1.65 (0.82–3.31)	0.16	1	1.36 (0.71–2.63)	0.36	1	0.69 (0.40–1.19)	0.18	1
+16896 G > T	Intron5	rs17205308		0.084	0.105	0.087	0.85 (0.52–1.38)	0.51	1	0.94 (0.58–1.52)	0.79	1	1.14 (0.84–1.54)	0.41	1
+19827 G > C	Intron9	rs721930		0.378	0.326	0.33	1.29 (0.96–1.72)	0.09	1	1.26 (0.95–1.67)	0.11	1	1.02 (0.84–1.24)	0.82	1
+20480 T > C	Intron9	rs2241046	.	0.158	0.137	0.157	1.17 (0.79–1.73)	0.44	1	0.99 (0.69–1.44)	0.97	1	0.88 (0.67–1.14)	0.32	1
+21699 A > G	Intron11	rs2241049	.	0.413	0.369	0.368	1.24 (0.93–1.65)	0.15	1	1.25 (0.94–1.66)	0.12	1	1.05 (0.87–1.27)	0.62	1
+23316 G > A	Exon13	rs2229151	L396L	0.297	0.347	0.335	0.78 (0.58–1.04)	0.09	1	0.80 (0.59–1.08)	0.15	1	1.04 (0.86–1.26)	0.68	1
+25108 A > G	Exon13	rs2895332	.	0.236	0.245	0.255	0.99 (0.72–1.36)	0.93	1	0.89 (0.65–1.22)	0.46	1	0.92 (0.74–1.13)	0.42	1
BL1_ht5			.	0.028	0.062	0.24	0.46 (0.21–1.00)	**0.05**	0.7						
BL2_ht1			.	0.276	0.337	0.229	0.74 (0.54–0.99)	**0.04**	0.7	0.97 (0.81–1.16)	0.73	1			
BL2_ht2			.	0.364	0.311	0.055	1.28 (0.96-1.71)	0.1	1						
BL2_ht3			.	0.154	0.128	0.665	1.22 (0.82–1.82)	0.32	1						
BL2_ht4			.	0.077	0.1	0.797	0.83 (0.50–1.36)	0.46	1						

The P values were obtained by logistic regression analysis, controlled for age (continuous value), sex (male = 0, female = 1), atopy status (non-atopy = 0, atopy = 1), BMI and smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2) as co-variables. Pc^{corr}; P values after multiple correction Bold faces mean the $P < 0.05$.

Please cite this article in press as: Park JS et al. Association of single nucleotide polymorphisms on Interleukin 17 receptor A (*IL17RA*) gene with aspirin hypersensitivity in asthmatics. Hum Immunol (2012), http://dx.doi.org/10.1016/j.humimm.2012.11.002

Table 3
Association analysis of IL17RA polymorphisms with the risk of asthma, controlling age, sex, smoking status and atopy in a Korean population.

Loci	Position	rs number	Amino acid change	BA (n = 554)	NC (n = 822)	OR (95%CI)	P	P^{corr}
−1075 A > G	Promoter	rs4819553		0.435	0.469	0.86(0.73–1.01)	0.06	0.93
−947 A > G	Promoter	rs4819554		0.435	0.469	0.86(0.73–1.01)	0.06	0.93
−50 C > T	Exon1	rs917864		0.413	0.449	**0.84(0.71–1.00)**	**0.04**	0.65
+1826 C > T	0	rs2241043		0.309	0.305	0.97(0.81–1.16)	0.75	1
+5374 G > A	Intron1	rs5748863		0.276	0.258	1.08(0.89–1.31)	0.42	1
+9819 A > G	Intron1	rs6518660		0.182	0.183	1.02(0.81–1.27)	0.88	1
+14413 C > T	Intron4	rs13053889		0.160	0.155	1.04(0.83–1.31)	0.73	1
+14832 T > C	Intron4	rs9606615		0.170	0.169	1.06(0.85–1.32)	0.63	1
+16795 C > T	Intron5	rs17807076		0.032	0.033	0.86(0.54–1.38)	0.54	1
+16896 G > T	Intron5	rs17205308		0.099	0.087	1.09(0.82–1.45)	0.55	1
+19827 G > C	Intron9	rs721930		0.339	0.330	1.08(0.91–1.29)	0.39	1
+20480 T > C	Intron9	rs2241046	.	0.143	0.157	0.92(0.73–1.17)	0.51	1
+21699 A > G	Intron11	rs2241049	.	0.380	0.368	1.09(0.92–1.30)	0.34	1
+23316 G > A	Exon13	rs2229151	L396L	0.334	0.335	0.97(0.81–1.16)	0.75	1
+25108 A > G	Exon13	rs2895332	.	0.242	0.255	0.93(0.76–1.12)	0.43	1
BL1_ht2			.	0.273	0.240	**1.27(1.06–1.53)**	**0.01**	0.17
BL1_ht3			.	0.242	0.229	1.07(0.87–1.30)	0.53	1
BL1_ht4			.	0.043	0.055	**0.65(0.44–0.96)**	**0.03**	0.47
BL2_ht1			.	0.659	0.665	0.97(0.81–1.16)	0.73	1
BL3_ht1			.	0.797	0.797	0.99(0.80–1.22)	0.90	1

The P values were obtained by logistic regression analysis, controlled for age (continuous value), sex (male = 0, female = 1), atopy status (non-atopy = 0, atopy = 1), BMI and smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2) as co-variables. P^{corr}; P values after multiple correction Bold faces mean the $P < 0.05$.

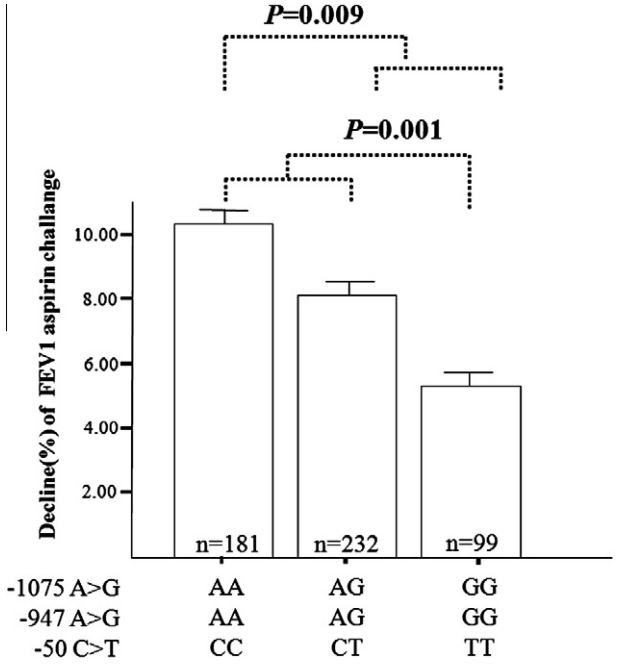


Fig. 2. The comparison of decline of FEV_1 by aspirin provocation between the subjects possessing *−1075 A > G*, *−947 A > G*, *−50 C > T* of *IL17RA*. *P*-value of codominant model was obtained by linear regression analysis, controlled for age (continuous values), sex (male = 0, female = 1), smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2) and atopy status (non-atopy = 0, atopy = 1) as co-variables.

tem (Applied Biosystems). The reactions were prepared with a 20 μL PCR mixture consisting of 10 μL master mix (iQ SYBR Green Supermix; Bio-Rad, Hercules, CA), 200 ng/μL cDNA template, and 1 μL each of the *IL17RA* gene primer pair. Reactions were denatured at 95 °C for 10 min and amplified for 40 cycles at 95 °C for 15 s, 58 °C for 32 s, and 72 °C for 32 s. A melting curve for all products was obtained immediately after amplification by increasing the temperature in 0.3 °C increments from 60 °C for 85 cycles by 15 s for each (Fig. 4b). Threshold cycles (Ct) for *IL17RA* or the endogenous reference gene were defined as the fractional cycle number at which the fluorescence of PCR products passed the fixed threshold. Using the $2^{-\Delta\Delta Ct}$ method, the data were presented as the fold change in gene expression, normalized to an endogenous reference gene (*PGK1*) and relative to a control (−1075AA, −947AA, −50CC genotype of *IL17RA*). For the control sample, ΔΔCt equaled zero and 2^0 equals 1, so that, by definition, the fold change in gene expression relative to the control equaled 1 [28]. For the test samples, evaluation of $2^{-\Delta\Delta Ct}$ indicated the fold change in gene expression relative to the control. The fold difference for the *IL17RA* mRNA expression for each patient was also calculated by comparing $2^{-\Delta\Delta Ct}$.

2.5. Statistical analyses

Widely used measures of linkage disequilibrium were applied to all pairs of biallelic loci: Lewontin's D' (|D'|) [29] and r^2. Haplotypes for each individual were inferred using the PHASE algorithm (ver. 2.0) developed by Stephens et al. [30]. The genotype and haplotype distribution was analyzed using logistic regression models with age (continuous value), gender (male = 0, female = 1), smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2), atopy (absence = 0, presence = 1), and BMI as covariates. Differences in the decline of FEV_1 following aspirin challenge among the genotypes were examined using independent T test. The data were managed and analyzed using the SAS software (ver. 9.1; SAS Inc., Cary, NC). A two-sided P value <0.05 was deemed to indicate statistical significance. The Bonferroni correction by number of polymorphisms was used for multiple comparisons. The IL7RA protein and m-RNA expressions are expressed as the mean ± standard error of the mean (SEM). Intergroup comparisons of IL7RA expression were assessed by the Kruskal wallis test and Mann–Witney U test. *P*-values less than 0.05 were regarded as being statistically significant.

3. Results

3.1. Characteristics of the study subjects

In total, 554 asthmatic patients and 825 normal controls were included. The clinical characteristics of the study subjects are summarized in Table 1. Significant differences in mean age and BMI were found between asthmatics and normal controls and between the AERD and the ATA groups ($P < 0.001$).

Please cite this article in press as: Park JS et al. Association of single nucleotide polymorphisms on Interleukin 17 receptor A (*IL17RA*) gene with aspirin hypersensitivity in asthmatics. Hum Immunol (2012), http://dx.doi.org/10.1016/j.humimm.2012.11.002

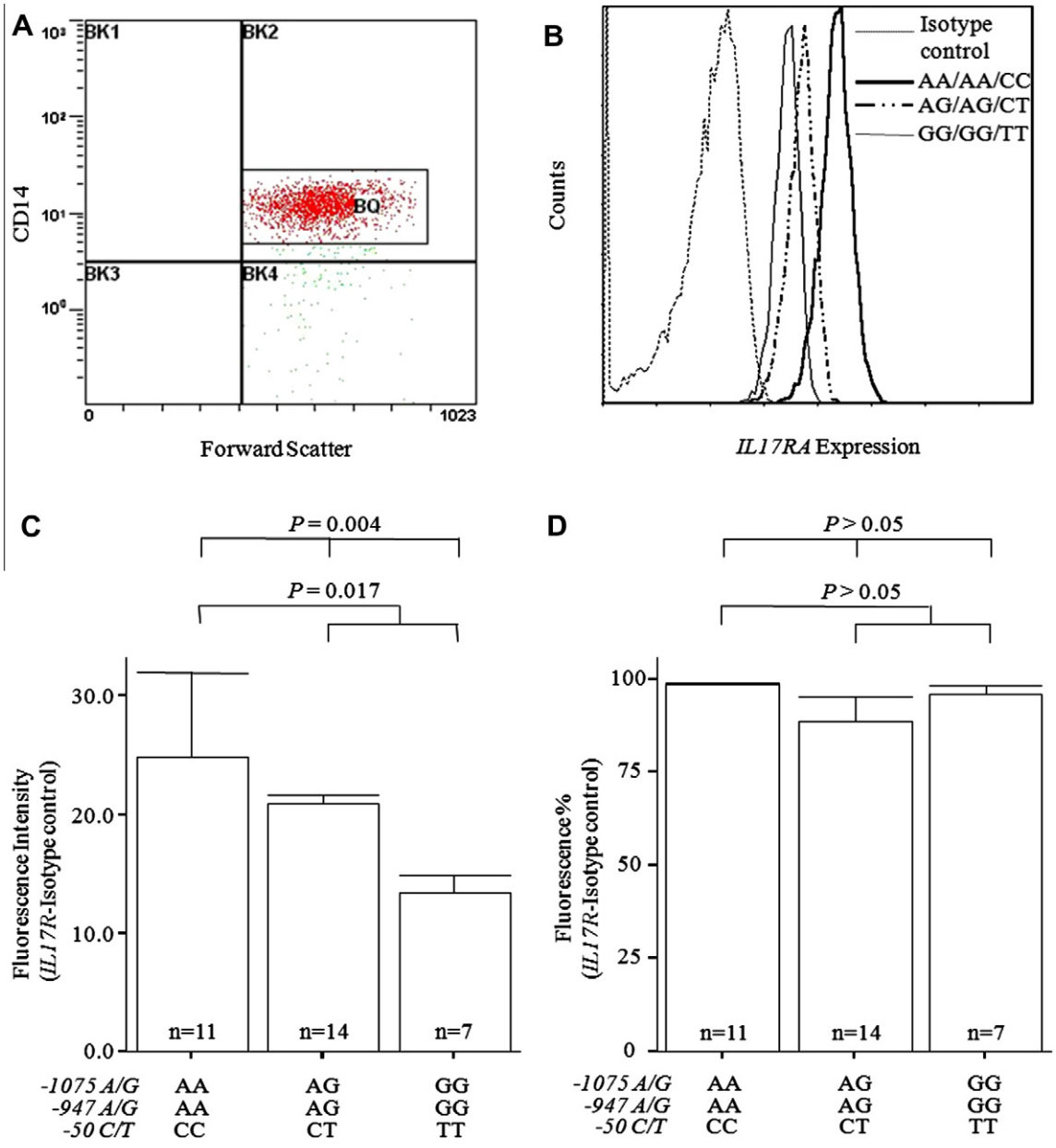


Fig. 3. Flow cytometric analysis of *IL17RA* expression on monocytes between *−1075 A > G*, *−947 A > G*, *−50 C > T* genotypes. Levels of *IL17RA* expression were compared between monocytes from asthmatics having *−1075 AA*, *−947 AA*, *−50 CC* ($n = 11$), *−1075 AG*, *−947 AG*, *−50 CT* ($n = 14$) and *−1075 GG*, *−947 GG*, *−50 TT* ($n = 7$). (A) Gating for CD14 positive cell among monocytes. (B and C) Levels of IL17RA expression were compared between monocytes from asthmatic patients having three SNPs. Each data was presented by the ratio of fluorescence intensities for IL17RA to the values of isotype-matched control. (D) Positive ratio (%) of IL17RA protein expression on CD14+ monocytes. *P* values were calculated by Mann–Whitney U test.

3.2. Minor allele frequencies (MAFs) and the Hardy-Weinberg equilibrium for IL17RA polymorphisms

In total, 15 polymorphisms for the *IL17RA* gene, validated by the National Center for Biotechnology Information (build 36) database, were selected and genotyped for the study subjects (Fig. 1A). The minor allele frequencies (MAFs) of these 15 SNPs for the all study subjects are summarized in Supplementary Table 2. The distributions of all loci were within Hardy–Weinberg equilibrium ($P > 0.01$). The linkage disequilibrium coefficients |D'| and r^2 among the *IL17RA* SNPs were calculated for all of the study participants (Fig. 1C)

3.3. Analysis of IL17RA polymorphisms with respect to development of asthma and aspirin intolerance in patients with asthma

In total, 15 polymorphisms of the *IL17RA* gene were analyzed using logistic models to assess their association with the risk of asthma and aspirin intolerance in 825 normal controls and 554 asthmatic patients: 143 AERD and 411 ATA subjects (Table 2). The analysis revealed that three SNPs (*−1075A > G* and *−947A > G* in the promoter, *−50 C > T* in the 5'-UTR) and one haplotype (Fig. 1B) (haplotype 1 in block 2) were associated with the risk of aspirin intolerance. The frequencies of minor alleles were significantly lower for AERD than ATA ($P = 0.0001$-0.0002). Statistical sig-

Please cite this article in press as: Park JS et al. Association of single nucleotide polymorphisms on Interleukin 17 receptor A (*IL17RA*) gene with aspirin hypersensitivity in asthmatics. Hum Immunol (2012), http://dx.doi.org/10.1016/j.humimm.2012.11.002

nificance was also maintained following correction for multiple comparisons (p^{corr} = 0.002-0.003). The frequency of one of the haplotypes (BL2-ht1) was found to be significantly lower for AERD than ATA (P = 0.001), and statistical significance was maintained following correction for multiple comparisons (p^{corr} = 0.02). The 15 polymorphisms of the *IL17RA* gene were analyzed using logistic models to assess their association with the risk of asthma in 554 asthmatic patients and 825 normal controls (Table 3) The frequencies of minor alleles of *−50 C > T* and haplotypes of *BL1_ht2* and *BL1_ht4* were different between the two groups (P = 0.01–0.04), however these differences were not significant following correction for multiple comparisons (P > 0.05).

Because the aspirin-induced decline in FEV_1 constitutes the most important parameter for the diagnosis of aspirin intolerance in asthmatics, we determined the association of *−1075A > G*, *−947A > G*, and *−50 C > T* with the rate of decline in FEV_1 following aspirin challenge (Fig. 2). Three SNPs were strongly associated with the rate of decline in FEV_1 following aspirin challenge, in a gene-dose dependent manner (*AAC*: 10.08 ± 13.03%, *AC/GT*: 8.53 ± 12.57%, *GGT*: 6.76 ± 12.60%; P = 0.004).

3.4. Comparison of IL17RA protein levels between −1075 A > G, −947 A > G and −50 C > T genotypes by flow cytometry

IL17RA protein expression was measured in peripheral blood $CD14^+$ monocytes obtained from asthmatics having *IL17RA −1075 AA*, *−947 AA*, *−50 CC* (n = 11), and those having *IL17RA −1075 GG*, *−947 GG*, *−50 TT* (n = 7) by flow cytometry (Fig. 3). IL17RA expression on the $CD14^+$ cells were significantly different between the two groups. The $CD14^+$ monocytes from asthmatic patients with AAC homozygotes for *−1075 A > G*, *−947 A > G*, *−50 C > T* showed significantly higher IL17RA expression than those of the GGT homozygotes group (*AAC;* 24.82 ± 23.86 vs. GGT; 13.38 ± 4.14 fluorescence intensity; P = 0.017; Fig 3C). More than 80% $CD14^+$ monocytes expressed *IL17RA* protein on their surface and the positive rate of *IL17RA* on $CD14^+$ monocytes was similar for the two groups (98.21 ± 2.10% vs. 95.73 ± 5.59%: Fig. 3D).

3.5. Comparison of IL17RA mRNA expression between −1075 A > G, −947 A > G, −50 C > T

To investigate the genetic effect of these polymorphisms on the mRNA expression of the *IL17RA* gene in PBMCs, we used real-time PCR to measure *IL17RA* mRNA levels in PBMCs derived from subjects carrying *−1075 A > G*, *−947 A > G*, *−50 C > T*. Quantification of the IL17RA mRNA level was done by measuring the intensity of the *IL17RA* real-time PCR product after correcting for the intensity of *PGK1*. The expression level of *IL17RA* mRNA in PBMCs was significantly higher in subjects having *−1075 AA*, *−947 AA*, *−50 CC* (n = 10) homozygotes than those having *−1075 GG*, *−947 GG*, *−50 TT* (n = 8) homozygotes (1.0 ± 0.27 vs. 0.39 ± 0.15 P = 0.010; Fig. 4C).

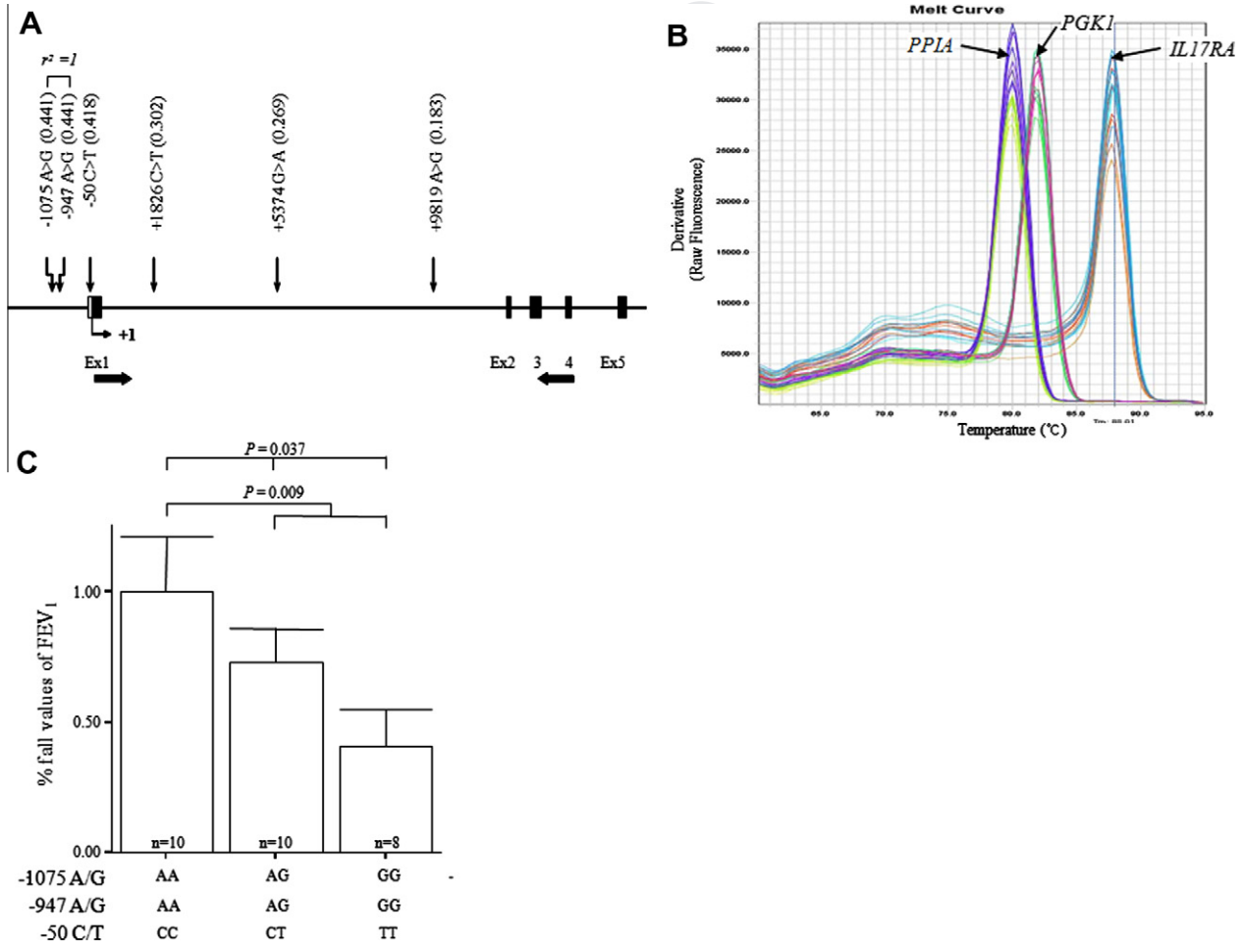


Fig. 4. mRNA expression of *IL17RA* gene according to *−1075 A > G*, *−947 A > G*, *−50 C > T* genotypes in hPBMCs of asthmatics. (A) Diagram of PCR primer. Product size is 317 bp. (B) Melting curve of *IL17RA* and *PGK1*. (C) mRNA expression of *IL17RA* gene according to *−1075 A > G*, *−947 A > G*, *−50 C > T* genotypes in hPBMCs of asthmatics. The cycle threshold (Ct) of each human *IL17RA* gene was normalized to the Ct for *PGK1*. Fold change was calculated relative to expression in common allele. Data are indicated means ± SE. The experiments were performed in duplicate.

Please cite this article in press as: Park JS et al. Association of single nucleotide polymorphisms on Interleukin 17 receptor A (*IL17RA*) gene with aspirin hypersensitivity in asthmatics. Hum Immunol (2012), http://dx.doi.org/10.1016/j.humimm.2012.11.002

4. Discussion

In the present study, for the first time, we identify the *IL-17RA* gene polymorphisms that are associated with the risk of AERD. The frequencies of the minor alleles of three SNPs (*−1075A > G and −947A > G* in the promoter, *−50 C > T* in the 5'-UTR) and one haplotype (haplotype-1 in block 2) were significantly lower in AERD than ATA. The human IL17RA gene is located at 22q11.22–22q11.23 and spans 13 exons, encoding a 866-amino acid type I membrane glycoprotein. This receptor is strongly expressed on CD33 myeloid cells, $CD14^+$ monocytes and peripheral blood cells [31]. Thus, we measured *IL17RA* protein levels on the surface of $CD14^+$ monocytes between *−1075 A > G, −947 A > G* and *−50 C > T* genotypes by flow cytometry as a functional validation study to evaluate the genetic effect of the SNPs. The IL17RA protein was significantly higher on the $CD14^+$ monocytes of common allele homozygotes of the SNPs than on those of minor allele. These data indicate that the minor alleles of the three SNPs may exert a protective effect against aspirin hypersensitivity by attenuating IL17RA protein expression. Interestingly, the IL17RA protein was expressed by more than 80% monocytes of the three genotypes. These data suggest that the genetic effect of the SNPs may determine the quantity of IL17RA protein expressed by the monocytes.

To determine the regulatory level of protein expression on the steps of transcription, we used real-time PCR using peripheral blood mononuclear cells (PBMCs), which are known to express a significant amount of IL17RA mRNA [32]. We found that the mRNA expression in PBMCs containing the common alleles homozygote were significantly higher than that of minor allele homozygote from the three SNPs. These data indicate that the three SNPs may exert their genetic effect at promoter sites that inducers or enhancers may act upon. When we searched the candidate transcriptional factors using the web sites TESS, the region around allele IL 17 RA *−1075 A > G, −947 A > G* and *−50 C > T* was found to contain consensus binding sites for NF-Zc (at bp −1071 to −1077), NF-Zz (at bp −1071 to −1077), Sp1 (at bp −941 to −947), AP-4 (at bp −943 to −948), E12 (at bp −943 to −948), HEN1 (at bp −943 to −948), and Pax-5 (at bp −40 to −55) (Supplementary Fig. 1). These data suggest that the three loci for the SNPs may serve as the sites for transcription factor binding.

Recent research on the potential involvement of *IL-17* cytokine family in pulmonary allergic responses has focused on *IL-17A*, *IL-17F*, and *IL-17E*, because the former two seem to take part in neutrophil recruitment and activation whereas *IL-17E* appears to be involved in the induction of TH2 cytokines and the recruitment of eosinophils through induction of eotaxin [16]. IL-17A signals through its receptor, IL-17RA, and induces the maturation of $CD34^+$ hematopoietic precursors into neutrophils. The activation of IL17RA may, therefore, be associated with neutrophilic inflammation. However, the associated inflammatory cell pattern has been regarded as eosinophils in AERD. We have no good explanation that accounts for the seeming discrepancy in these data. As well as neutrophilic inflammation, IL17 induces NF-κB and prostaglandin E2 [33]. Moreover, in addition to prostaglandin metabolism, IL17A induces the production of cytokines (IL-6, TNF-α, IL-1β), chemokines (RANTES, MCP-1, MIP-2/IL-8, MIP-3α, GROα), cell-surface markers (RANKL and ICAM-1), and proinflammatory mediators (nitric oxide, COX-2) [34]. All are important mediators in the development of aspirin hypersensitivity in asthma. Thus, differentially regulated IL-17 RA protein expression may alter the synthesis of cytokines, chemokines, and prostaglandin E2 upon stimulation of IL-17, which may lead to aspirin hypersensitivity in asthma.

Whereas IL17RA is a well-known gene in innate-adaptive immune responses, few studies have been carried out on diseases associated with IL17RA gene polymorphisms. Common haplotypes consisting of two intronic SNPs (rs5748864 and rs6518660) in *IL17RA* have been reported to be associated with susceptibility to Crohn's disease [35]. Although asthma shares a common immunopathology with Crohn's disease, rs6518660 was not associated with asthma of the present study.

In summary, we identified three SNPs associated with the development of aspirin intolerance in asthmatics. Additionally, the associations of *−1075 A > G, −947 A > G, −50 C > T* are more pronounced when paired with functional studies demonstrating protein and gene levels by real-time PCR and flow cytometry. This information on genetic polymorphisms with regard to the *IL17RA* gene may be useful in developing new methods for diagnosing AERD, and for devising new strategies for its control.

5. Conclusions

We genotyped 15 SNPs on *IL17RA* and examined their link to aspirin-intolerant in asthmatics. Logistic analysis showed that *IL17RA −1075 A > G, −947 A > G and −50 C > T* are associated with aspirin hypersensitivity in asthma. In addition, these associations of *−1075 A > G, −947 A > G and −50 C > T* are more pronounced when validated with the measurement of protein and mRNA expression in $CD14^+$ peripheral blood monocytes and mononuclear cells. This information on genetic polymorphisms of the important *IL17RA* gene may be useful in the development of new methods to diagnose aspirin intolerance, and new strategies for the control of aspirin intolerance.

Acknowledgments

The DNA 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. The English in this manuscript has been checked by textcheck (www.textcheck.com/certificate/RfXz47).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2012.11.002.

References

[1] Samter M, Beers Jr RF. Concerning the nature of intolerance to aspirin. J Allergy 1967;40:281–93.
[2] Stevenson DD, Sanchez-Borges M, Szczeklik A. Classification of allergic and pseudoallergic reactions to drugs that inhibit cyclooxygenase enzymes. Ann Allergy Asthma Immunol 2001;87:177–80.
[3] Sousa AR, Parikh A, Scadding G, Corrigan CJ, Lee TH. Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. N Engl J Med 2002;347:1493–9.
[4] Szczeklik A. The cyclooxygenase theory of aspirin-induced asthma. Eur Respir J 1990;3:588–93.
[5] Sanak M, Simon HU, Szczeklik A. Leukotriene C4 synthase promoter polymorphism and risk of aspirin-induced asthma. Lancet 1997;350:1599–600.
[6] Choi JH, Park HS, Oh HB, Lee JH, Suh YJ, Park CS, et al. Leukotriene-related gene polymorphisms in ASA-intolerant asthma: an association with a haplotype of 5-lipoxygenase. Hum Genet 2004;114:337–44.
[7] Kim SH, Oh JM, Kim YS, Palmer LJ, Suh CH, Nahm DH, et al. Cysteinyl leukotriene receptor 1 promoter polymorphism is associated with aspirin-intolerant asthma in males. Clin Exp Allergy 2006;36:433–9.
[8] Park JS, Chang HS, Park CS, Lee JH, Lee YM, Choi JH. Association analysis of cysteinyl-leukotriene receptor 2 (CYCLTR2) polymorphisms with aspirin intolerance in asthmatics. Pharmacogenet Genomics 2005;15:483–92.
[9] Kim SH, Choi JH, Park HS, Holloway JW, Lee SK, Park CS, et al. Association of thromboxane a2 receptor gene polymorphism with the phenotype of acetyl salicylic acid-intolerant asthma. Clin Exp Allergy 2005;35:585–90.
[10] Leff AR. Regulation of leukotrienes in the management of asthma: biology and clinical therapy. Annu Rev Med 2001;52:1–14.

UNCORRECTED PROOF

[11] Dahlen SE, Malmstrom K, Nizankowska E, Dahlen B, Kuna P, Kowalski M, et al. Improvement of aspirin-intolerant asthma by montelukast, a leukotriene antagonist: a randomized, double-blind, placebo-controlled trial. Am J Respir Crit Care Med 2002;165:9–14.
[12] Park JS, Jang AS, Park SW, Lee YM, Uh ST, Kim YH, et al. Protection of leukotriene receptor antagonist against aspirin-induced bronchospasm in asthmatics. Allergy Asthma Immunol Res 2010;2:48–54.
[13] Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis, diagnosis, and management. J Allergy Clin Immunol 2003;111:913–21. quiz 922.
[14] Kim SH, Yang EM, Lee HN, Cho BY, Ye YM, Park HS. Combined effect of IL-10 and TGF-beta1 promoter polymorphisms as a risk factor for aspirin-intolerant asthma and rhinosinusitis. Allergy 2009;64:1221–5.
[15] Oh SH, Park SM, Park JS, Jang AS, Lee YM, Uh ST, et al. Association analysis of peroxisome proliferator-activated receptors gamma gene polymorphisms with asprin hypersensitivity in asthmatics. Allergy Asthma Immunol Res 2009;1:30–5.
[16] Kawaguchi M, Adachi M, Oda N, Kokubu F, Huang SK. IL-17 cytokine family. J Allergy Clin Immunol 2004;114:1265–73. quiz 1274.
[17] Louten J, Boniface K, de Waal Malefyt R. Development and function of th17 cells in health and disease. J Allergy Clin Immunol 2009;123:1004–11.
[18] Gaffen SL. Structure and signalling in the IL-17 receptor family. Nat Rev Immunol 2009;9:556–67.
[19] Yao Z, Fanslow WC, Seldin MF, Rousseau AM, Painter SL, Comeau MR, et al. Herpesvirus saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity 1995;3:811–21.
[20] Miljkovic D, Cvetkovic I, Vuckovic O, Stosic-Grujicic S, Mostarica Stojkovic M, Trajkovic V. The role of interleukin-17 in inducible nitric oxide synthase-mediated nitric oxide production in endothelial cells. Cell Mol Life Sci 2003;60:518–25.
[21] Wong CK, Cheung PF, Ip WK, Lam CW. Interleukin-25-induced chemokines and interleukin-6 release from eosinophils is mediated by p38 mitogen-activated protein kinase, c-jun n-terminal kinase, and nuclear factor-kappa b. Am J Respir Cell Mol Biol 2005;33:186–94.
[22] Jung JS, Park BL, Cheong HS, Bae JS, Kim JH, Chang HS, et al. Association of IL-17rb gene polymorphism with asthma. Chest 2009;135:1173–80.
[23] Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: gina executive summary. Eur Respir J 2008;31:143–78.
[24] Nizankowska-Mogilnicka E, Bochenek G, Mastalerz L, Swierczynska M, Picado C, Scadding G, et al. EAACI/GA2LEN guideline: aspirin provocation tests for diagnosis of aspirin hypersensitivity. Allergy 2007;62:1111–8.
[25] Chang Hun Soo, Park Jong-Sook, Jang An-Soo, Park Sung-Woo, Uh Soo-Taek, Kim Young Hoon, et al. Diagnostic value of clinical parameters in the prediction of aspirin-exacerbated respiratory disease in asthma. Allergy Asthma Immunol Res 2011;3(4):256–64.
[26] Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal 1999;14:143–9.
[27] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–9.
[28] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta c(t)) method. Methods 2001;25:402–8.
[29] Hedrick PW. Gametic disequilibrium measures: proceed with caution. Genetics 1987;117:331–41.
[30] Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89.
[31] Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, et al. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci U S A 2004;101:6062–7.
[32] O'Connor Jr W, Kamanaka M, Booth CJ, Town T, Nakae S, Iwakura Y, et al. A protective function for interleukin 17A in T cell-mediated intestinal inflammation. Nat Immunol 2009;10:603–9.
[33] Paradowska A, Masliniski W, Grzybowska-Kowalczyk A, Lacki J. The function of interleukin 17 in the pathogenesis of rheumatoid arthritis. Arch Immunol Ther Exp (Warsz) 2007;55:329–34.
[34] Dong C. Diversification of T-helper-cell lineages: finding the family root of IL-17-producing cells. Nat Rev Immunol 2006;6:329–33.
[35] McGovern DP, Rotter JI, Mei L, Haritunians T, Landers C, Derkowski C, et al. Genetic epistasis of IL23/IL17 pathway genes in crohn's disease. Inflamm Bowel Dis 2009;15:883–9.

UNCORRECTED PROOF

Please cite this article in press as: Park JS et al. Association of single nucleotide polymorphisms on Interleukin 17 receptor A (*IL17RA*) gene with aspirin hypersensitivity in asthmatics. Hum Immunol (2012), http://dx.doi.org/10.1016/j.humimm.2012.11.002