

Original article

Association between single nucleotide polymorphisms and wheat-dependent exercise-induced anaphylaxis in Chinese population

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Keywords: wheat-dependent exercise-induced anaphylaxis; single nucleotide polymorphism; association study

Background Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a complex disease resulting from interaction of environmental and genetic factors. The aim of this study was to investigate the association of three single nucleotide polymorphisms (SNPs) (*IL-4*-C590T, *IL-4RA* A1727G and *IL-10*-A627C) with WDEIA.

Methods SNP genotyping was conducted among the case subset composing 51 patients with WDEIA and four control subsets by sequencing DNA yielded from polymerase chain reaction (PCR). Statistical analysis of genotype/allele's frequencies between cases and controls were carried out through Fisher's exact test with the software of SPSS16.0.

Results For *IL-4*-C590T, there were statistically significant differences of genotype frequencies in case-control 1 ($P=0.03$) and case-control 4 ($P=0.001$) and statistically significant differences of allele frequencies in three case-control models (case-control 1: $OR=4.27$ (95% $CI=1.40-13.07$), $P=0.009$; case-control 3: $OR=1.99$ (95% $CI=1.13-3.50$), $P=0.02$; case-control 4: $OR=2.39$ (95% $CI=1.49-3.84$), $P=0.001$). All other association studies showed no statistically significant ($P>0.05$).

Conclusions *IL-4*-C590T may be related to the susceptibility of WDEIA, and the minor allele C might be a potential risk factor accounting for WDEIA. *IL-4RA* A1727G and *IL-10*-A627C might not be involved in the occurrence of WDEIA.

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Food-dependent exercise-induced anaphylaxis (FDEIA) is a peculiar form of food allergy where food-intake combined with exercises after ingesting the causative food will induces clinical symptoms.¹ The clinical symptoms usually include urticaria, angioedema, hypotension and possibly even anaphylaxis.² In 1979, Maulitz et al³ reported the first case of FDEIA, whose causative allergen was shellfish. Wheat-dependent exercise-induced anaphylaxis (WDEIA), a distinct wheat allergy, is the main form of FDEIA in Japanese patients.⁴ Susceptible population will suffer from WDEIA if they do exercises within 1–6 hours after ingesting wheat products. In 1985, Kushimoto et al⁵ published the first case report of WDEIA. The first report of WDEIA in Chinese population was reported by Yin and Wen⁶ in 2009.

WDEIA is a multiple-factor allergic disease resulting from interaction of environmental factors and genetic factors. The relative environmental factor, generally referring to the causative allergen, has been demonstrated to be ω -5-gliadin.⁷ Nevertheless, the genetic component accounting for the molecular mechanism of WDEIA is still unknown. Genetic association study is considered to be a powerful method on investigating the genetic factors underlying complex diseases these years.⁸ Thus, this study was conducted under this way.

In the present study, we performed a genetic association study to investigate the relationship between single

nucleotide polymorphisms (SNPs) and WDEIA. We chose three SNPs to be functionally important in each gene and to be associated with atopic diseases before,⁹⁻²⁰ which were *IL-4*-C590T (rs2243250), *IL-4RA* A1727G (rs1801275), and *IL-10*-A627C (rs1800872). For instance, *IL-4*-C590T was demonstrated to be associated with mite allergy in German children,¹¹ *IL-4RA* A1727G had influence on allergic asthma in Chinese population,¹⁹ and *IL-10*-A627C had an association with food allergy in Japanese population.¹⁸

To date, no study has been published to explore the genetic background of WDEIA. The aim of this study was to analyze the association of SNPs in three cytokine-related genes with WDEIA, and then tried to partly explain the human genetic factors of WDEIA.

METHODS

Study populations

This study consisted of five subsets. The first subset

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contained 51 WDEIA patients whose causative allergens were only wheat related food, and only had WDEIA without other allergic diseases. Inclusion criteria²¹ for this subset were: (1) skin symptoms, respiratory manifestations, and cardiovascular system symptoms were involved as clinical manifestations, and digestive symptoms might also be combined; (2) these symptoms above would be onset when patients did exercises within 1–6 hours after eating wheat products; (3) acute allergy was diagnosed through simultaneously detecting the concentration of allergens' specific immunoglobulin E (sIgE) to wheat, gluten and ω -5-gliadin; (4) patients would not suffer from WDEIA if they avoided eating wheat products or did not do exercises within 6 hours after eating wheat related foods; (5) FDEIA caused by other food rather than wheat related was excluded. Patients recruited into this subset met all of the five inclusion criteria. The second subset was regarded as Control1 including 17 patients with WDEIA and also other allergic disease. The third subset, Control2, consisted of 18 patients with FDEIA, whose causative allergens were other foods. The fourth subset was defined as Control3 comprising 72 patients with other allergic disease, such as pollen allergy, drug allergy rather than WDEIA or FDEIA. The fifth subset contained 203 healthy persons without any allergic diseases, and was named as Control4. Healthy people were enrolled based on a copy of questionnaire. Four criteria were used for recruiting Control4: (1) no allergic rhinitis; (2) no allergic asthma and cough; (3) no skin system allergic manifestations; (4) no food allergy. When people met all of the four criteria, they could be enrolled as candidates. Once candidates met that every concentration of sIgE to wheat, gluten or ω -5-gliadin is under 0.35 kU_A/L detected by fluorescent enzyme immunoassay (Phadia250 Detection System, ImmunoCAP, Phadia AB, Sweden), they could be qualified health control.

Ethics approval

This study was approved by the Ethics Committee of Peking Union Medical College Hospital of Chinese Academy of Medical Sciences.

sIgE antibody detection

A combination of detecting sIgE to wheat, gluten, and ω -5-gliadin in study populations was performed based on fluorescent enzyme immunoassay following manufactory instructions (Phadia250 Detection System).

DNA preparation and SNP genotyping

Genomic DNA was extracted from EDTA-anticoagulated whole blood samples using MiniBEST Universal Genomic DNA Extraction Kit Ver.3.0 (TaKaRa, Japan) according to the instruction manual, and then stored at –20°C until use.

Target DNA fragments for the three SNPs were amplified by polymerase chain reaction (PCR) using 2×EasyTaq PCR SuperMix (TransGen Biotech, China) according to

the manufacturer's protocol and subjected to automatic DNA sequencing. Primers for PCR reaction were reported before¹⁶ and shown in Table 1.

SNP genotyping was conducted by analyzing target DNA fragments' sequence with the software of Chromas 2.22 (Techelysium, Austrilia).

Statistical analysis

All statistical analysis was performed with the statistical software of SPSS 16.0 (SPSS Inc., USA). The chi-squared (χ^2) test was used to test Hardy-Weinberg Equilibrium (HWE) in study populations. Fisher's exact test was used to analyze statistical differences of genotype and allele frequencies between cases and controls. Binary Logistic regression was used to analyze the effect of different allele on sIgE levels (sIgE to wheat, gluten, and ω -5-gliadin) in WDEIA subjects. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Demographic characteristics

To explore the relationship of *IL-4*-C590T, *IL-4RA* A1727G and *IL-10*-A627C with WDEIA, we recruited and genotyped 51 cases with only WDEIA (case-subset), 17 patients with not only WDEIA but also other allergic disease (control 1), 18 patients with FDEIA (control 2), 72 patients with other allergic disease (control 3), and 203 healthy people without any allergic diseases (control 4). Age for the case-subset is the age of firstly triggering the onset of WDEIA, and age for control 1, control 2, control 3, and control 4 is the visiting age when they were recruited into this study.

Demographic characteristics for every subset were summarized in Table 2 including gender, age, and mean sIgE levels (wheat-sIgE, gluten-sIgE and ω -5-gliadin-sIgE).

Table 1. Forward and reverse primer sequences for the three SNPs

SNPs	Primer forward (F) and reverse (R)
<i>IL-4</i> -590C/T	F: 5'-CACTAAACTTGGGAGAACATGGT-3' R: 5'-TGCTTTGCATAGAAGGGA-3'
<i>IL-4RA</i> 1727A/G	F: 5'-CCCCACCAGTGGCTACC-3' R: 5'-GCCTTGTAACCAGCCTCTCCT-3'
<i>IL-10</i> -627A/C	F: 5'-GAAACATGTGCCTGAGAATCC-3' R: 5'-TTAGGCAGTCACCTTAGGTCTC-3'

Table 2. Demographic characteristics between the cases and controls

Characteristics	Case	Control 1	Control 2	Control 3	Control 4
Male (n)	25	9	6	36	93
Female (n)	26	8	12	36	110
Male:female	0.96:1	1.125:1	0.5:1	1:1	0.85:1
Age range (years)	12–69	12–71	5–47	5–71	21–77
Age (mean±SD, years)	37±14	34±17	22±10	35±14	37±11
Wheat-sIgE (kU _A /L)	1.53	2.66	0.22	1.29	0
Gluten-sIgE (kU _A /L)	3.70	3.78	0.06	0.53	0
ω -5-Gliadin-sIgE (kU _A /L)	13.63	7.31	0	0.17	0

Concentrations of sIgE to wheat, gluten and ω -5-gliadin in different subset were shown as a scatter diagram (Figure 1). Relevant value was regarded as 0 kU_A/L when sIgE level was below 0.35 kU_A/L among all study populations.

Genotype and allele frequencies

Genotype and allele frequencies were calculated in each subset (Table 3).

HWE in study populations

All subsets were in HWE with regard to *IL-4*-C590T, *IL-4*RA A1727G, and *IL-10*-A627C (data not shown). Thus, the association analysis could be performed among study populations.

IL-4-C590T is significantly associated with WDEIA

Genotype association analysis was summarized (Table 4). Statistically significant differences in genotype frequencies of *IL-4*-C590T existed between case and control 1 ($P=0.03$) and case and control 4 ($P=0.001$) under the model of CC vs. CT vs. TT. Comparing with healthy people, the homozygote CC (CC vs. (CT+TT)) conferred risk effect on inducing WDEIA ($OR=5.22$ (95% $CI=1.67-16.31$), $P=0.006$). Tests of homozygote of TT (TT vs. (CC+CT)) revealed that TT genotype offered significantly protection for WDEIA cases as compared to control 1, control 3, and control 4, respectively (case-control 1: $OR=0.22$ (95% $CI=0.06-0.75$), $P=0.02$; case-control 3: $OR=0.42$ (95% $CI=0.20-0.88$), $P=0.03$; case-control 4: $OR=0.39$ (95% $CI=0.21-0.72$), $P=0.004$), but not for WDEIA cases when compared with control 2 ($P>0.05$). However, all other genotype association studies showed no statistically significant ($P>0.05$).

Table 3. Genotype and allele frequencies in study populations (n (%))

SNP and genotypes/alleles	Case	Control 1	Control 2	Control 3	Control 4
<i>IL-4</i> -C590T (5q31.1)					
CC	7 (14)	0 (0)	0 (0)	5 (7)	6 (3)
CT	23 (45)	4 (24)	9 (50)	22 (31)	66 (32)
TT	21 (41)	13 (76)	9 (50)	45 (62)	131 (65)
CC+CT	30 (59)	4 (24)	9 (50)	27 (38)	72 (35)
CT+TT	44 (86)	17 (100)	18 (100)	67 (93)	197 (97)
C	37 (36)	4 (12)	9 (25)	32 (22)	78 (19)
T	65 (64)	30 (88)	27 (75)	112 (78)	328 (81)
<i>IL-4</i> RA A1727G (16p12.1)					
AA	35 (69)	13 (76)	13 (72)	49 (68)	135 (66)
AG	15 (29)	4 (24)	3 (17)	21 (29)	64 (32)
GG	1 (2)	0 (0)	2 (11)	2 (3)	4 (2)
AA+AG	50 (98)	17 (100)	16 (89)	70 (97)	199 (98)
AG+GG	16 (31)	4 (24)	5 (28)	23 (32)	68 (34)
A	85 (83)	30 (88)	29 (81)	119 (83)	334 (82)
G	17 (17)	4 (12)	7 (19)	25 (17)	72 (18)
<i>IL-10</i> -A627C (1q32.1)					
AA	22 (43)	6 (35)	4 (22)	26 (36)	95 (47)
AC	23 (45)	8 (47)	11 (61)	40 (56)	86 (42)
CC	6 (12)	3 (18)	3 (17)	6 (8)	22 (11)
AA+AC	45 (88)	14 (82)	15 (83)	66 (92)	181 (89)
AC+CC	29 (57)	11 (65)	14 (78)	46 (64)	108 (53)
A	67 (66)	20 (59)	19 (53)	92 (64)	276 (68)
C	35 (34)	14 (41)	17 (47)	52 (36)	130 (32)

Table 4. Genotype analysis of *IL-4*-C590T in case-control studies

Case-Control	CC vs. CT vs. TT (P values)	CC vs. (CT+TT)		TT vs. (CC+CT)	
		P values	OR (95% CI)	P values	OR (95% CI)
1	0.03*	0.18	0.86 (0.77-0.96)	0.02*	0.22 (0.06-0.75)
2	0.32	0.18	0.86 (0.77-0.96)	0.59	0.70 (0.24-2.06)
3	0.06	0.23	2.13 (0.64-7.14)	0.03*	0.42 (0.20-0.88)
4	0.001*	0.006*	5.22 (1.67-16.31)	0.004*	0.39 (0.21-0.72)

* $P<0.05$.

The results of allele association analysis were

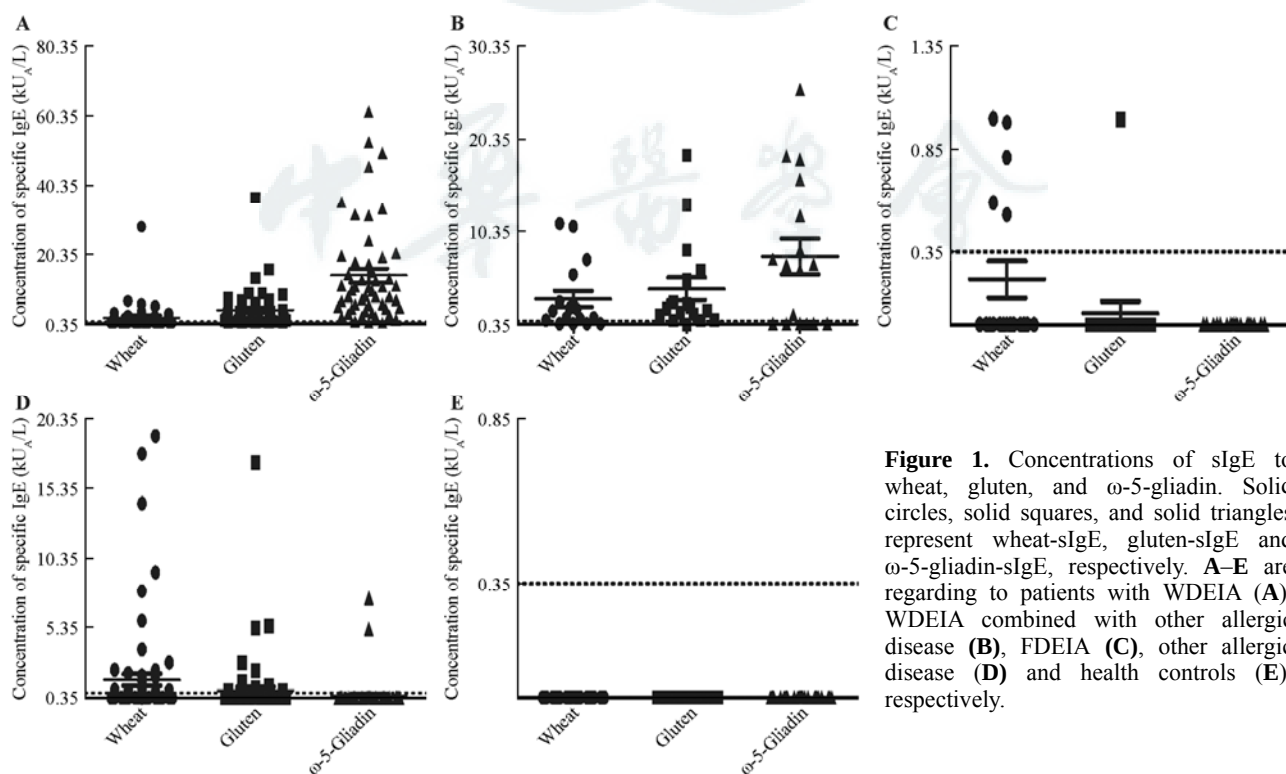


Figure 1. Concentrations of sIgE to wheat, gluten, and ω -5-gliadin. Solid circles, solid squares, and solid triangles represent wheat-sIgE, gluten-sIgE and ω -5-gliadin-sIgE, respectively. A-E are regarding to patients with WDEIA (A), WDEIA combined with other allergic disease (B), FDEIA (C), other allergic disease (D) and health controls (E), respectively.

summarized (Table 5). There were statistically significant differences of allele frequencies distribution between case and control 1, case and control 3, and case and control 4 (case-control 1: $OR=4.27$ (95% $CI=1.40-13.07$), $P=0.009$; case-control 3: $OR=1.99$ (95% $CI=1.13-3.50$), $P=0.02$; case-control 4: $OR=2.39$ (95% $CI=1.49-3.84$), $P=0.001$). Therefore, the minor allele C might be the potential risk factor for WDEIA. No significant association was observed in case-control 2 ($P>0.05$).

Neither *IL-4RA* A1727G nor *IL-10-A627C* shows association with WDEIA

No statistically significant differences of genotype or allele frequencies of *IL-4RA* A1727G or *IL-10-A627C* were observed in any model of case-control studies ($P>0.05$) (Tables 6–9).

Three SNPs are not associated with sIgE levels in WDEIA subjects

We then compared sIgE levels (sIgE to wheat, gluten, and ω -5-gliadin, respectively) between the group with only minor allele (CC for *IL-4-C590T*, GG for *IL-4RA* A1727G, and CC for *IL10-A627C*, respectively) and the group with major allele (CT+TT for *IL-4-C590T*, AA+AG for *IL-4RA* A1727G, and AA+AC for *IL10-A627C*, respectively), and found that there were no statistical differences in sIgE levels between the two

Table 5. Allele analysis of *IL-4-C590T* in case-control studies

Case-control	C vs. T (<i>IL-4-C590T</i>)	
	P values	OR (95% CI)
1	0.009*	4.27 (1.40–13.07)
2	0.30	1.71 (0.73–4.02)
3	0.02*	1.99 (1.13–3.50)
4	0.001*	2.39 (1.49–3.84)

* $P<0.05$.

Table 6. Genotype analysis of *IL-4RA* A1727G in case-control studies

Case-control	AA vs. AG vs. GG (<i>P</i> values)	studies			
		AA vs. (AG+GG)		GG vs. (AA+AG)	
		<i>P</i> values	<i>OR</i> (95% <i>CI</i>)	<i>P</i> values	<i>OR</i> (95% <i>CI</i>)
1	0.82	0.76	0.67 (0.19–2.39)	1.00	0.98 (0.94–1.02)
2	0.17	1.00	0.84 (0.26–2.76)	0.17	0.16 (0.01–1.88)
3	1.00	1.00	1.03 (0.48–2.22)	1.00	0.70 (0.06–7.93)
4	0.94	0.87	1.10 (0.57–2.13)	1.00	1.00 (0.11–9.10)

Table 7. Allele analysis of *IL-4RA* A1727G in case-control studies

Case-control	A vs. G (<i>IL-4RA</i> A1727G)	
	P values	OR (95% CI)
1	0.59	0.67 (0.21–2.14)
2	0.80	1.21 (0.46–3.20)
3	1.00	1.05 (0.53–2.07)
4	0.89	1.08 (0.60–1.92)

Table 8. Genotype analysis of *IL-10-A627C* in case-control studies

Case-control	AA vs. AC	AA vs. (AC+CC)		CC vs. (AA+AC)	
	vs. CC (P values)	P values	OR (95% CI)	P values	OR (95% CI)
1	0.74	0.78	1.39 (0.45–4.34)	0.68	0.62 (0.14–2.82)
2	0.30	0.16	2.66 (0.77–9.19)	0.69	0.67 (0.15–3.00)
3	0.51	0.46	1.34 (0.64–2.80)	0.55	1.47 (0.45–4.84)
4	0.88	0.75	0.86 (0.46–1.60)	0.81	1.10 (0.42–2.87)

Table 9. Allele analysis of *IL-10-A627C* in case-control studies

Case-control	A vs. G (<i>IL-10-A627C</i>)	
	P values	OR (95% CI)
1	0.54	1.34 (0.61–2.97)
2	0.23	1.71 (0.79–3.71)
3	0.79	1.08 (0.64–1.84)
4	0.72	0.90 (0.57–1.43)

different groups in the first subset (Figure 2).

DISCUSSION

In the present study, we performed the study to examine the association of three SNPs (*IL-4-C590T*, *IL-4RA* A1727G and *IL-10-A627C*) with WDEIA. We found that *IL-4-C590T* was associated with WDEIA, while neither genotype frequencies distribution nor allele frequencies distribution were observed to have statistically significant differences between cases and controls for *IL-4RA* A1727G or *IL-10-A627C*.

Morita et al⁴ reviewed that WDEIA was the main form of FDEIA and held 57% in Japanese patients with FDEIA. Of all WDEIA patients observed in outpatient clinic, some only suffered from WDEIA, while others had not only WDEIA but also other allergic diseases, such as pollen disease, food allergy, drug allergy, and so on. We assumed that the molecular mechanisms underlying the development of WDEIA, WDEIA combined with other allergic diseases, FDEIA (causative allergens were not wheat related products), and other allergic diseases were not the same. Based on this hypothesis, we distributed patients and healthy controls into five different subsets, when statistical analysis was performed to investigate the relationship of three SNPs with WDEIA under different models of case-control studies.

Our analysis results showed that *IL-4-C590T* had an association with WDEIA. Statistically significant differences of genotype frequencies were observed between case and control 1. Homozygote TT might play a protective role in these patients with WDEIA combined with other allergic disease, but from those patients only with WDEIA. No significant differences were observed between case and control 2 either for genotype frequencies or for allele frequencies. Between case and control 3, significant differences were observed and were only under the model of TT vs. (CC+CT), but not under the other two models (CC vs. CT vs. TT and CC vs. (CT+TT)) (Table 4). We observed that minor allele C should be a risk factor and major allele T be a protective factor for the onset of WDEIA (Tables 4 and 5).

We also analyzed the association between the *IL-4-C590T* genotypes and serum specific IgE levels (sIgE to wheat, sIgE to gluten, and sIgE to ω -5-gliadin, respectively) in WDEIA patients, but found no significant association (Figure 2). So multi-factors might be related to the onset of WDEIA, which is a complex allergic disease resulting from interaction of environmental factors and genetic

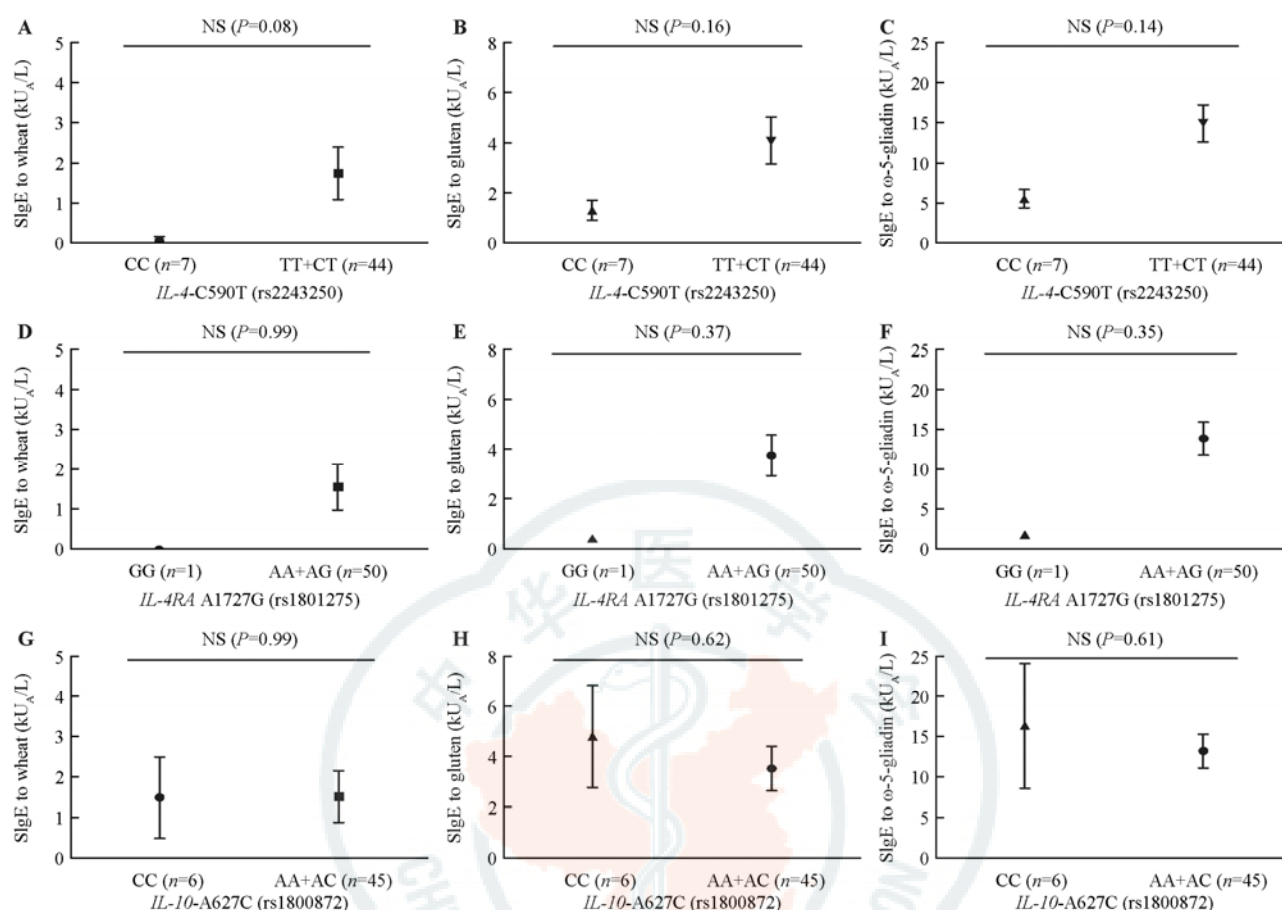


Figure 2. Comparison of sIgE levels between the group with only minor allele and the group with major allele. **A**, **B**, and **C** were respect to *IL-4-C590T*; **D**, **E**, and **F** were respect to *IL-4RA A1727G*; **G**, **H**, and **I** were respect to *IL10-A627C*. NS: nonsignificant.

factors. Serum specific IgE levels of wheat specific IgE, gluten specific IgE and ω -5-gliadin specific IgE are related to WDEIA and they have the value of 0.35 kU_A/L as the threshold for this disease. However, serum total IgE level is not directly associated with WDEIA and it has no elevated total IgE values for this disease. For example, a patient with WDEIA might have a less serum total IgE level than a healthy person without any kind of allergic disease. Therefore, we did not analyze the relationship between the *IL-4-C590T* genotypes and serum total IgE levels, though in other studies¹² *IL-4-C590T*, as a genetic factor of atopic allergy, is associated with serum total IgE levels.

The relationship between *IL-4-C590T* and allergic diseases has been studied in many researches before; however, the results were contradictory. Liu et al¹¹ found that -C590T had the effect on sensitization to mite depending on Der p 1 allergen concentration's levels in German children and underlined the importance of gene-environment interaction for this polymorphism role in the development of specific sensitization. Studies from de Guia et al¹² demonstrated that -C590T was a potential risk factor to and correlated with atopic allergy in Philippine allergic individuals. Amirzargar et al²² reported that -C590T was associated with bronchial asthma and did contribute to the association between haplotypes and serum total IgE level in Iran population. However,

Korzycka-Zaborowska et al²³ did not find the association of -C590T with atopy in Polish population, and Lesiak et al²⁴ did not observe the association with severity of atopic dermatitis (AD) in Australian population, either. These contrary results yielded from different studies might partly result from the complexity of allergic diseases. Other reasons, inclusion criteria and exclusion criteria e.g., might be taken into account, too.

Early studies have reported that *IL-4RA A1727G* and *IL-10-A627C* were associated with allergic diseases, such as *IL-4RA A1727G* with allergic asthma,^{14,19} and *IL-10-A627C* with food allergy^{16,18} and β -lactam allergy,¹⁷ and so on. However, in our study, *IL-4RA A1727G* and *IL-10-A627C* were not found to be associated with WDEIA, a distinct kind of allergic diseases. Several reasons, such as differences of race, stratification of samples, size of sample, study method chosen, and so on, may explain the different statistical results between our study and earlier studies.

In addition to the study of genotype and allele frequencies differences between cases and controls, we also investigated the effect of different genotype on sIgE levels among WDEIA subjects. When comparing sIgE levels between the group with major allele and the group with only minor allele in the first subset, no association was observed for all of the three SNPs. The result

consists with Dizier et al's study in 1999, which excluded the contribution of *IL-4-C590T* to IgE variation by linkage analysis in 234 families including 1020 subjects.²⁵ Data presented by Walley et al²⁶ and Korzycka-Zaborowska et al²³ also shown that *IL-4-C590T* was not associated with sIgE concentrations. More interesting and meaningful results might be yielded if we further perform the association of different genotype with different severity degree of WDEIA's clinical manifestation in a larger size of samples.

Several studies have been carried out to explain the mechanism of WEDIA.²⁷⁻³² Of all these findings, antibody (IgE/IgG1)-mediated mechanism is thought to be the most pivotal one.³⁰ In terms of this theory, when allergic patients did exercises after ingestion of wheaten foods, exercises could enhance the absorption of allergens into the gastrointestinal tract,³³ followed by the producing of larger amounts of antibodies. Because of sufficient allergens and allergen-antibody complexes, both IgE-mediated classical pathway and IgG1-mediated activating pathway could elicit systemic anaphylaxis.³⁰ The concentration of IgE/IgG1 in circulate humor was adjusted by some cytokines. Some studies demonstrated that the regulatory cytokine of IL-10 could down-regulate IgE/IgG1 concentration, while IL-4 and IL-5 would confer up-regulation effect;³⁴ according to animal model researches, IL-4 also could exacerbate the cross-linking of FcεRI or FcγRIII, and then induce severe allergic reaction with the cooperation of IL-4Rα and Stat6.³⁵ Thus, *IL-4*, *IL-4RA*, *IL-5*, *IL-10*, and *Stat6* were important candidate genes for investigating the genetic factors underlying the development of WDEIA. So, three SNPs (*IL-4-C590T*, *IL-4RA* A1727G and *IL-10-A627C*) in this study were appropriate choice. Though *IL-4RA* A1727G or *IL-10-A627C* did not have association with WDEIA, the statistically significant may be observed under case-control studies based on larger samples or under a family-based study.

Elucidating the genetic background underlying WDEIA is helpful for better understanding the pathogenesis of this disease. However, for the study of complex diseases like WEDIA, investigating relationships between gene-gene interaction and gene-environment interaction might be more effective methods rather than single locus association analysis. Meanwhile, exome sequencing and whole-genome sequencing maybe considered as more effective tools for uncovering the roles of human genetic factors for the onset of WDEIA in further studies.^{36,37}

In conclusion, our present study showed a SNP (*IL-4-C590T*) associated with WDEIA, which was the first association study ever performed between WEDIA and susceptible locus, and partly explored the molecular mechanism underlying the development of WDEIA.

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