

Association between a C+33T polymorphism in the IL-4 promoter region and total serum IgE levels

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Summary

Background Susceptibility to asthma and other atopic diseases is known to be associated with elevated total IgE levels. Several investigators have linked the interleukin-4 (IL-4) gene and nearby markers located on chromosome 5q to elevated total IgE levels. A single nucleotide polymorphism in the IL-4 gene promoter region (C+33T) has recently been identified.

Objective As part of an effort to identify genetic variants contributing to the susceptibility to elevated total serum IgE levels, an association analysis of a newly identified promoter polymorphism (C+33T) with total serum IgE levels was conducted.

Methods and results The study was conducted using 240 Japanese subjects (120 asthmatics and 120 healthy controls). The IL-4 C+33T polymorphism was genotyped by PCR-restriction fragment length polymorphism analysis. The frequency of the T allele was 0.675 in asthmatic subjects and 0.671 in healthy controls. An ANOVA model adjusted for age, sex and disease status suggested a genetic association of C+33T polymorphism with elevated total serum IgE levels ($P < 0.05$).

Conclusion The data suggest that IL-4 promoter C+33T polymorphism may be one of the genetic polymorphisms that explain genetic linkage or association between elevated total serum IgE levels and markers on chromosome 5q.

Keywords: total serum IgE levels, IL-4 promoter, chromosome 5q, single nucleotide polymorphism, association analysis

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Introduction

An increased total serum IgE level is an important predisposing factor for developing atopic diseases, including bronchial asthma, allergic rhinitis and atopic dermatitis [1,2]. Total serum IgE levels appear to be determined by a combination of genetic and environmental factors, and it is anticipated that most of the genetic variance of total IgE levels will be encoded by alleles at several genes. There is significant evidence for a linkage between IgE phenotypes and chromosome 5q [3]. A single nucleotide (C to T at –589 bp relative to the open reading frame) polymorphism in the IL-4 promoter region has been associated with elevated total serum IgE levels [4].

Other studies, however, have failed to find any association of this promoter polymorphism with total serum IgE levels or asthma [5,6]. These conflicting findings may suggest the presence of other potential functional variants in the IL-4 promoter region that presumably influence IgE variation. A nucleotide substitution, for example, was identified within the OAP40 element located just upstream of the NF-AT site which appears to be largely responsible for the increased expression of the IL-4 gene in humans [7].

A new genetic polymorphism has recently been identified in the 5' flanking region of the IL4 gene [8]. As part of an effort to identify genetic variants contributing to the susceptibility to total serum IgE levels, an association analysis between a newly identified promoter polymorphism and total serum IgE levels in a Japanese population was conducted.

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Materials and methods

In this study, 240 Japanese subjects (120 asthmatic subjects and 120 healthy controls) were investigated. Unrelated subjects with bronchial asthma were recruited from the pulmonary clinic at the First Department of Medicine, Hokkaido University Hospital, and were included on the basis of history (the presence of cough, wheeze or dyspnea) with a demonstrated reversible airflow limitation (15% variability in forced expiratory volume in 1 second [FEV1.0] or in peak expiratory flow rate), increased airway responsiveness to methacholine, or both. Healthy controls, who had requested annual physical examinations, were included if they met the following criteria: (i) no history of bronchial asthma or any other allergic diseases and (ii) no medications. None of the subjects ($n = 240$) had smoked for at least 2 years prior to the start of the study, and 230 of the subjects had never smoked.

Total serum IgE levels were measured by a radio-immunosorbent test (IgE RIST) in all subjects. Specific IgE levels to house dust mite (HDM), *Dermatophagoides farinae*, were also determined in 120 asthmatic subjects. A specific IgE Ab level (IgE CAP RAST) equal to or more than 0.35 UA/mL was considered positive. Screening for the C+33T polymorphism (C to T at +33 bp relative to transcription initiation site) was performed by PCR-RFLP with restriction enzyme Mnl I, as described previously [8]. Screening was carried out for the C+33T polymorphism in a total of 480 chromosomes in Japanese subjects.

Statistical analysis

An allelic frequency distribution was tested according to a Hardy–Weinberg equilibrium by the χ^2 test.

A strong linkage disequilibrium had previously been reported between the +33T allele and the –589T allele in a Japanese population [8], and furthermore, it has been observed that the –589T allele is associated with elevated levels of total serum IgE [4]. Accordingly, differences in total IgE levels were examined between +33C/C and

+33C/T or +33T/T genotypes using a factorial ANOVA on a model which included disease status (asthmatic or healthy) and disease status*C+33T polymorphism in addition to age, sex and the C+33T polymorphism (SYSTAT, SYSTAT Inc., Evanston, IL, USA, 1992). Differences in total serum IgE levels between +33C/C and +33C/T or +33T/T genotypes were then examined using a similar multiple linear regression model in healthy controls and asthmatic subjects separately. Total serum IgE levels were log-transformed to normalize the distribution.

The genetic effects of the C+33T promoter polymorphism on asthma severity, as indicated by FEV1.0% at the initial visit, was also examined as a significant association has been described between the C-589T variant and FEV1.0 in asthmatic subjects in a Caucasian population (9).

Results

The C+33T genotypes were distributed according to a Hardy–Weinberg equilibrium ($P > 0.05$) in the whole population, indicating that asthmatic subjects and healthy controls were recruited from the same population. There was no difference in the frequency of the T allele between asthmatic subjects (0.675) and healthy controls (0.671).

Forty-six (38%) of the 120 asthmatic subjects in the study showed no responsiveness to HDM-antigen. The mean serum IgE levels were 2.40 log IU/mL (SD 0.62) in the 120 subjects with asthma, and 1.75 log IU/mL (0.6) in the 120 healthy subjects ($P < 0.001$) (Table 1). The combined +33T/T and +33C/T group ($n = 219$) had a mean total IgE level of 2.11 log IU/mL (SD 0.48), which was significantly higher than that of +33C/C homozygotes (1.89, SD 0.47; $P = 0.016$) (Table 2a). A factorial ANOVA performed on a model including sex, age, the C+33T genotype, disease status and genotype*disease status showed no interaction between the C+33T polymorphism and disease status (Table 2a). When the two groups were analysed separately, the C+33T polymorphism was found to be associated with total IgE levels ($P = 0.039$, Table 2b)

Table 1. Characteristics of 120 subjects with bronchial asthma and 120 healthy controls according to the C+33T genotype*

	Subjects with asthma			Healthy controls		
	C/C	C/T	T/T	C/C	C/T	T/T
No of subjects	11	56	53	10	59	51
Age (years)	51.7 $\pm$ 16.5	49.2 $\pm$ 17.5	47.8 $\pm$ 16.9	35.7 $\pm$ 13.0	44.2 $\pm$ 13.5	43.6 $\pm$ 14.2
Gender (F/M)	7/4	30/26	33/20	7/3	36/23	30/21
Total serum IgE (log IU/ml)	2.05 $\pm$ 0.48	2.40 $\pm$ 0.52	2.47 $\pm$ 0.60	1.56 $\pm$ 0.52	1.81 $\pm$ 0.60	1.83 $\pm$ 0.65

*Data are means $\pm$ SD.

Table 2. ANOVA models for total IgE levels*

(a) C/C vs C/T and T/T in a whole subject				(b) C/C vs C/T and T/T in 120 asthmatic subjects				(c) C/C vs C/T and T/T in 120 healthy controls			
Variables	Mean squares	F ratio	P	Variables	Mean squares	F ratio	P	Variables	Mean squares	F ratio	P
Age	1.793	5.683	0.018	Age	0.414	1.431	0.234	Age	1.755	5.116	0.026
Sex	4.803	15.225	< 0.001	Sex	1.872	6.465	0.012	Sex	2.88	8.399	0.004
C+33T	1.852	5.871	0.016	C+33T	1.264	4.367	0.039	C+33T	0.803	2.341	0.129
polymorphism†				polymorphism‡				polymorphism§			
Disease status¶	6.64	21.036	< 0.001								
C+33T polymorphism*	0.02	0.078	0.781								
disease status											

*The difference in total IgE levels according to C+33T genotypes was examined in two models (a) and (b, c) using ANOVA. †In model (a), log (total IgE) (IU/ml) was 1.81 ± 0.56 for C/C and 2.12 ± 0.55 for C/T and T/T. ‡In model (b), log (total IgE) (IU/ml) was 2.07 ± 0.54 for C/C and 2.43 ± 0.54 for C/T and T/T. §In model (c), log (total IgE) (IU/ml) was 1.52 ± 0.59 for C/C and 1.82 ± 0.59 for C/T and T/T. ¶Disease status: asthmatic subjects or healthy controls.

in the 120 asthmatics, but not in the 120 healthy controls ($P = 0.129$, Table 2c).

An unpaired *t*-test revealed no statistically significant relationship between the C+33T genotype and FEV1.0% (Mean FEV1.0% [SD] were 64.6 [13.6] and 66.9 [13.1], in subjects with +33C/C and +33C/T or +33T/T genotypes, respectively; $P > 0.1$).

Discussion

A major goal in these genetic studies is to understand the role of common genetic variants in the regulation of total serum IgE levels. Single nucleotide polymorphisms (SNPs) in the IL-4 promoter region are considered potential candidates to explain the linkage or association found between total serum IgE levels and chromosome 5q [3,4]. A mutation was recently described in the 5' untranslated region (UTR) of the IL4 gene [8] while in the present study, it was tested directly for any association with total serum IgE levels. One advantage of an association study is the potentially greater statistical power compared with linkage-based designs, and the present data demonstrated that the C+33T polymorphism was weakly associated with total serum IgE levels in a subset of the Japanese population. Given the nature of total serum IgE as a multifactorial phenotype [10], and a significant biological linkage between total serum IgE levels and IL-4 [11], the findings support the previous contention that IL-4 promoter polymorphisms may contribute to genetic regulation of total serum IgE levels [4].

The 5' UTRs of mRNA may contain *cis*-acting elements that control the translation, degradation and localization of transcripts. The 5' terminal nuclear modification of eukaryotic mRNAs, the cap[⁷mGpppN], also plays a critical role in both mRNA translation and stability [12]. Therefore, although the mechanisms underlying the association between the C+33T polymorphism and total serum IgE levels are not yet clear, the sequence variation of the C+33T polymorphism may affect the efficiency of either transcription or translation of the IL-4 gene, as well as the stability of mRNA. It has been hypothesized that frequent genetic variants may contribute significantly to the genetic risk for common and complex phenotypes [13] and that therefore, high prevalence of the +33T allele also provides a strong biological rationale for the involvement of this promoter polymorphism in the regulation of total serum IgE levels.

A transition from -589C to -589T at the IL-4 promoter region is associated with a greater luciferase report, and electrophoretic mobility shift assay (EMSA) has revealed that the pattern of protein binding of a -589C oligonucleotide is altered by a -589T oligonucleotide [4]. Therefore, another explanation for these findings may be the strong

linkage disequilibrium between the +33T allele and the -589T allele in the Japanese population [8]. Moreover, functional interactions between these two variants may also play some role in the regulation of IgE responsiveness in humans. In order to sort out these complex interactions, it is crucial to relate IgE phenotypes to haplotypes in these two loci using a large number of subjects, in addition to conducting a functional analysis of the C+33T polymorphism in relation to the C-589T polymorphism.

When the two groups were analysed separately, the C+33T polymorphism was still found to be associated with total IgE levels in the 120 asthmatics, but not in the 120 healthy controls. The lack of an interaction between the C+33T polymorphism and disease status suggested that the genetic effect of the C+33T polymorphism on total serum IgE levels is independent of disease status. Therefore, the weaker significance levels of the genetic association in two separate analyses may be partly due to decreased statistical power. There is, however, the possibility that the genetic effect of IL-4 promoter polymorphism might be stronger in asthmatic subjects than it is in healthy controls, and that several genetic and environmental factors that are unique to bronchial asthma are required for the polymorphism to influence total serum IgE levels.

Noguchi *et al.* previously reported an absence of an association between the C-589T polymorphism and total serum IgE levels using another subset of a Japanese population [14]. Asthma is believed to be a relatively heterogeneous disease in terms of its genetic and environmental background. There is considerable variation in age at onset of bronchial asthma, suggesting the presence of genetic heterogeneity in relation to age at onset. The mean age of asthmatic subjects in the present study was much higher than that in Noguchi's study (49.2 years vs 12.1 years). Moreover, asthmatic subjects in Noguchi's study showed very high IgE responsiveness to HDM antigen (mean log [HDM-specific IgE] is 1.54), while almost 40% of asthmatic subjects in our study showed no responsiveness to HDM-antigen. These differences between the asthmatic subjects of the two studies likely explain the conflicting results. Furthermore, additional genetic and environmental factors appear to influence the expression of genetic effects of the IL-4 promoter polymorphism on total serum IgE levels.

Although a significant association between the C-589T variant and asthma severity indicated by FEV1 in asthmatic subjects has been reported, this finding is limited to one Caucasian population [9]. Together with the present results, the genetic effect of the C+33T polymorphism on asthma severity appears to be dependent on other genetic or environmental backgrounds that are unique to Caucasian populations.

In conclusion, these findings suggest that the C+33T polymorphism in the 5' untranslated region of the IL-4 gene may be one of the genetic variants that, in part, explain the linkage or association found between total serum IgE levels and genetic polymorphisms on chromosome 5q.

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