

Polymorphism of the immune-braking gene CTLA-4 (+49) involved in gender discrepancy of serum total IgE levels and allergic diseases

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Summary

Background A variety of genes are related to allergic disorders in different ethnic populations. The genetic basis for the gender discrepancy of allergic diseases remains to be determined.

Objective This study was conducted to investigate whether IL-4 promoter (– 590 C/T) and cytotoxic T lymphocyte antigen 4 (CTLA-4) (+49 A/G) polymorphisms were correlated with a gender discrepancy of total IgE levels and allergic diseases in a Chinese population.

Methods A total of 1333 participants aged 19–49 years were enrolled in this study. Allergic diseases were recognized by the presence of asthma, rhinitis or atopic dermatitis in conjunction with detectable specific IgE in the blood. Polymorphisms of IL-4 promoter (– 590) and CTLA-4 (+49) were determined by restriction fragment length polymorphism.

Results Males or females with allergic diseases had higher total IgE levels than those without ($P = 0.000$). Females with the A/A genotype in the CTLA-4 (+49) position had significantly higher total IgE levels than those with A/G, and those with the G/G genotype had the lowest IgE levels (154.9 vs. 107.1 vs. 79.8 KU/L; mean log values: 1.79 vs. 1.65 vs. 1.54, $P < 0.001$). However, males with different genotypes in the CTLA-4 (+49) position exhibited no difference in the total IgE levels. Females with allergic rhinitis had a significantly higher frequency of the A/A genotype in the CTLA-4 (+49) polymorphism than those without atopic diseases ($P = 0.016$). In contrast, males with and without allergic disorders exhibited no significant difference in the CTLA-4 (+49) polymorphisms ($P > 0.05$). The IL-4 promoter (–590) polymorphisms, however, had no correlation with the total IgE levels or allergic diseases in either females or males.

Conclusion In females only, the CTLA-4 (+49), but not the IL-4 promoter (– 590), polymorphism was significantly associated with elevation of total IgE levels and allergic rhinitis. Here, we have, for the first time, demonstrated a gender-linked genetic relationship with allergic disease.

Keywords atopic diseases, gender, polymorphism, total IgE, IL-4, CTLA-4

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Introduction

Globally, the prevalence of allergic disorders has increased significantly in the last two decades [1–3]. Results from epidemiological studies suggest that atopic diseases are hereditary since monozygotic twins are more frequently affected than heterozygotic twins [4], and the offspring of affected parents also have a higher risk for developing the disease [5]. Cookson et al. [6] first proposed a linkage of chromosome 11q13 with asthma detectable only through the maternal line in certain families in Oxford. Later, many studies worldwide and in different ethnic populations demonstrated that there are at least 23 loci in 18 chromosomes associated with inheritance of asthma [7–9]. Asthma is frequently associated with allergic rhinitis or atopic dermatitis, suggesting that different atopic disorders are influenced

by the same genes. Other studies, however, have shown that atopic dermatitis may be due to unique genetic factors [10], and the gene–environmental interactions for asthma and allergic rhinitis may or may not be co-morbid [11].

The prevalence of allergic disorders is higher in males than in females [12]. The severity of allergic diseases, however, is higher in females [13]. The molecular basis of these gender discrepancies is not clear. Recently, cytotoxic T lymphocyte antigen 4 (CTLA-4), an important immune-braking molecule, has been shown to mediate the inhibition of allergic reactions [14, 15]. In a functional study, subjects with the CTLA-4 49(G/G) genotype were found to have a reduced CTLA-4 cell surface expression upon T cell activation [16]. There is no reference describing any functional discrepancy of the CTLA-4 gene between males and females. In this study, we propose that certain genes might be associated with gender discrepancy of allergic reactions. We investigated male/female discrepancies in the relationship between polymorphisms of the genes, including IL-4 (– 590) and CTLA-4 (+49), related to the serum total IgE levels and the presence of allergic disorders.

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Materials and methods

Subjects and materials studied

A total of 1333 adults were recruited for this study after informed consent was obtained. The sex ratio was even (667 male and 666 female). Subjects were recruited between September 1999 and September 2001 at Chang Gung Memorial Hospital (Kaohsiung, Taiwan). A research nurse was trained to explain the purpose of the study to eligible participants as they were having blood samples taken for routine physical check-ups. Allergic diseases including asthma, rhinitis or atopic dermatitis were reported by the participants in a short questionnaire. The content of this questionnaire included demographic data (age, gender and smoking history) as well as allergic history (asthma, allergic rhinitis and atopic dermatitis). These allergic entities were further recognized by detectable specific IgE to at least one of the six common allergens in Taiwan, including *Dermatophagoides pteronyssinus* (Der p), cockroach, dog dander, shrimp, peanut and pollen mixture in the blood. This choice of six common allergens was based on the results of a pilot study with 200 subjects to explore the popular allergens in the general Taiwanese population. The presence of specific IgE to common allergens ($\geq 5\%$ positive rate) was found as follows: *Dermatophagoides pteronyssinus* (Der p) (35.0%), cockroach (17.6%), dog dander (15.0%), shrimp (14.6%), peanut (7.0%) and pollen mixture (6.5%). Serum samples from these participants were separated by a centrifuge at 1600g for 15 min and stored at -70°C until analysis. The blood cells after serum collection were subjected to DNA extraction for the determination of CTLA-4 and IL-4 gene polymorphisms as described below.

Detection of total IgE and specific IgE levels

Serum total IgE levels and specific IgE antibodies in the sera from the participants were determined by the Pharmacia CAP system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden), which is a better *in vitro* test to detect allergic sensitization [17]. A full range of the serum total IgE detecting system was applied to measure the IgE levels from the participants.

Extraction of genomic DNA and analysis of CTLA-4 and IL-4 promoter polymorphism

Blood cells harvested after serum collection were subjected to DNA extraction with 0.5% SDS lysing buffer followed by proteinase K (1 mg/mL) digestion of nuclear protein for 4 h at 60°C [18]. Total DNA was harvested by standard phenol-chloroform extraction followed by 70% alcohol precipitation. As modified from our previous report [18], we amplified the PCR reactions (50 μL) of the CTLA-4 (+49 A/G) polymorphism using the forward primer 5'-GCT CTA CTT CCT GAA GAC CT-3' and the reverse primer 5'-CAA GCC AGA TTG AGT TTT TAC-3' for 35 cycles. The PCR products were incubated with the *Bbv*I restriction enzyme (New England Biolabs, Boston, MA, USA) at 37°C for 2 h before being subjected to a 2% agarose gel electrophoresis with ethidium bromide staining (Sigma, Inc., St Louis, MO, USA). The resultant bands of A/G genotypes showed one of three

possible results. There may have been a non-digested higher molecular weight single band, or two heterozygous bands representing partial digestion, or a complete digestion with a low molecular weight band. These represent the genotypes A/A, A/G and G/G, respectively.

Similarly, the IL-4 promoter (-590 C/T) polymorphism was also studied by restriction fragment length polymorphism. We amplified the PCR reactions with the forward primer 5'-ACT AGG CCT CAC CTG ATA CG-3' and the reverse primer 5'-GTT GTA ATG CAG TCC TCC TG-3' in a total 35 cycles of amplification. The PCR products were incubated with the *Bsm*FI restriction enzyme (New England Biolabs) at 37°C for 2 h before being subjected to a 2% agarose gel electrophoresis with ethidium bromide staining. Again, there were three possible band results. The C/C genotype samples appeared as a non-digested higher molecular weight single band, the C/T genotype samples were partially digested and appeared as two heterozygous bands, and the T/T samples were completely digested, appearing as a low molecular weight band.

Data analysis and biostatistics

A questionnaire interview provided each subject's history of asthma, rhinitis or atopic dermatitis. Allergic sensitization was further recognized by detectable specific IgE to at least one of six common allergens. Differences in the total IgE levels in subjects with different CTLA-4 (+49) and IL-4 (-590) genotypes were presented both with mean total IgE and mean log-transformed IgE levels with standard deviation (SD). The rationale for the presentation with log-transformed IgE levels is that the log-transformed IgE values, but not the serum total IgE levels, are normal distribution data. These normal distribution data are suitable for the biostatistic test by ANOVA followed by Pearson's correlation. In searching for a cut-off point of elevated serum total IgE levels suitably to present a high-risk group to atopy in the general population, we set up the cut-off at ≥ 100 KU/L, which is located above the 70th percentile in this study population, and in a previous report [19]. Associations of the CTLA-4 (+49) and IL-4 (-590) polymorphisms to subjects with atopic diseases and subjects with elevation of total IgE levels were assessed by the χ^2 test. A *P*-value < 0.05 was regarded as being statistically significant. SPSS (version 8.0, Chicago, IL, USA) statistical analysis software was used.

Results

Prevalence of allergic diseases in males and females

In this cross-section surveillance of adult participants, we found a 23.3% (310/1333) prevalence of allergic diseases defined by positive allergic history in conjunction with detectable specific IgE to at least one of six common allergens. The allergic disease prevalence was not significantly different between males and females (23.4% vs. 23.2%). The prevalence of adult asthma was 3.5% (46/1333), allergic rhinitis was 17.9% (239/1333) and atopic dermatitis was 5.0% (66/1333). Male and female subjects with detectable specific

IgE to at least one of six common allergens were 37.7% (257/667) and 32.2% (215/666), respectively.

Effects of gender, age and smoking on serum IgE levels

The subjects studied included 667 males and 666 females, aged between 19 and 49 years. Male subjects had a significantly higher total IgE level than female subjects, both in terms of mean total IgE levels and its mean log-transformed values ($P < 0.001$; Table 1). Subjects who smoked, but not younger or older, had significantly higher

Table 1. Influence of gender, age and smoking on total IgE and log-transformed IgE levels

Mean $\pm$ SD (KU/L)	Mean total IgE	Mean log IgE
Male subjects	149.51 $\pm$ 253.21	1.82 $\pm$ 0.55
Female subjects	100.39 $\pm$ 158.26	1.63 $\pm$ 0.59
<i>P</i> -values	<0.001	<0.001
Younger age (19–30 years)	115.22 $\pm$ 161.52	1.69 $\pm$ 0.61
Older age (31–49 years)	125.63 $\pm$ 208.64	1.72 $\pm$ 0.58
<i>P</i> -values	= 0.413	= 0.435
Non-smoking	112.17 $\pm$ 171.19	1.66 $\pm$ 0.61
Smoking	184.73 $\pm$ 282.63	1.90 $\pm$ 0.58
<i>P</i> -values	= 0.003	<0.001

The total subjects studied were 1333: 667 males and 666 females, respectively. Younger and older subjects were 612 and 721, respectively. Smoking and non-smoking subjects were 245 and 1088, respectively.

Table 2. Different IgE levels between allergic and non-allergic males and females

Mean $\pm$ SD (KU/L)	Male	Female	<i>P</i> -values
Non-allergic participants	511	512	
Mean total IgE	106.39 $\pm$ 216.03*	65.40 $\pm$ 126.67**	<0.001
Mean log IgE	1.68 $\pm$ 0.52	1.45 $\pm$ 0.54	<0.001
Allergic participants	156	154	
Mean total IgE	298.71 $\pm$ 313.38*	205.55 $\pm$ 193.59**	<0.001
Mean log IgE	2.29 $\pm$ 0.39	2.15 $\pm$ 0.39	<0.001

P-values were determined by Student's *t*-test. *Significant difference ($P < 0.001$) between male subjects with and without atopic diseases. **Significant difference ($P = < 0.001$) between female subjects with and without atopic diseases.

IgE levels (Table 1). Subjects with atopic disease had significantly higher total IgE levels than those without ($P < 0.001$; Table 2). Males with atopic diseases had significantly higher total IgE levels than females with atopic diseases ($P < 0.001$; Table 2).

Gender-dependent association of CTLA-4 polymorphism with serum total IgE levels

Analysis of the serum total IgE levels in subjects with different genotypes of the CTLA-4 (+49) polymorphism showed that females with the A/A genotype had higher total IgE levels than those with the A/G genotype, and those with the G/G genotype had the lowest mean total IgE levels (154.9 vs. 107.1 vs. 79.8 KU/L). Males with different (+49, A/A, A/G and G/G) genotypes, however, had no significant difference in the total IgE levels. Since the distribution of the serum total IgE levels studied is not a normal distribution, we performed a log transformation of total IgE levels, which reveals a normal distribution suitable for testing differences between or among subjects with different genotypes. As shown in Table 3, females with an A allele of the CTLA-4 (+49) polymorphism had significantly higher total IgE levels than those without ($P = 0.001$). Either atopic females ($P = 0.048$) or non-atopic females ($P = 0.002$) with the A/A genotype had significantly higher IgE levels than those with heterozygous G/A or homozygous G/G genotypes (no A allele). In contrast, male subjects with different CTLA-4 (+49) genotypes did not have significantly different total IgE levels ($P > 0.05$). As shown in Table 1, where subjects who smoked had higher IgE levels, we further analysed whether smoking had different effects on subjects with different CTLA-4 (+49) and IL-4 (–590) polymorphisms. We could only analyse smoking male subjects because 36.2% (242/667) of males had smoked, but only 0.4% (3/666) of females had smoked. We therefore analysed whether smoking and non-smoking male subjects had different IgE levels in different CTLA-4 (+49) and IL-4 (–590) polymorphisms. As shown in Table 4, smoking male subjects with different polymorphisms, although having higher IgE levels than non-smoking subjects, revealed no difference of IgE levels among subjects with different genotypes.

When we cut off the total IgE levels at ≥ 100 KU/L (≥ 70 th percentile) to reflect elevated IgE level or a risk to hypersensitivity, female subjects with elevated IgE

Table 3. Correlations of CTLA-4 (+49) polymorphisms with log IgE levels in males and females

	CTLA-4 (+49) in males				CTLA-4 (+49) in females			
	A/A	A/G	G/G	<i>P</i> -value	A/A	A/G	G/G	<i>P</i> -value
Total participants	1.82	1.87	1.76	0.071	1.79	1.65	1.54	0.001
SD	0.58	0.55	0.55		0.66	0.58	0.59	
Non-allergic	1.68	1.74	1.62	0.080	1.54	1.49	1.40	0.048
SD	0.57	0.53	0.51		0.62	0.53	0.54	
Allergic	2.27	2.28	2.27	0.961	2.33	2.21	2.05	0.002
SD	0.36	0.41	0.38		0.34	0.36	0.44	

Results are presented with log-transformed IgE levels with standard deviation (SD) since log-transformed IgE values instead of total IgE levels are normal distribution data. *P*-values were determined by ANOVA followed by Pearson's correlation.

Table 4. Influence of CTLA-4 and IL-4 polymorphisms on the IgE levels in smoking and non-smoking male subjects

	CTLA-4 (+49)			<i>P</i> -value	IL-4 promoter (–590)			<i>P</i> -value
	A/A	A/G	G/G		T/T	T/C	C/C	
Non-smoking	1.71	1.83	1.72	0.259	1.71	1.77	1.94	0.615
SD	0.57	0.55	0.58		0.57	0.64	0.59	
Smoking	1.82	1.98	1.81	0.227	1.93	1.90	1.80	0.936
SD	0.62	0.61	0.59		0.65	0.57	0.27	

Male smoking subjects were 36.2% (242/667), while female smoking subjects were only 0.4% (3/666). The influence of smoking on the IgE levels in subjects with different genotypes was analysed in male subjects only.

Table 5. Correlations of IL-4 promoter (–590) polymorphisms with log IgE levels in males and females

	IL-4 promoter (–590) in males			<i>P</i> -value	IL-4 promoter (–590) in females			<i>P</i> -value
	T/T	T/C	C/C		T/T	T/C	C/C	
Total participants	1.81	1.81	1.89	0.805	1.55	1.67	1.49	0.294
SD	0.57	0.60	0.39	0.826	0.64	0.61	0.57	0.166
Non-allergic	1.69	1.65	1.91		1.36	1.54	1.27	
SD	0.56	0.57	0.44	0.228	0.57	0.61	0.43	0.791
Allergic	2.32	2.31	1.88		2.02	1.95	2.04	
SD	0.33	0.40	0.24		0.44	0.32	0.41	

Data presented are log-transformed mean total IgE levels ($\pm$ SD). *P*-values were analysed by ANOVA followed by Pearson's correlation.

(≥ 100 KU/L) levels had a significantly higher A/A (15.3% vs. 7.8%) but lower G/G (34.4% vs. 47.1%) genotype than those without elevated IgE levels ($P = 0.002$). The genotype distribution was not significantly different between male subjects with and without elevated IgE levels ($P > 0.05$). As shown in Table 5, the IL-4 promoter (–590) C/T polymorphisms were not correlated with total IgE levels in male or female subjects. When the elevation of total IgE levels was cut off at ≥ 100 KU/L (≥ 70 th percentile), the IL-4 promoter (–590) polymorphism was not different between male or female subjects with and without elevated IgE ($P > 0.05$). There was no significant linkage disequilibrium between the CTLA-4 (+49) and IL-4(–590) polymorphisms.

Gender-dependent association of CTLA-4 polymorphism with different atopic diseases

We next examined correlations between the CTLA-4 (+49) or IL-4 promoter (–590) polymorphism and the presence of allergic rhinitis, asthma or atopic dermatitis. The A/A genotype frequency of the CTLA-4 (+49) polymorphism was significantly higher in females with allergic rhinitis ($P = 0.016$), but not asthma or atopic dermatitis, than in females without allergic diseases (Table 6). In contrast, the A/A genotype frequency of the CTLA-4 (+49) polymorphism was not significantly different in males with allergic rhinitis, asthma or atopic dermatitis ($P > 0.05$). The genotype spectrum of the IL-4 promoter (–590 C/T) polymorphism was not correlated with allergic rhinitis, asthma or atopic dermatitis in either male or female subjects ($P > 0.05$).

Table 6. Gender-dependent association of CTLA-4 (+49) polymorphisms with atopic diseases

	CTLA-4 (+49) in males			CTLA-4 (+49) in females		
	A/A	A/G	G/G	A/A	A/G	G/G
Total participants	86	312	269	65	312	289
Non-allergic*	65	236	211	44	240	227
Allergic	21	76	58	21	72	62
Asthma	5	12	6	4	12	7
Rhinitis*	13	52	46	21	53	54
Dermatitis	8	18	11	3	16	10

A total of 1333 (male, 667; female, 666) participants were studied. Females with allergic rhinitis tended to have a higher frequency of the A/A genotype in the CTLA-4 (+49) polymorphism than those without allergic disease, as indicated by an asterisk ($P = 0.016$; χ^2 test).

Discussion

Although a gender association has long been clinically recognized in certain autoimmune diseases such as Graves' disease and systemic lupus erythematosus, no genetic basis for this gender discrepancy has been demonstrated. Similarly, it has not been described if any genetic polymorphism is involved in the gender discrepancy of total IgE levels or atopic diseases. Certain autoimmune diseases had previously been linked with the CTLA-4 polymorphism at position 49 (codon 17) of exon 1 [20–22]. The G/G genotype at this

position has been associated with a higher frequency of diabetes, Graves' disease and Addison disease [20–22].

Here, we have, for the first time, demonstrated a gender-linked genetic relationship with allergic disease. The elevations of total IgE levels and allergic rhinitis are significantly associated with the presence of the A/A genotype in the CTLA-4 (+49) polymorphism in females, but not in males. Females, but not males, with the G/G genotype in the CTLA-4 (+49) polymorphism had the lowest total IgE levels, suggesting that the G/G genotype in the CTLA-4 position 49 may have an inhibiting effect on certain allergic reactions, or the A allele may augment allergic reactions only in females. Recently, a controversy has arisen after many studies with asthma subjects were performed to explore the association between the CTLA-4 polymorphism and both serum total IgE and asthma [23–25]. In a genome-wide scanning for asthma genes, the CTLA-4 locus in the chromosome 2q33 was shown to be a candidate locus for asthma [26]. Some other studies, however, showed that the CTLA-4 locus was not associated with asthma in Japanese and German populations [23, 24]. In contrast, Howard et al. [27] have recently shown that the +49 A/G single-nucleotide polymorphism was linked to elevated IgE levels in Dutch, White, African-American and Hispanic populations. A significant association was also found in the CTLA-4, -1147 C/T polymorphism with asthma and hyper-responsiveness in that study [27]. Taken together, these results indicate that correlations of the CTLA-4 polymorphisms to allergic disorders should be different in different genders and ethnic populations.

The elevation of serum IgE levels has long been implicated in allergic diseases [28, 29]. However, some studies have shown that the genes for IgE production and asthma are not completely co-localized [30]. IL-4 and IL-13 genes are known to regulate IgE production [31, 32]. Studies from IL-4 and IL-4/IL-13 receptor- α knockout mice, however, showed that allergic reaction could occur in these knockout mice [33, 34]. This suggests that there must be more than one pathway contributing to the induction of allergic diseases. In support of this probability, evidence has shown that male subjects have a higher prevalence of allergic disorders, while female subjects have greater severity [12, 13]; and we found that females with the CTLA-4, 49A/A genotype had almost the same total IgE levels as males, whereas females with the CTLA-4, 49G/G genotype had the lowest total IgE levels. These results suggest that there are many genes involved in the direct control and indirect regulation of IgE production as well as the occurrence and severity of asthma in males and females. The CTLA-4 gene may be one of the regulatory genes modulating IgE production in females, while the IgE regulatory gene for males and the IgE control gene(s) for both males and females have not yet been clarified. In addition, this study also highlights that the CTLA-4, 49G/G genotype in females may interact with gender-related genes or hormones to down-regulate IgE production and allergic reactions.

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