Original Article
Allergy Asthma Immunol Res. 2009 October;1(1):36-40.
doi: 10.4168/aair.2009.1.1.36
pISSN 2092-7355 • eISSN 2092-7363

AAIR
Allergy, Asthma & Immunology Research

No evidence of association between interleukin-13 gene polymorphism in aspirin intolerant chronic urticaria

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Aspirin-intolerant chronic urticaria (AICU) is a common condition among the chronic urticaria population, but the genetic mechanism is not yet understood. In this study, the genotypes and haplotypes of three interleukin (IL)-13 polymorphisms, –1510 A>C, –1055C>T, and Arg110Gln (110G>A), as well as their respective clinical phenotypes were examined to determine whether genetic variants of *IL-13* play a role in AICU. Single-nucleotide polymorphism (SNP) genotyping was used to compare *IL-13* genotype and allele frequencies among 135 patients with AICU, 146 with aspirin-tolerant chronic urticaria (ATCU), and 430 normal controls (NC). Relationships among the AICU phenotype, atopy, and total IgE level were also investigated. The results failed to show a significant difference in the allele or genotype frequencies between the AICU group and either the ATCU or NC group (P>0.05, respectively). Haplotype analysis confirmed that there was no significant difference among the three study groups (P>0.05), nor was there a significant difference in atopy or total IgE level according to the three genetic polymorphisms (P>0.05, respectively). Our data lead to the conclusion that there is no evidence supporting genetic polymorphisms in *IL-13* as a genetic risk factor for the development of AICU.

Key Words: aspirin; *IL-13*; urticaria

INTRODUCTION

Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) cause symptoms in 20–30% of patients with chronic urticaria (CU).[1,2] In the Korean population, 35.7% of CU patients are affected, as confirmed by oral provocation testing.[3] Several genes are likely to be involved in the etiology and pathogenesis of aspirin-intolerant urticaria (AIU), including both the acute and chronic forms as well as intermediate phenotypes. The candidate genes include HLA-DRB1 *1302-DQB1*0609,[4] ALOX5,[5] FCER1A,[6] HNMT,[7] and FCER1G.[8]

The cytokine interleukin (IL)-13 is produced primarily by activated Th2 cells[9] and is thought to play a role in the mechanisms of several atopic conditions. *IL-13* promotes B cell differentiation and is capable of inducing isotype-switching in B cells, resulting in the production of IgG4 and IgE.[10] Human lung mast cells express *IL-13*Rα1, and activation of these cells by the cytokine for 5 days increased FcεRI expression and proliferation.[11] *IL-13* maps to chromosome 5q31, which has been implicated in the development of asthma and atopy.[12] It is a critical effector in the induction and maintenance of IgE production and IgE-mediated allergic airway responses.[13]

Analyses of *IL-13* gene polymorphisms have produced various results depending on the specific study population. For example, genetic association studies in German[14] and Chinese populations have suggested a role for the IL-13 variant Arg-110Gln in the increased IgE production and atopic sensitization that characterizes allergic rhinitis, whereas this allele was found to be associated with asthma in British and Japanese populations.[15] Previous studies have reported that AICU patients had a significantly higher rate of atopy than ATCU patients and normal controls, suggesting that atopy is a risk factor for AICU.[3,8] A study on FcER1α promoter polymorphisms found that the prolonged exposure of AICU patients to IgE induces the persistent accumulation and activation of mast cells and basophils via increased surface expression of high-affinity IgE receptors.[6]

To identify genetic factors in the development of AICU, we an-

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Received: June 24, 2009; Accepted: September 2, 2009
• There are no financial or other issues that might lead to conflict of interest.

alyzed the genotypes and haplotypes of three polymorphisms of *IL-13* to determine whether genetic variants of *IL-13* play an important role in AICU.

MATERIALS AND METHODS

Study subjects and phenotyping

Two groups of CU patients, AICU (n=135) and ATCU (n=146), who were identified based on the results of n oral provocation test with up to 500 mg of aspirin (Rhonal; KunWha Pharmaceutical Co., Seoul, Korea), were enrolled in the study, as described previously.[4] AICU patients who had aspirin-intolerant asthma (AIA) were excluded. Normal healthy controls (NC; n=430) with no personal or family history of allergic diseases, or aspirin or drug hypersensitivity were recruited from the general Korean population. Informed consent was obtained from all participants, and the institutional review board of Ajou University Hospital approved this study. Skin-prick tests were performed with 55 common aeroallergens (Bencard Co., West Sussex, UK), and atopy was defined as one or more positive reactions to common inhalant allergens. Total IgE concentrations were measured using a UniCAP system (Phadia, Uppsala, Sweden), according to the manufacturer's instructions. Anti-thyroglobulin levels in serum were measured using anti-Tg (B.R.A.H.M.S Aktiengesellschaft, Hennigsdorf, Germany) and serum antimicrosomal antibodies with a thyroid peroxidase IgG ELISA (Zeus Scientific, Zierikzee, Netherlands).

SNP identification and genotyping

Peripheral blood samples from 40 healthy Korean volunteers were used to identify single nucleotide polymorphisms (SNPs). Genomic DNA was prepared from the blood samples using a Puregene DNA purification kit (Gentra, Minneapolis, MN, USA), according to the manufacturer's protocol.

The *IL-13* gene was examined for SNPs using an ABI Prism 3100 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Three polymorphisms of *IL-13*, –1510 A>C, –1055C>T, and Arg-110Gln (110G>A), were genotyped using a primer-extension method and a SNAPshot ddNTP primer-extension kit (Applied Biosystems). The primers used were: for *IL-13* -1510A>C, forward (F) 5′-TGTGGGAGATGCCGTGGG-3′, reverse (R) 5′-TCTGACTCCCAGAAGTCTGC-3′, and extension 5′-TACAGATTAGGAAACAGGCCCGTAG-3′; for *IL-13*–1055C>T, (F) 5′-AGAGAGGGTGGGAATGAC-3′, (R) 5′-CCAGTCTCTGCAGGATCA-3′, and extension 5′-TGTCGCCTTTTCCTGCTCTTCCCTC-3′; and for *IL-13* 110G>A, (F) 5′-AGTTTGTAAAGGACCTGCTCTTAC-3′, (R) 5′-TCAGGTCCTGTCTCTGCAA-3′, and extension 5′-TTAAAGAAACTTTTTCGCGAGGGAC-3′.

Statistical analysis

Chi-squared tests were used to detect significant departures in genotype frequency from the Hardy-Weinberg equilibrium at each SNP. Haplotypes of *IL-13* were analyzed using Haploview v2.0, based on the expectation maximization algorithm. Differences in the mean values of phenotypic characteristics among AICU patients were compared using the chi-squared test, or Fisher's exact test and independent *t*-test. Continuous variables that did not have a normal distribution, such as the levels of serum total IgE, were log-transformed. Differences in genotype frequency between groups were examined with the chi-squared test, and three logical regression models (co-dominant, dominant, recessive) were used after accounting for age and gender covariates. The level of statistical significance was set at $P<0.05$. SPSS 12.0 for Windows software (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses.

RESULTS

In our study, genetic associations among three genetic polymorphisms of *IL-13*, –1510A>C, –1055C>T, and Arg110Gln (110G>A), and the three study groups (AICU, ATCU, and NC) were examined. No significant association was detected between the *IL-13* polymorphisms and susceptibility to AICU ($P>0.05$).

Significant differences in AICU phenotypes, based on the presence of atopy and log serum total IgE levels, were found between the AICU, ATCU, and NC groups ($P<0.001$, respectively) (Table 1). However, there were no significant differences be-

Table 1. Clinical characteristics of study subjects

	AICU	ATCU	NC	*P* value		
				AICU vs ATCU	AICU vs NC	ATCU vs NC
Sex (male/total)	66/135 (48.9%)	68/146 (46.6%)	206/430 (47.9%)	0.394	0.844	0.428
Atopy (presence/total)	97/134 (72.4%)	71/145 (49.0%)	32/277 (11.6%)	**<0.001**	**<0.001**	**<0.001**
Log (serum total IgE [IU/mL]/number of subjects)	5.17±1.07/117	4.67±1.11/137	3.63±1.34	**<0.001**	**<0.001**	**<0.001**
Anti-thyroglobulin antibody (positive/total)	12/94 (12.8%)	23/130 (17.7%)	NA	0.355	NA	NA
Antimicrosomal antibody (positive/total)	7/94 (7.4%)	20/130 (15.4%)	NA	0.096	NA	NA

Values in bold indicate significant *P* value. The value was presented as means±S.D.
AICU, aspirin-intolerant chronic urticaria; NC, normal controls; ATCU, aspirin-tolerant chronic urticaria; NA, not applicable.

Table 2. Allele and genotype frequencies of aspirin-intolerant chronic urticaria

	Genotype	AICU (n=135)	ATCU (n=146)	NC (n=430)	*P* value		
					AICU vs ATCU	AICU vs NC	ATCU vs NC
-1510A>C	AA	68 (50.4%)	72 (49.3%)	235 (54.7%)	0.728	0.799	0.37
	AC	58 (43.0%)	61 (41.8%)	156 (36.3%)	0.438	0.42	0.858
	CC	9 (6.7%)	13 (8.9%)	39 (9.1%)	0.983	0.438	0.286
	q	0.281	0.298	0.272	0.438	0.42	0.858
-1055C>T	CC	91 (67.4%)	103 (70.5%)	295 (68.6%)	0.514	0.692	0.438
	CT	41 (30.4%)	39 (26.7%)	112 (26.0%)	0.944	0.155	0.299
	TT	3 (2.2%)	4 (2.7%)	23 (5.3%)	0.447	0.864	0.632
	q	0.174	0.161	0.184	0.944	0.155	0.299
Arg110Gln (110 G>A)	GG	66 (48.9%)	70 (47.9%)	17 (47.9%)	0.848	1	0.836
	GA	52 (38.5%)	59 (40.4%)	17 (40.5%)	0.652	0.735	0.858
	AA	17 (12.6%)	174 (11.6%)	50 (11.6%)	0.974	0.827	0.867
	q	0.319	0.318	0.328	0.652	0.735	0.858

Each *P* value was calculated with co-dominant, dominant, and recessive models. Logistic regression analysis was applied to control for age and sex as covariables. q; Minor allele frequency.
AICU, aspirin-intolerant chronic urticaria; NC, normal controls; ATCU, aspirin-tolerant chronic urticaria; n, number of patients.

Table 3. Comparison of the log total IgE according to the genotype of IL-13 in aspirin-intolerant chronic urticaria

SNPs	AA vs CC	AA+AC vs CC	AA vs AC+CC
-1510A>C	5.14±1.17/56	5.17±1.05/109	5.14±1.17/56
	5.10±1.41/8	5.10±1.41/8	5.19±0.99/61
P value	0.93	0.863	0.819
-1055C>T	5.12±1.15/78	5.17±1.07/114	5.12±1.15/78
	5.08±1.62/3	5.08±1.62/3	5.26±0.91/39
P value	0.954	0.888	0.502
Arg110Gln (110G>A)	5.08±1.13/59	5.14±1.09/102	5.08±1.13/59
	5.36±0.93/15	5.36±0.93/15	5.26±1.02/58
P value	0.372	0.452	0.373

The value was presented as means±S.D./number of patients.

Table 4. Comparison of the atopy (presence/total) according to the genotype of IL-13 in aspirin-intolerant chronic urticaria

SNPs	AA vs CC	AA+AC vs CC	AA vs AC+CC
-1510A>C	50/68 (73.5%)	91/125 (72.8%)	50/68 (73.5%)
	6/9 (66.7%)	6/9 (66.7%)	47/66 (71.2%)
P value	0.698	0.707	0.848
-1055C>T	69/91 (75.8%)	95/131 (72.5%)	69/91 (75.8%)
	2/3 (66.7%)	2/3 (66.7%)	28/43 (65.1%)
P value	1	1	0.218
Arg110Gln (110G>A)	51/66 (77.3%)	86/118 (72.9%)	51/66 (77.3%)
	11/16 (68.8%)	11/16 (68.8%)	46/68 (67.6%)
P value	0.522	0.768	0.249

tween the two CU groups with respect to allele and genotype frequencies ($P>0.05$) (Table 2). Furthermore, the three polymorphisms of *IL-13* were not in linkage disequilibrium.

Using Haploview 2.0 and the expectation maximization algorithm, five common haplotypes were constructed: ht1 (ACG), ht2 (CTA), ht3 (ACA), ht4 (CCG), and ht5 (CTG). Haplotype analysis further confirmed that there were no significant differences among the three study groups ($P>0.05$) (data not shown).

The relationship between the investigated genotypes of the three polymorphisms and AICU phenotype was not significantly different for atopy (Table 4) or total IgE (Table 3) according to the three different methods of analysis ($P>0.05$).

DISCUSSION

This study evaluated three AICU-susceptibility loci in the *IL-13* gene: two SNPs in the promoter region (–1510 A>C and –1055C>T) and one nonsynonymous SNP (Arg110Gln, 110G >A). In a case control study, we evaluated the three polymorphisms for evidence of association with AICU and related phenotypes. Based on the results, no evidence was found to support a significant association between these three SNPs and the diagnosis of AICU.

The cytokines *IL-13* and *IL-4* share biological properties such as isotype switching to IgE and expression of endothelial cell adhesion molecules.[16] Accordingly, it was reasonable to propose that in chronic urticaria, *IL-13* increases mast cell FcεR1 expression and mediator release,[17] leading to the development of hives and the enhancement of cutaneous inflammation. In a previous report, patients with more frequent itch were found to have higher cytokine levels, suggesting that *IL-13* may serve as a serological marker of disease activity.[18]

Owing to the lack of previously published data on *IL-13* SNPs in patients with AICU, our results cannot be compared directly with others. However, several studies showed a linkage between *IL-13* and a high serum total IgE level,[19,20] including an association of *IL-13* Arg110Gln with serum IgE levels in patients with asthma[15,21] and atopic dermatitis.[22] *IL-13* variants have

been associated with airway responsiveness[23] and with eosinophil count.[24] Recently, *IL-13* Arg110Gln was reported to be significantly associated with reduced lung function in Korean children and adolescents.[25] Nevertheless, we found no significant association of either of the promoter SNPs or the coding SNP with AICU traits, including serum total IgE and atopy. Our findings indicate that a genetic variation in the form of any of the three SNPs at the *IL-13* locus is not likely to be involved in the development of AICU.

This result is consistent with an earlier report showing that there was no relationship between the coding SNP and allergic rhinitis induced by artemisia pollen and/or DerpI in a Chinese population. Marginal significance was found for atopy-related phenotypes associated with the Arg110Gln variant and in vitro specific IgE responses to common inhalant allergens.[26] In our previous study, two SNPs, at FcεR1β E237G and FcεR1γ, were associated with atopy in AICU patients, but not in ATCU patients, and were proposed to explain the increased release of histamine from basophils and subsequent development of the AICU phenotype.[8] Furthermore, other studies have noted a significant association of the FcεR1β-109T>C and FcεR1β E237G polymorphisms with serum IgE levels in asthmatic patients,[27,28] and the FcεR1α –344C>T polymorphism in AICU.[6] However, in our study, *IL-13* genetic polymorphisms were not related to either atopy or total IgE levels in AICU patients, implying that neither the two SNPs in the functional promoter nor the SNP in the coding sequence contribute significantly to AICU susceptibility. This is the first detailed investigation showing the absence of a genetic association between *IL-13* and AICU and its phenotypes, *i.e.*, serum total IgE and atopy. There is no evidence that these three SNPs are associated with AICU; instead, SNPs of *IL-13* may confer a greater susceptibility to asthma or other inflammatory diseases.

ACKNOWLEDGMENTS

This study was supported by a grant of the Korean Health 21 R&D Project, Ministry For Health, Welfare and Family Affairs, Republic of Korea (A050571 and A030001).

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