

Lack of Association between the *IL13* Variant Arg110Gln and Susceptibility to Cedar Pollinosis in a Japanese Population

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Key Words

Allergic rhinitis · Candidate gene · Hay fever · Interleukin-13 · Japanese cedar pollinosis · Single nucleotide polymorphism

Abstract

Background: Interleukin (IL)-13 has come to be appreciated as a molecule critically involved in allergic inflammatory responses. Recent studies revealed that a common variant in the coding region of the *IL13* gene, Arg110Gln, has been implicated in the development of asthma and atopy. **Methods:** To assess whether the *IL13* variant Arg110Gln is associated with cedar pollinosis, one of the most common atopic diseases in the Japanese population, we examined the Arg110Gln variant using PCR-RFLP to compare the genotype and allele frequencies between 95 patients with cedar pollinosis and 95 healthy control subjects. Relationships between the

Arg110Gln variant and the pollinosis-related traits, e.g. rhinitis severity, eosinophil counts in nasal secretion and serum total and allergen-specific IgE levels, were also investigated. **Results:** The frequencies of the minor allele Gln110 were 25.8% in patients with cedar pollinosis and 30.9% in healthy control subjects ($p > 0.05$). There was also no significant difference in the genotype frequencies between cases and controls ($p > 0.05$). In addition, we found no significant association of the Arg110Gln variant with any of the pollinosis-related phenotypes ($p > 0.05$). **Conclusions:** Our data suggest lack of evidence for identifying the variant Arg110Gln at the *IL13* locus as a genetic risk factor involved in the development of Japanese cedar pollinosis.

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Introduction

The origin of allergy may be strongly influenced by a variety of environmental exposures; however, host susceptibility and a variety of genes are also likely to be involved in the etiology and pathogenesis of allergic diseases such as asthma and hay fever [1–3]. Japanese cedar pollinosis (JCP) is a springtime hay fever caused by inhalation of the pollen of Japanese cedar (*Cryptomeria japonica*), representing a major health problem in Japan because of its high prevalence, severe symptoms, impairment of the patient's quality of life and expenses in controlling the disease [4–6]. Recently, several candidate genes such as the *FCER1B* gene [7], the *IL4RA* gene [8], the *EPO* gene [8, 9] and the *ADAM33* gene [10], have been reported to underlie JCP and its intermediate phenotypes, suggesting a contributory role of genetic factors in the development of this common atopic disorder.

The type 2 cytokine IL-13, which shares signaling pathways and many biological activities with IL-4, plays a pivotal role in the generation of allergic airway inflammation [11–13]. To date, numerous genetic analyses have indicated that the gene encoding human IL-13 (located on chromosome 5q31) is implicated in the development of asthma and atopy [14, 15]. Of the *IL13* gene, Arg110Gln, which is a functional single nucleotide polymorphism (SNP) in the coding region [16, 17], has been comprehensively studied and has been found to be associated with asthma phenotypes in ethnically diverse populations [18–23]. Furthermore, recent genetic association studies in the German population [24] and Chinese samples [25] suggested a potential role of the *IL13* variant Arg110Gln (referred to as Arg130Gln in their reports) for heightened IgE production and atopic sensitization in allergic rhinitis/hay fever. To address whether this coding SNP affects susceptibility to JCP, the most common hay fever in Japan, we performed a case-control study in a Japanese population.

Subjects and Methods

Subjects and Phenotypes

The present study was performed with the approval of the Ethical Committee of the RIKEN Yokohama Institute, and written informed consent was obtained from all participants. 95 unrelated adult individuals with JCP and 95 age-matched unrelated healthy controls were enrolled in the study. All subjects were from the population of the Kinki area (west Japan).

The phenotypic characteristics of recruited subjects have been described in detail elsewhere [10] and are summarized in table 1. Briefly, of 95 patients with JCP, 5 cases (5.3%) were mild, 22 cases

Table 1. Phenotypic characteristics of recruited subjects

Category	Cases n = 95	Controls n = 95
Total serum IgE levels (means $\pm$ SD)		
log IU/ml	2.21 $\pm$ 0.51	1.48 $\pm$ 0.51
RAST positive to Japanese cedar pollen	95 (100%)	0 (0.0%)
RAST positive to house dust mites	43 (45.3%)	0 (0.0%)
Eosinophil positive in nasal secretions	82 (86.3%)	NA
Rhinitis severity		
Mild	5 (5.3%)	NA
Moderate	22 (23.2%)	NA
Severe	68 (71.6%)	NA

(23.2%) were moderate and 68 cases (71.6%) were diagnosed as severe according to the scores of three main nasal symptoms (sneezing, rhinorrhea and nasal obstruction) based on the clinical severity classification for allergic rhinitis (Okuda's method), as previously described [26]. None of the patients had a history of asthma and allergen-specific immunotherapy. The control subjects were all symptom free, had no history of atopic disorders and had negative allergen-specific IgE (<0.7 arbitrary unit/ml) in serum against house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), Japanese cedar pollen and three other common pollens in the study area (orchard grass, ragweed and *Artemisia*). The geometric mean of serum total IgE levels was 162.5 (range 5.3–10,000) IU/ml in cases and 30.0 (range 3.2–240) IU/ml in healthy controls. Patients with JCP had higher total IgE levels than control subjects (mean $\pm$ SD: 2.21 $\pm$ 0.51 vs. 1.48 $\pm$ 0.51 log IU/ml; $p < 0.0001$, t test).

Genotyping

DNA samples were extracted from whole peripheral blood of study subjects by standard methods. PCR reaction was performed with 5 ng of template genomic DNA, in a 10- μ l solution consisting of 13.75 pmol of each primer of 5'-tgacctcttgcctgcag-3' for forward and 5'-tgatgcttcgaagttcagtagatc-3' for reverse (italic nucleotides modified to create a *Bgl*II restriction site), 1.1 μ l of 10 $\times$ Vogelstein buffer (pH 8.8), 0.55 μ l of 78 mM MgCl₂, 0.55 μ l of 25 mM each dNTPs and 0.55 U of Ex-*Taq* DNA polymerase (TaKaRa Bio, Otsu, Japan). Thermocycling started with an initial denaturation step for 2 min at 95°C, and then 37 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 55°C and extension for 30 s at 72°C, with a final extension step for 7 min at 72°C. A 263-bp PCR fragment including the Arg110Gln polymorphism was then digested by addition of 3 U *Bgl*II (TaKaRa Bio) overnight at 37°C. The digestion products were visualized on a 4% agarose gel stained with ethidium bromide.

Statistics

Statistical analysis was performed using SPSS 10.0J for Windows (SPSS, Chicago, Ill., USA). The Hardy-Weinberg equilibrium was assessed by χ^2 test. The genotype and allele frequencies for the *IL13* variant Arg110Gln in cases and control subjects were compared using Pearson's χ^2 test. If an expected number was less than 5, Fisher's exact test was used. Quantitative traits relating to rhini-

Table 2. Genotype and allele frequencies for Arg110Gln variant

Category	Cases n = 95	Controls n = 94	Odds ratio (95% CI)	p value
Genotype				
Arg/Arg	0.568	0.479	–	
Arg/Gln	0.347	0.426	0.69 (0.38–1.26)	0.226
Gln/Gln	0.084	0.096	0.74 (0.26–2.08)	0.568
Allele				
Arg	0.742	0.691	–	
Gln	0.258	0.309	0.78 (0.50–1.22)	0.275
Major homozygote	0.568	0.479	–	
Minor homozygote + heterozygote	0.432	0.521	0.70 (0.39–1.24)	0.217
Major homozygote + heterozygote	0.916	0.904	–	
Minor homozygote	0.084	0.096	0.87 (0.32–2.36)	0.782

In healthy controls, 94 samples were successfully genotyped. The reference category was assigned an odds ratio of 1.00. CI = Confidence interval.

Table 3. Arg110Gln genotypes and total serum IgE levels

Category	Cases n = 95	Controls n = 94	Total n = 189
Total IgE (means $\pm$ SD), log IU/ml			
Arg/Arg	2.196 $\pm$ 0.442	1.486 $\pm$ 0.490	1.873 $\pm$ 0.583
Arg/Gln	2.263 $\pm$ 0.585	1.539 $\pm$ 0.544	1.866 $\pm$ 0.666
Gln/Gln	2.097 $\pm$ 0.632	1.213 $\pm$ 0.459	1.629 $\pm$ 0.697
p value	0.676	0.231	0.188 ^a

In healthy controls, 94 samples were successfully genotyped.

^a Analysis using a general liner model incorporating disease status (case or control) as a covariate.

tis severity and nasal eosinophila in patients with JCP were also analyzed with χ^2 test. Association of the Arg110Gln genotypes with total serum IgE levels (logarithm transformed) and cedar pollen-RAST scores was examined by ANOVA and general liner model. Nonparametric tests were employed to analyze associations between Arg110Gln genotypes and cedar pollen-specific IgE values in patient sera. Two-tailed p values of less than 0.05 were considered statistically significant.

Results

In our study population, the distributions of Arg110Gln genotypes of the *IL13* gene were in Hardy-Weinberg equilibrium, and the overall allele frequencies for Arg110 and Gln110 were 0.717 (271/378) and 0.283 (107/378), respectively. No significant association was detected between the Arg110Gln variant and susceptibility to JCP ($p > 0.05$, table 2). Moreover, this variant was not sig-

nificantly associated with rhinitis severity and nasal eosinophila in patients with JCP (outlined in table 1). The frequency of the minor allele Gln110 was 0.278 in severe cases compared to 0.250 in mild-to-moderate cases (odds ratio = 1.15, 95% confidence interval = 0.57–2.35; $p > 0.05$), and was 0.308 in patients with eosinophil-positive compared to 0.250 in those with eosinophil-negative nasal secretion (odds ratio = 1.33, 95% confidence interval = 0.54–3.30; $p > 0.05$).

An analysis was also carried out on the relationship between investigated genotypes and IgE measurements. We did not find significant differences in total serum IgE levels among the Arg110Gln genotypes in JCP patients, healthy controls and both groups combined ($p > 0.05$, table 3). There was no correlation between the Arg110Gln genotype and cedar pollen-RAST scores in our study population ($p > 0.05$). The Arg110Gln variant was also not significantly associated with cedar pollen-specific IgE val-

ues in sera from patients ($p > 0.05$). A tendency to lower cedar pollen-RAST scores was observed in those homozygous for Gln110 compared to those homozygous for Arg110 and heterozygous combined, but statistical significance was not reached ($p = 0.067$). In addition, we analyzed the allele and genotype frequencies of the Arg110Gln variant in JCP-affected individuals with or without sensitization to house dust mites and did not observe any significant association ($p > 0.05$).

Discussion

The *IL13* gene encodes a T-lymphocyte-derived cytokine, IL-13, which is produced primarily by activated Th2 cells. IL-13 has been shown to be an important and unique mediator of allergic processes such as IgE production, eosinophilic inflammation, mucus hypersecretion and airway hyperresponsiveness [27]. Recently, numerous SNPs have been identified at the *IL13* locus, and a significant association has been found between these SNPs and asthmatic and/or allergic phenotypes in several populations of distinct ethnic background [18–23, 28–32]. The role of a common coding SNP in the fourth exon that causes a substitution of the amino acid arginine by glutamine at position 110 of the mature protein (Arg110Gln) in the development of asthma and atopy has been widely investigated in ethnically diverse groups; however, less attention was directed to the genetic influence of this functional SNP on the risk of allergic rhinitis/hay fever.

This study represents an evaluation of the Arg110Gln variant in the *IL13* gene as a susceptibility locus for JCP, one of the most common seasonal allergic diseases in the Japanese population. Using a case-control study, we evaluated the Arg110Gln variant for evidence of association to JCP and related phenotypes. Based on the results, we found no evidence to support a significant association between the Arg110Gln variant and the diagnosis of JCP. We also noticed no significant association between this coding SNP and cedar-pollinosis-related traits including serum levels of total and allergen-specific IgE, eosinophil counts in nasal secretion and clinical severity of rhinitis. Our findings might indicate that genetic variation in Arg110Gln at the *IL13* locus is not likely to be involved in the development of JCP.

Of course, the lack of association in our study could reflect a type II error. However, a previous case-control study has shown no significant association of the *IL13* variant Arg110Gln with self-reported hay fever in a large

cohort of Germans [24]. Moreover, there was no relationship between this coding SNP and the diagnosis of allergic rhinitis due to *Artemisia* pollen and/or Der p 1 in a Chinese population [25]. For atopy-related phenotypes, Nieters et al. [24] found a marginal significance for the association ($p = 0.046$) of the Arg110Gln variant with in vitro specific IgE responses to common inhalant allergens in their study subjects, being almost completely of Caucasian origin, while Wang et al. [25] showed a borderline effect ($p = 0.039$) of this SNP on serum total IgE levels, but not on specific IgE concentrations against either *Artemisia* pollen or Der p 1, in Chinese patients with allergic rhinitis. Most recently, Miyazawa et al. [33] also reported a negative association of this SNP with JCP susceptibility and anti-Cry j 1 antibody titers in a small Japanese study cohort. Combined with the results of our study, these facts suggest that the Arg110Gln variant in *IL13* is unlikely to represent a major determinant in the development of hay fever and allergic sensitization in ethnically diverse populations.

Hay fever is a typical atopic disease characterized by type I hypersensitivity reactions following induction of IgE-sensitized mast cell release by allergenic pollens. Although T-cell activation is also a characteristic feature of allergic disorders in the upper and lower airways, increased T-cell activation is not consistently found in hay fever [34, 35]. This may explain the lack of association between the Arg110Gln variant of the *IL13* gene (encoding cytokine IL-13 produced principally by activated Th2 cells) and susceptibility to hay fever seen in our study as well as in others [24, 25, 33].

It should also be mentioned that several previous case-control association studies did not reveal any relationship between the *IL13* variant Arg110Gln and asthma per se or asthma-related traits including bronchial hyperresponsiveness, elevated total IgE levels and positive allergen skin tests [28, 36, 37]. However, these data were in contrast to studies showing an association of this SNP with asthma susceptibility [18, 23] or allergy phenotypes [20, 21, 23]. The effects of racial and ethnic differences in environmental and/or genetic risk factors on the development of complex common diseases [38] may account for the conflicting findings. Interestingly, a significant correlation was well demonstrated between the Gln110 variant and high total IgE levels [19, 29, 30], but not specific IgE against common allergens [19, 31], in an unselected population of German children. Consequently, it might be deduced that rather than controlling allergen-specific IgE responses, the Arg110Gln variant at the *IL13* locus may play a potential role in total serum IgE production during the early life.

In summary, we used a candidate gene approach to assess the possible association of the Arg110Gln variant in *IL13* with cedar pollinosis in a Japanese population. Our results indicate that this functional coding SNP does not contribute significantly to JCP susceptibility. It is worthy of note that, to our knowledge, this study is the first detailed investigation showing a genetic association between the *IL13* variant Arg110Gln and hay fever and its related traits, i.e. serum levels of total and specific IgE, nasal eosinophilia as well as disease severity. Further studies will be required to determine the relevance of other polymorphisms in the *IL13* gene as well as *IL13* haplotypes to the development of JCP in order to more accu-

rately define the potential causative polymorphism underlying the pathogenesis of the disease.

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