

IL-13 and *IL-17A* gene polymorphisms in Japanese patients with aspirin-exacerbated respiratory disease

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Background: The role of interleukin (IL) 13 and IL-17A in aspirin-exacerbated respiratory disease (AERD) remains unknown.

Objective: To analyze the *IL-13* and *IL-17A* gene polymorphisms in Japanese patients with AERD.

Methods: The single-nucleotide polymorphisms in each gene were examined in patients with AERD, patients with aspirin-tolerant asthma (ATA), and healthy controls.

Results: Frequencies of the TT/CT genotype of the *IL-13* -1111C>T gene were higher than frequencies of the CC genotype in AERD patients compared with ATA patients ($P < .001$). In female patients with AERD, frequencies of the TT/CT genotype were higher than those of the CC genotype compared with female patients with ATA ($P < .001$). However, genotype frequencies of *IL-13* Arg110Gln did not differ between AERD and ATA patients. Frequencies of the CC genotype of the *IL-17A* -737C>T gene were higher than those of the TT/CT genotype in AERD patients compared with ATA patients ($P = .02$). In female patients with AERD, frequencies of the CC genotype were higher than those of the TT/CT genotype compared with female patients with ATA ($P = .03$). Forced expiratory volume in 1 second (percentage predicted) in AERD patients with the CC genotype of the *IL-13* -1111C>T gene was lower than that in the patients with the TT/CT genotype. AERD patients with the TT/CT genotype of the *IL-17A* -737C>T gene had a higher peripheral total eosinophil count compared with the patients with the CC genotype. The comparison of the clinical characteristics according to the *IL-13* Arg110Gln gene polymorphism showed no difference.

Conclusions: These findings suggest that the *IL-13* -1111C>T and *IL-17A* -737C>T gene sequence variations might have a role in the development of AERD.

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INTRODUCTION

Aspirin-exacerbated respiratory disease (AERD), so-called aspirin-intolerant asthma, is a clinical syndrome characterized by severe asthmatic attacks after ingestion of aspirin and/or nonsteroidal anti-inflammatory drugs (NSAIDs).¹ The inhibitory action of aspirin and NSAIDs on cyclooxygenase activity may cause diversion to the 5-lipoxygenase pathway, which leads to the overproduction of cysteinyl leukotrienes (LTs).² A general consensus exists that increased levels of cysteinyl LTs are key inflammatory mediators in AERD. However, a study of Japanese asthmatic patients demonstrated that prostaglandin D₂ was overproduced during aspirin-intolerant bronchoconstriction, and the urinary concentrations of

LTE₄ and metabolites of prostaglandin D₂ correlatively increased during the reaction.³

Because aspirin intolerance is found only in a specific population, genetic predisposition is considered a crucial determinant for the development of AERD. Candidate genes have concentrated on LT-related genes.^{4–7} However, conflicting results have been reported,^{8,9} and other genetic determinants remain to be identified. We have recently reported that the Arg16Gly β_2 -adrenergic receptor gene polymorphism in AERD is different from that in the patients with aspirin-tolerant asthma (ATA).¹⁰

AERD is known to be associated with higher female incidence, adult onset of asthma, less atopic tendency, persistent eosinophilic infiltration in the airway mucosa, and a more severe clinical course.¹ CD4⁺ T_H lymphocytes produce a number of cytokines, such as interleukins (ILs), and ILs secreted from T_H2 lymphocytes play a key role in bronchial asthma.¹¹ One of the critical cytokines produced by T_H2 lymphocytes is IL-13, which is involved in eosinophilia and airway hyperresponsiveness.¹² IL-13 overexpression in bronchial biopsy specimens and its increase in sputum are characteristics in severe asthmatic patients.¹³ T_H17 lymphocytes have recently been recognized as a distinct subset of T_H lymphocytes and secrete IL-17.¹⁴ The IL-17 family is composed of 6 members designated IL-17A through F, but T_H17

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lymphocytes particularly produce IL-17A and IL-17F.¹⁵ Accumulation of IL-17A and IL-17F messenger RNA has been shown in the bronchial submucosa of patients with moderate to severe asthma.¹⁶ Also, it has been reported that elevation of plasma IL-17A level is associated with asthma severity,¹⁷ and airway hyperresponsiveness positively correlates with IL-17A levels in the sputum of asthma patients.¹⁸ Recently, it has been demonstrated that human T_H17 cells, like mice,¹⁹ express IL-13 α_1 -receptor and that IL-13 attenuates IL-17A production.²⁰ Finally, a polymorphism in *IL-17F* that results in a loss-of-function mutation has been shown to be inversely related to asthma risk.²¹ However, there are few reports on the association of *IL-17A* gene polymorphisms with asthma.

The *IL-13* gene is located on chromosome 5q31-q33, a region frequently linked to asthma.^{22,23} Two of the most characterized single-nucleotide polymorphisms (SNPs) in *IL-13* include a promoter SNP ($-1111C>T$) and a coding SNP in exon 4 (Arg130Gln). The *IL-13* Arg130Gln polymorphism is associated with an elevated eosinophil count and high total serum IgE levels.^{24–26} Functional studies support a regulatory role associated with allergic inflammation for the $-1111C>T$ variant.^{26,27} Only 1 report has investigated the association between the *IL-13* gene polymorphism and AERD, the findings of which indicated that the allele and genotype frequencies of 2 promoter polymorphisms of the *IL-13* $-1510A>C$ and $-1055C>T$ gene and Arg110Gln polymorphism were not associated with AERD in a Korean population.²⁸

The *IL-17A* gene is located on chromosome 6p12.1, the genomic region associated with different types of asthma.^{29–31} A study on the association between asthma susceptibility and *IL-17A* gene polymorphisms in a Taiwanese population has shown that among 9 SNPs investigated, only 1 SNP ($-737C>T$) was associated with asthma, and the risk genotype of the SNP was the CC genotype.³² However, to our knowledge, no studies have evaluated its association with AERD. Taking this into account, we hypothesized that the *IL-13* and *IL-17A* gene polymorphisms might be involved in susceptibility to AERD.

To our knowledge, this is the first report of *IL-13* and *IL-17A* gene polymorphisms in Japanese patients with

AERD. The minor allele frequency of *IL-13* $-1510 A>C$ in an Asian population, including Japanese patients, is unknown in the dbSNP database of the National Center for Biotechnology Information. Therefore, we selected the polymorphisms of *IL-13* $-1111C>T$, *IL-13* Arg110Gln, and *IL-17A* $-737C>T$ as representative SNPs of the genes for the analyses in the present study.

METHODS

Patients and Clinical Assessment

This study was performed with the approval of the Institutional Ethics Committee of Gunma Institute for Allergy and Asthma, Gunma Hospital for Allergic and Respiratory Diseases, and written informed consent was obtained from each individual before the study commenced.

All study participants were nonsmoking, Japanese patients who were recruited from the outpatient clinics of Gunma Hospital for Allergic and Respiratory Diseases, Dokkyo Medical University Koshigaya Hospital, Yukawa Clinic of Internal Medicine, and Hiroshima Allergy and Respiratory Clinic in Japan. Characteristics of the study population are given in Table 1. Diagnosis of bronchial asthma was confirmed using the criteria of the Global Initiative for Asthma guidelines. All patients showed clinical symptoms that met the criteria for asthma, and their conditions were diagnosed by experienced pulmonologists. Forced expiratory volume in 1 second (FEV₁) was measured with a spirometer, and airway reversibility was defined as a greater than 12% and greater than 200-mL increase in volume in the first second of forced expiration from baseline after inhalation of short-acting β_2 -adrenergic bronchodilators. The diagnosis of AERD was made on the basis of either a positive result on lysine-aspirin challenge test⁵ or apparent history of more than 1 self-reported episode of bronchial response to aspirin or NSAID ingestion. The provocation test could not be applied to the participants who did not give written informed consent. Patients with AERD combined with aspirin-intolerant urticaria were excluded from this study. ATA was defined as bronchial asthma with no history of NSAID-induced asthma attack. All patients were in a stable clinical condition and had not been

Table 1. Clinical Characteristics of the Study Participants

Characteristic	AERD patients	ATA patients	Controls
No. of participants	95	300	100
Age, mean (SD), y	51.4 (13.4)	49.8 (15.9)	47.1 (13.6)
Male, No. (%)	26 (27.4)	107 (35.7)	34 (34.0)
FEV ₁ , mean (SD), % predicted	73.3 (11.5)	75.5 (24.5)	NA
Total serum IgE, mean (SD), IU/mL ^a	204.7 (233.6)	458.5 (651.5)	NA
Eosinophil, mean (SD), μ L ^b	712.2 (822.9)	321.0 (326.6)	NA
Atopy, No. (%) ^c	21 (22.1)	129 (43.0)	NA

Abbreviations: AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; FEV₁, forced expiratory volume in 1 second; NA, not applicable.

^a $P < .001$ for AERD patients vs ATA patients by the Welch *t* test.

^b $P < .001$ for AERD patients vs ATA patients by the Welch *t* test.

^c $P < .001$ for AERD patients vs ATA patients by the χ^2 test.

treated with oral corticosteroids. A total of 100 nonsmoking individuals with no history of bronchial asthma or other respiratory symptoms were selected from healthy volunteers who visited our clinic for annual routine physical examinations and comprised the healthy controls. The serum levels of total IgE and specific IgE antibodies were measured by the CAP system (Phadia, Uppsala, Sweden), and the patients with positive scores for house dust mite were classified as atopy. The sensitization was defined as a specific IgE level of 0.35 IU/mL or greater with a detection limit of 0.1 IU/mL as previously described.^{33,34} The total eosinophil count was measured in peripheral blood using a flow cytometer (Coulter Maxm; Beckman-Coulter Inc, Fullerton, California).

Genotyping of *IL-13* and *IL-17A* Polymorphisms

DNA in the specimens obtained by rubbing buccal mucosa by a cotton swab was extracted by using QIAamp 96 DNA blood kits (Qiagen, Hilden, Germany). The target DNA sequence of the *IL-13* -1111C>T was amplified using a set of primers (forward: 5'-TGGGGGTTTCTGGAGGAC-3', reverse: 5'-GCAGAATGAGTGCTGTGGAG-3') and that of Arg110Gln was amplified using a set of primers (forward: 5'-GGTCCTGTCTCTGCAAATAATG-3', reverse: 5'-GTTTCCAGCTTGCATGTCC-3'). The target DNA sequence of the *IL-17A* -737C>T was amplified using a set of primers (forward: 5'-CCCCCATCATGTCTCCTCTCC-3', reverse: 5'-CCAA-GCAACTTGGTGTTTTGAGG-3'). Allelic discrimination assay for SNPs relating to the expressions of *IL-13* -1111C>T, *IL-13* Arg110Gln, and *IL-17A* -737C>T (rs1800925, rs20541, and rs8193036, respectively) was performed by a SNP detective system as previously described.¹⁰ All study participants and investigators remained unaware of the genotype until the final analysis.

Statistical Analysis

Data are presented as mean (SD) or number (percentage) of observations, unless stated otherwise. Differences in the mean value of the phenotypic characteristics within the groups were compared using either the analysis of variance test or *t* test, and qualitative data were compared by the χ^2 test. Allele frequencies were estimated by the gene counting

method. Significant departures of genotype frequency from the Hardy-Weinberg equilibrium at each SNP were tested by the χ^2 analysis. Differences in minor allele frequencies of *IL-13* -1111C>T, *IL-13* Arg110Gln, and *IL-17A* -737C>T in AERD patients and healthy controls were compared with those of ATA patients by the χ^2 test. Logistic regression analysis was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Each gene polymorphism related to the asthma phenotype was examined by multivariable logistic regression models with adjustment for covariates, namely with the asthma phenotype as the dependent variable and independent variables that included age (continuous value), sex (male = 0, female = 1), and 2 alternative genotype models that combined the homozygous TT genotype and heterozygous CT genotype group and homozygous CC genotype. In addition, subgroup analyses with sex of the multivariable logistic regression analysis were performed, and the interactions between sex and the genotype were tested using the Wald statistic. Statistical analyses were undertaken using SPSS statistical software for Windows, version 17 (SPSS Inc, Chicago, Illinois). Our study is exploratory in nature. Therefore, no correction for multiple testing was performed. $P < .05$ was considered significant.

RESULTS

The clinical characteristics of the study participants are summarized in Table 1. No significant difference was found between AERD patients and ATA patients in terms of age, sex, and FEV₁ (percentage predicted). The levels of total serum IgE in AERD patients were lower than those in ATA patients ($P < .001$). AERD patients had a higher peripheral total eosinophil count compared with ATA patients ($P < .001$). A significant difference was found in the prevalence of atopy between AERD and ATA patients ($P < .001$).

Table 2 indicates the frequencies of the *IL-13* -1111C>T and Arg130Gln genotypes and the T minor allele in each group. The genotype distribution fulfills the Hardy-Weinberg equilibrium in each group. The frequencies of the T allele of the *IL-13* -1111C>T genotype in ATA patients (frequency of allele [q] = 0.120) and controls (q = 0.165) were similar,

Table 2. Genotype Frequencies of the *IL-13* Gene in Each Group^a

SNP loci	Genotype, No. (%)			Allele frequency	P value	HWE P value
	CC	CT	TT			
-1111C>T						
AERD patients (n = 95)	52 (54.7)	41 (43.2)	2 (2.1)	0.237	<.001	.06
ATA patients (n = 300)	232 (77.3)	64 (21.3)	4 (1.4)	0.120	–	.86
Controls (n = 100)	70 (70.0)	27 (27.0)	3 (3.0)	0.165	.10	.84
Arg130Gln						
AERD patients (n = 95)	60 (63.2)	29 (30.5)	6 (6.3)	0.216	.08	.34
ATA patients (n = 300)	210 (70.0)	84 (28.0)	4 (2.0)	0.160	–	.47
Controls (n = 100)	66 (66.0)	30 (30.0)	4 (4.0)	0.190	.32	.80

Abbreviations: AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; HWE, Hardy-Weinberg equilibrium.

^a Minor alleles in patients with AERD and controls were compared with those in patients with ATA by means of the χ^2 test. Values in bold indicate significant *P* values.

Table 3. Multivariable Logistic Regression Analysis and Subgroup Analysis With Sex of the *IL-13* Genotype in Japanese Patients With Aspirin-Exacerbated Respiratory Disease Compared With Those With Aspirin-Tolerant Asthma^a

SNP loci and genotype	OR (95% CI)		
	Multivariable	Male	Female
–1111 C>T			
CC	1.0	1.0	1.0
TT/CT	2.8 (1.7–4.6)	1.8 (0.7–4.4)	3.5 (1.9–6.3)
<i>P</i> value	<.001	.22	<.001
Arg130Gln			
CC	1.0	1.0	1.0
TT/CT	1.4 (0.8–2.2)	1.3 (0.5–3.1)	1.4 (0.8–2.5)
<i>P</i> value	.22	.62	.27

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

^a Multivariable logistic regression analysis was applied for age and sex and age as covariables. Values in bold indicate significant *P* values.

whereas the frequency in AERD patients was increased ($q = 0.237$). The frequency of the T allele in AERD patients was higher than that in ATA patients ($P < .001$). On the other hand, the frequencies of the T allele of the *IL-13* Arg130Gln genotype did not differ among the 3 groups.

Table 3 presents the results of multivariable logistic regression analysis of the *IL-13* –1111C>T and Arg130Gln genotypes, controlling for age and sex in AERD patients compared with those with ATA. Frequencies in the combined homozygous TT and heterozygous CT genotype group of *IL-13* –1111C>T were higher than those of the homozygous CC genotype in AERD patients compared with those with ATA ($P < .001$). The OR of AERD patients compared with ATA patients associated with the combined TT and CT genotype group compared with those with the CC genotype was 2.8 (95% CI, 1.7–4.6). Frequencies in the combined TT and CT genotype group of *IL-13* Arg130Gln were not different from those of the CC genotype group in AERD patients compared with ATA patients. Subgroup analyses with sex showed the positive association between asthma phenotype and the *IL-13* –1111C>T genotype in female patients but not in male patients, and in female patients with AERD the frequencies of the combined TT and CT genotype group were higher than those of the CC genotype group compared with

female ATA patients (OR, 3.5; 95 % CI, 1.9–6.3; $P < .001$) (Table 3). The interactions between sex and genotype were not significant ($P = .21$).

Frequencies of the *IL-17A* –737C>T genotype and the T minor allele in each group are given in Table 4. The genotype distribution fulfills the Hardy-Weinberg equilibrium in each group. No differences were found between the frequencies of the T allele in ATA patients ($q = 0.385$) and those of healthy controls ($q = 0.360$), whereas the frequency in AERD patients ($q = 0.279$) was lower than that in ATA patients ($P = .008$).

Table 5 gives the results of multivariable logistic regression analysis of the *IL-17A* –737C>T genotype, controlling for age and sex in AERD patients compared with those with ATA. Frequencies of the CC genotype were higher than those of the combined TT and CT genotype group in AERD patients compared with ATA patients ($P = .02$), and the OR for the CC genotype group compared with the combined TT and CT genotype group in AERD patients compared with ATA patients was 1.8 (95% CI, 1.1–2.9). Subgroup analyses with sex showed the positive association between asthma phenotype and the genotype in female but not in male patients, and in female patients with AERD the frequencies of the CC genotype were higher than those of the combined TT and CT genotype group compared with those with ATA (OR, 1.9; 95% CI, 1.1–3.2; $P = .03$) (Table 5). The interactions between sex and genotype were not significant ($P = .74$).

Comparison of the clinical characteristics in AERD patients according to the *IL-13* and *IL-17A* gene polymorphisms revealed that FEV₁ in the patients with the homozygous CC genotype of the *IL-13* –1111C>T gene was lower than that in the patients with the combined TT and CT genotype group ($P = .048$) but not those with the *IL-13* Arg130Gln gene polymorphism. The levels of total serum IgE and the prevalence of atopy were not different between the polymorphisms in each gene. AERD patients with the CC genotype of the *IL-17A* –737C>T gene had a lower peripheral total eosinophil count than did the patients in the combined TT and CT genotype group ($P = .03$) (Table 6).

DISCUSSION

We investigated the frequencies of the *IL-13* –1111C>T and Arg130Gln genotypes in 3 groups of patients (AERD patients, ATA patients, and healthy controls). The frequency of the T allele of the *IL-13* –1111C>T genotype, but not the

Table 4. Genotype Frequencies of the *IL-17A* –737 C>T Gene in Each Group^a

	Genotype, No. (%)			Allele frequency	<i>P</i> value	HWE <i>P</i> value
	CC	CT	TT			
AERD patients (n = 95)	47 (49.5)	43 (45.3)	5 (5.3)	0.279	.008	.22
ATA patients (n = 300)	107 (35.7)	155 (51.7)	38 (12.6)	0.385	–	.12
Controls (n = 100)	39 (39.0)	50 (50.0)	11 (11.0)	0.360	.53	.40

Abbreviations: AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; HWE, Hardy-Weinberg equilibrium.

^a Minor alleles in patients with AERD and controls were compared with those in patients with ATA by means of the χ^2 test. Values in bold indicate significant *P* values.

Table 5. Multivariable Logistic Regression Analysis and Subgroup Analysis With Sex of the *IL-17A* -737 C>T Genotype in Japanese Patients With Aspirin-Exacerbated Respiratory Disease Compared With Those With Aspirin-Tolerant Asthma^a

Genotype	OR (95% CI)		
	Multivariable	Male	Female
TT/CT	1.0	1.0	1.0
CC	1.8 (1.1–2.9)	1.6 (0.7–3.9)	1.9 (1.1–3.2)
P value	.02	.29	.03

Abbreviations: CI, confidence interval; OR, odds ratio.

^a Multivariable logistic regression analysis was applied for age and sex and age as covariables. Values in bold indicate significant *P* values.

Arg130Gln genotype, in AERD patients was higher than that in ATA patients. Frequencies of the combined TT and CT genotype group of *IL-13* -1111C>T were higher than those of the CC genotype group in AERD patients compared with ATA patients. In female patients with AERD but not in male patients, frequencies of the combined TT and CT genotype group of *IL-13* -1111C>T were higher than those of the CC genotype group compared with female ATA patients. It is well known that AERD is more commonly found in females,¹ and our data may correspond to this clinical finding. However, the interactions between sex and genotype were not significant.

One report from Korea indicated that AERD patients with the CC genotype of -1055C>T had a lower FEV₁ than those with the TT genotype.²⁸ In this study, we evaluated the association between *IL-13* -1111C>T polymorphism and AERD and found that FEV₁ in AERD patients with the CC genotype of the *IL-13* -1111C>T gene was lower than that in the patients with the combined TT and CT genotype group, suggesting that the *IL-13* -1111C>T polymorphism might

have a role in the development of AERD in a Japanese population. This result may support a regulatory role for the -1111C>T variant in asthma as reported.²⁷

As the second part of this study, we investigated the polymorphisms of *IL-17A* -737C>T in the 3 groups. The frequency of the T allele of *IL-17A* -737C>T in AERD patients was lower than that in ATA patients. Frequencies of the CC genotype of *IL-17A* -737C>T were higher than those of the combined TT and CT genotype group in AERD patients compared with ATA patients. In female patients with AERD but not in male patients, frequencies of the CC genotype of *IL-17A* -737C>T were higher than those of the combined TT and CT genotype group compared with ATA patients. However, the interactions between sex and genotype were not significant. This is the first report, to our knowledge, to suggest an association between the *IL-17A* gene polymorphisms and AERD.

The peripheral eosinophil count but not the serum IgE level in AERD patients has been shown to be higher than in ATA patients,^{1,35} corresponding to the results of this study. The present study provides evidence that AERD in the patients with the combined TT and CT genotype group of the *IL-17A* -737C>T gene had a higher peripheral total eosinophil count than the patients with the CC genotype. Although it has been demonstrated that *IL-13* Arg130Gln polymorphism is associated with elevated eosinophil count and high IgE level,^{25–27} no association was found between the clinical markers and the *IL-13* Arg130Gln gene polymorphism in this study. This work did not describe the mechanism linking the *IL-17A* genotype and the peripheral total eosinophil count. However, we may propose some possibilities. Namely, AERD is well known to be less atopic than ATA,¹ and this study demonstrated that the allele and genotype frequencies of the *IL-13* Arg110Gln gene were not associated with AERD in a Japanese population, suggesting that the *IL-13* Arg130Gln poly-

Table 6. Comparison of the Clinical Characteristics According to *IL-13* and *IL-17A* Gene Polymorphisms in Aspirin-Exacerbated Respiratory Disease Patients^a

SNP loci and genotype	FEV ₁ , mean (SD), % predicted	Total IgE, mean (SD), IU/mL	Eosinophils, mean (SD), /μL	Atopy, %
<i>IL-13</i> -1111C > T				
CC	71.2 (12.9)	201.0 (257.7)	653.8 (694.4)	15.8
TT/CT	75.8 (9.1)	209.3 (203.8)	783.0 (960.9)	6.3
P value	.048	.88	.48	.08
<i>IL-13</i> Arg130Gln				
CC	73.3 (11.1)	215.3 (249.1)	608.8 (684.7)	16.8
TT/CT	73.3 (12.4)	182.9 (200.3)	908.5 (1,020.9)	5.3
P value	.98	.56	.16	.16
<i>IL-17A</i> -737C > T				
TT/CT	72.7 (11.3)	243.1 (290.5)	905.1 (1,078.5)	11.6
CC	73.9 (11.9)	166.4 (152.0)	519.4 (362.5)	10.5
P value	.59	.14	.03	.85

Abbreviations: FEV₁, forced expiratory volume in 1 second; SNP, single-nucleotide polymorphism.

^a Differences in the mean value of the phenotypic characteristics within the patients were compared by *t* test, and qualitative data were compared by the χ^2 test. Values in bold indicate significant *P* values.

morphism may not be highly implicated in AERD. Interestingly, recent investigations have shown that the number of *IL-17A*-positive cells correlated with eosinophilic tissue inflammation, and *IL-17A* may play an important role in the eosinophil accumulation in tissues.^{36,37} It has also recently been demonstrated that human T_H17 cells express the *IL-13* α_1 -receptor and that *IL-13* attenuates *IL-17A* production.²⁰ So, with the results of this study, we hypothesize that the interaction between *IL-13* -1111C>T and *IL-17A* -737C>T gene sequence variations might be involved in the process to induce allergic inflammation associated with eosinophilic inflammation in AERD.

The present study has certain limitations. First, the number of the study participants was limited. The larger population, the more one can generalize the results. Second, the diagnosis of AERD was made on the basis of either a positive result on a lysine-aspirin challenge test⁹ or an apparent history of more than 1 self-reported episode of bronchial response to aspirin or NSAID ingestion. The clinical history indicated that drug hypersensitivity appeared less than 24 hours between administration of the suspected drug and the onset of reactive symptoms as recommended by the European Network for Drug Allergy.³⁸ Although placebo-controlled oral challenge tests are considered the best approach, the suspected drugs were not applied to the participants in this study because of patients' refusal and our safety policy of inpatient provocations only.³⁹ ATA was defined as bronchial asthma with no history of NSAID-induced asthma attack. Ideally, everyone in the study should have had the challenge test to be certain about aspirin intolerance. However, many of the patients with AERD were recruited from the outpatient clinics of the experienced pulmonologists in Japan. Further studies are required, including replication studies in another population, and it is obvious that generalizing is necessary for forming coherent interpretations in different situations.

In conclusion, we were the first to analyze the *IL-13* and *IL-17A* gene polymorphisms in Japanese patients with AERD and suggest that *IL-13* -1111C>T and *IL-17A* -737C>T gene sequence variations might have a role in the development of AERD. The results seem to be specifically relevant for female patients; however, interactions between sex and genotypes were not demonstrated.

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