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Genetic variation at the *interleukin-18* locus is associated with wheat-dependent exercise-induced anaphylaxis in the Han Chinese population

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Running Title: Genetic variation at the *IL-18* gene and WDEIA

Abstract

Objective: To investigate the association of single nucleotide polymorphisms (SNPs) at the *interleukin (IL)-18* gene locus with wheat-dependent exercise-induced anaphylaxis (WDEIA) in the Han Chinese population.

Method: 130 patients with WDEIA and 600 healthy subjects were recruited for this study. Three tag SNPs (rs360729, rs360717 and rs1946518) were selected and genotyped, and total IgE and sIgE levels were measured using the ImmunoCAP system.

Results: After Bonferroni correction, the frequency of the G-allele in rs1946518 in the WDEIA group was significantly higher than that in control group ($P = 0.0015$). Genotypic distributions of rs1946518 significantly differed between WDEIA and control groups, and compared with the TT genotype, the homozygote GG genotype was associated with a higher risk of WDEIA ($P = 0.0031$). At position rs1946518, the log-transformed total IgE values were significantly higher in the group with the heterozygous GT genotype than in TT genotype group ($P = 0.0024$). Haplotype AGG formed by three SNPs alleles occurred significantly more frequently in WDEIA group than in control group ($P = 0.003$).

Conclusion The minor allele G at position rs1946518 might serve as a marker for the risk of WDEIA.

Key words: Wheat-dependent exercise-induced anaphylaxis; *Interleukin-18*; Single nucleotide polymorphisms; Immunoglobulin E

1. Introduction

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a relatively rare but potentially severe immunoglobulin E (IgE)-mediated food allergy. WDEIA is clinically characterized by anaphylactic reactions, including urticaria, angioedema, dyspnea, hypotension, and shock. WDEIA is a special form of food-dependent exercise-induced anaphylaxis (FDEIA) that includes reactions to many other foods, such as shellfish, vegetables, nuts, and rice [1,2]. In a recent study on the Chinese population, 57% of severe episodes of food anaphylaxis were triggered by wheat [3]. There is a general agreement that IgE-dependent mast cell degranulation and histamine release are key factors involved in WDEIA [4,5]; however, the precise underlying mechanisms and genetic background of the disease still remain unclear.

Interleukin-18 (IL-18) belongs to the IL-1 superfamily of proinflammatory cytokines, which has been known to be involved in allergic inflammatory reactions by influencing the balance of Th1/Th2 immune response [6,7]. Serum levels of IL-18 in patients with allergic rhinitis (AR) [8], allergic asthma (AA) [9], and atopic dermatitis (AD) [10] are higher than those in healthy controls. Several important polymorphic sites in the *IL-18* gene, including two in the promoter region (-607C/A and -137G/C) have been reported in different allergic diseases, including AA, AR, AD, and drug allergies [11]. However, the role of *IL-18* gene polymorphisms in WDEIA or FDEIA has not been reported yet. In the present study, in order to understand the exact role of *IL-18* in the development of WDEIA and food anaphylaxis, we determined the distribution of three single nucleotide polymorphisms (SNPs) of the *IL-18* gene (rs360729, rs360717, and rs1946518) in Han Chinese patients with WDEIA and controls. Serum total IgE and antigen-specific IgE (sIgE) levels were measured and analyzed for regulatory effects of *IL-18* genotypes on IgE production.

2. Material and methods

2.1. Subjects

130 patients with WDEIA and 600 healthy control subjects were included in this study. WDEIA samples were collected from patients who were examined at the Peking Union Medical College Hospital in Beijing, China, from 2008 to 2015. Diagnosis of WDEIA was standardized based on a definitive episode plus confirmation of sIgE levels using the following inclusion criteria [12]: (1) skin symptoms, respiratory manifestations, and cardiovascular system symptoms were involved as clinical manifestations and may occur in combination with digestive symptoms; (2) symptoms mentioned in (1) were triggered when patients engaged in physical exercise within 1–6 h after wheat product ingestion; (3) an acute allergy was diagnosed by simultaneous detection of the concentration of sIgE to wheat, gluten, and ω -5-gliadin. Specially, patients did not suffer from WDEIA if they avoided ingesting wheat products or did not exercise within 6 h after wheat product ingestion; moreover, patients who showed FDEIA caused by foods other than wheat were excluded. All the control subjects were healthy individuals who had no history of food allergy, AR, allergic asthma and cough, AD and any other allergic manifestations, who were recruited from individuals on regular physical check-ups in the same hospital and from healthy volunteers who were living or working in Beijing. The present study was approved by the Ethics Committee of the Peking Union Medical College Hospital of the Chinese Academy of Medical Sciences. Written informed consent was obtained from all participants.

2.2. Total IgE and sIgE detection

Peripheral blood was collected from all participants, and total IgE and sIgE levels were measured using the ImmunoCAP system (Phadia250 Detection System, Phadia AB, Uppsala, Sweden) according to the manufacturer's instructions [13]. The

sIgE antibody levels were measured for three allergens: wheat, gluten, and ω -5-gliadin. Subjects who showed level > 0.35 kU_A/L for at least one of the three sIgE measured were defined as patients with WDEIA.

2.3. Tag-SNP selection

To comprehensively measure polymorphisms at *IL-18*, tag SNPs were selected based on the positions of the SNPs in genes from NCBI dbSNP (<https://www.ncbi.nlm.nih.gov/projects/SNP>) and genotypic data of the Han Chinese in Beijing (CHB) available from the HapMap project on the SNPinfo Web Server (<https://snpinfo.niehs.nih.gov>). The tagger function of the SNPinfo Web Server was used to evaluate minor allele frequencies (MAF) and linkage disequilibrium (LD) of the three SNPs selected for analysis. SNPs were selected as follows: First, the selection criteria for SNPs were set to MAF $> 1\%$ in the Han Chinese population. Next, LD between SNPs was evaluated using the squared correlation coefficient (r^2). For SNPs with strong LD ($r^2 > 0.8$), one SNP could be chosen as a representative of the other SNPs. Finally, we selected two tag SNPs (rs1946518 and rs360717) in the regulating regions and one (rs360729) in the intron region of *IL-18* (Table 1).

2.4. DNA extraction and SNP genotyping

Genomic DNA was extracted from Ethylene Diamine Tetraacetic Acid EDTA-anticoagulated whole blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan), according to the manufacturer's instructions. SNP genotyping was performed using the Sequenom MassARRAY System (Sequenom, San Diego, CA, USA). Primers for the multiplex assay were designed using the MassARRAY Assay Design 4.0 software (Sequenom, San Diego, CA, USA), and the sequences were listed in supplementary table 1. The sample was first amplified by multiple PCR. The reaction system (5 μ l) contained 1 μ l (50 ng/ μ l) of DNA sample,

1.9 μl of H_2O , 0.5 μl of PCR Buffer (10 $\times$), 0.4 μl (25 mmol/L) of MgCl_2 , 0.1 μl (5 units/ μl) of Qiagen Hot Star Taq, 0.1 μl (25 mmol/L) of dNTP mix, and 1 μl of the PCR primer mix. PCR reaction conditions were as follows: 45 cycles of 4 min at 94°C, 20 s at 94°C, 30 s at 56°C, and 1 min at 72°C, followed by 3 min at 72°C and hold at 4°C. Next, locus-specific single-base extension reaction and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) were performed. Finally, genotypes were identified using MassARRAY Typer software (Sequenom, San Diego, CA, USA) on the basis of the mass of PCR products after the extension reaction.

2.5 Validation analysis

For further validation of our findings, the serum levels of *IL-8* were also determined in a subgroup of 60 clinical WDEIA samples and 60 normal serum samples from healthy controls by ELISA kit (Sangon Biotech Company, Shanghai, China).

2.6. Statistical analysis

Statistical analyses were performed using SPSS software (version 24.0, SPSS & IBM, Inc, Chicago, IL, USA) and GraphPad Prism software (La Jolla, CA, USA). Demographic data were compared between WDEIA and control groups using χ^2 analysis. SNPstats software (<http://bioinfo.iconcologia.net/snpstats/start.htm>) was used to calculate the Hardy–Weinberg equilibrium, compare genotypic frequencies between WDEIA and control groups under co-dominant, log-additive, dominant and recessive models of inheritance, and calculate odds ratios (OR) and 95% CIs. Statistic power was calculated by online software Genetic Power Calculator [14]. The χ^2 and Fisher's exact tests were used to compare allele frequencies between groups. Haploview 4.2 software was used for calculating pairwise LD of SNPs and estimating

haplotype frequencies in the case-control analysis [15]. sIgE levels and log-transformed serum total IgE values were compared in patients with WDEIA with different genotypes using ANOVA or Kruskal–Wallis test. A $P < 0.05$ was considered to be statistically significant. For multiple allelic frequency comparisons, a positive threshold was set at a corrected p-value of 0.0167 (Bonferroni corrected p-value of 0.05 for multiple testing of three SNPs). And the Bonferroni correction was also applied to genetic associations considering different genetic models and haplotypes.

3. Results

3.1. Demographic characteristics

Demographic characteristics of all patients were summarized in Table 1. The results showed that, the difference of total IgE between WDEIA and control groups was significant ($P < 0.0001$). But no significant differences in terms of gender ($P = 0.54$) and age ($P = 0.11$) were observed. Additionally, the wheat sIgE, gluten sIgE and ω -5-Gliadin sIgE in the control group were negative.

3.2. Hardy–Weinberg equilibrium in study populations

As illustrated in Table 2, the distributions of individual *IL-18* alleles in the control group followed the Hardy–Weinberg equilibrium (all, $P > 0.05$). Successful genotyping rates for the three selected SNPs were in the range of 98%–100%. The accuracy of genotyping has been further confirmed by Sanger’s sequencing in 40 randomly selected cases. Furthermore, the power analysis showed that the statistical power was $>80\%$ to detect a genotype relative risk of 1.8 at an alpha level of 0.05 for variants with an MAF of 45%, assuming a WDEIA prevalence of 0.01% of in Chinese.

3.3. Allele frequencies in WDEIA and control groups

According to the results of the allele association analysis (Table 2), the frequency of the G-allele in rs1946518 of WDEIA group was significantly higher than that in the control group (OR = 1.547, 95% CI = 1.177–2.025, $P = 0.0015$) after Bonferroni correction, suggesting the minor allele G of rs1946518 is a potential risk factor for WDEIA. However, no significant differences in allele frequencies of rs360729 and rs360717 were observed between the WDEIA and the control groups after Bonferroni correction (both, $P > 0.0167$).

3.4. Genotype frequencies in WDEIA and control groups

As shown in Table 3, significant differences in genotype frequencies of rs1946518 between the WDEIA and control groups were observed (OR = 1.56, 95% CI = 1.18–2.04, $P = 0.0014$) after Bonferroni correction under log-additive model. In addition, compared with the TT genotype, the homozygote GG genotype was associated with a higher risk of WDEIA (OR = 2.34, 95% CI = 1.38–3.98, $P = 0.0031$) under co-dominant model. Significant differences in genotypes frequencies of rs1946518 were observed under recessive model rather than dominant model. However, the associations between other two SNPs and risk of WDEIA were not significant ($P > 0.05$) after Bonferroni correction.

3.5. Linkage disequilibrium (LD) and case-control haplotype analysis

Next, we calculated LD and constructed haplotype blocks for the three SNPs (Fig. 1). After haplotypes analysis using the three SNPs, the haplotypes with frequencies in the two groups were listed in Table 4. The results showed the AGG haplotype formed by rs360729, rs360717, and rs1946518 alleles occurred more frequently in the WDEIA than that in control group ($P = 0.003$, OR = 1.511, 95% CI = 1.153–1.980) after Bonferroni correction. But the frequency of the AGT haplotype

in the WDEIA group was significantly lower than that in the control group ($P = 0.001$, $OR = 0.624$, $95\% CI = 0.468-0.832$).

3.6. Association of rs1946518 genotypes with total IgE and sIgE levels in the WDEIA group

To determine whether *IL-18* SNPs were related with IgE production, log-transformed total IgE values at position rs1946518 among different genotypes were measured. As shown in Fig. 2, the log-transformed IgE values significantly differed among patients with TT, GT, and GG genotypes ($P = 0.0036$). Log-transformed IgE values were significantly lower in the group with the major allele homozygous TT genotype than in the group with the heterozygous GT genotype (2.145 ± 0.466 vs. 2.52 ± 0.437 , $P = 0.0024$). However, sIgE levels (sIgE to wheat, gluten, and ω -5-gliadin) did not significantly differ among patients with WDEIA with TT, GT, and GG genotypes ($P > 0.05$, Fig. 2).

3.7. Validation analysis of association of rs1946518 polymorphisms and serum level of *IL18*

To confirm the reliability of the role of rs1946518 polymorphisms of *IL18* in WDEIA, the association of rs1946518 polymorphisms and serum level of *IL18*. As illustrated in Figure 3A, the *IL18* serum levels in WDEIA patients were significantly higher than that in normal controls ($P=0.012$). More importantly, at rs1946518, the serum *IL18* level in WDEIA group was significantly different between TT, GT and GG genotypes groups ($P = 0.045$), and the serum *IL18* level in GT group was higher than that in TT and GG group (Figure 3B).

4. Discussion

WDEIA is considered to be a complex disease that is influenced by genetic predisposition and environmental factors [16]. The human *IL-18* gene has been

regarded as an important regulator of innate and acquired immune responses [17], indicating *IL-18* may have allergy-promoting and anti-allergic effects [18]. Various SNPs of *IL-18* are associated with allergic diseases, such as AR [19], AA [20], and AD [21]. However, the role of *IL-18* in allergic diseases still remains controversial [20,22]. Till now, many studies have shown that polymorphisms at the *IL-18* promoter (in particular *IL-18*-607C/A and -137G/C) influence IL18 production and activity [19,23]. In addition, a functional polymorphism of *IL-18* is involved in severe asthma and might contribute to enhanced innate immunity and Th1- and Th2-driven immune responses [24]. In the present study, our data showed that the significant differences in allele and genotype frequencies of rs1946518 of *IL-18* were observed between WDEIA and control groups. Moreover, the minor allele G of rs1946518 is a risk factor for WDEIA. Compared with homozygote TT, genotypes with the minor allele G (GG and GT) increased the risk of WDEIA. Moreover, the verification analysis revealed that the serum *IL18* level in patients with GT genotype was higher than that in patients with TT and GG genotypes. The reason might be that a change from T to G at this position alters the activity of the *IL-18* promoter leading to abnormal expression of *IL-18*. This promotes the Th2 response, thereby leading to the development of allergic diseases.

Recent evidences showing the involvement of IL-18 in ultimately IgE production from B cells have shed a new insight on the dual effects of IL-18 on inflammatory responses [10,25]. In our study, in keeping with studies from Kruse [26] and Sebelova [17], we found that the serum total IgE levels of individuals with the GT genotype of rs1946518 were significantly higher than that in individuals with the TT genotype. We speculated that rs1946518 polymorphisms upregulated IgE production, thereby contributing to the development of the WDEIA phenotype. Additionally, several other

studies have shown that *IL-18* polymorphisms are associated with sIgE levels to mite allergens among patients with asthma [27] and AR [19]. However, our data showed no association between rs1946518 genotypes and serum sIgE levels (sIgE to wheat, gluten, and ω -5-gliadin) in patients with WDEIA.

Furthermore, several studies have reported significant associations of *IL-18* haplotypes with AA [28] and AR [29]. In the present study, although the SNPs of rs360729 and rs360717 did not show any association with WDEIA, the haplotype AGG formed by rs360729, rs360717, and rs1946518 alleles was associated with a high risk of WDEIA. Taken together, all these results in our study indicated that SNPs in the *IL-18* gene might have functional interactions and could play a role in WDEIA of Han Chinese population. And the *IL-18* rs1946518 polymorphism is a biomarker for predicting the susceptibility of a Han Chinese to WDEIA. However, there were several limitations in our study. Firstly, the diagnosis of WDEIA in our study was based on clinical history, physical examination, sIgE levels, and elimination diets. However, the gold standard for diagnosing WDEIA with food allergies, the double-blind, placebo-controlled oral food challenges (DBPCFC) is not allowed in China, because it places patients at risk of a severe anaphylactic reaction. Therefore, we assessed the relevant sIgE levels (to wheat, gluten and ω -5-gliadin) using the Pharmacia CAP System, which should provide similar predictive values at lower risk for the patient. Secondly, our sample was relatively small, and hence future studies should enlarge the sample sizes and select more susceptible gene polymorphisms for analysis.

In summary, the present study showed that the rs1946518 polymorphism in the *IL-18* promoter region might be associated with WDEIA and the total IgE levels. To the best of our knowledge, this was the first association study to explore the role of

IL-18 gene polymorphisms in Chinese patients with WDEIA. The results of this study will be helpful in understanding the pathogenesis of WDEIA and possibly FDEIA and guide diagnosis and treatment of this allergic disease.

Conflict of interest

The authors declare no conflict of interest.

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References

1. Morita E, Kunie K, Matsuo H (2007) Food-dependent exercise-induced anaphylaxis. *J Dermatol Sci* 47: 109-117.
2. Maulitz RM, Pratt DS, Schocket AL (1979) Exercise-induced anaphylactic reaction to shellfish. *J Allergy Clin Immunol* 63: 433-434.
3. Jiang N, Yin J, Wen L, Li H (2016) Characteristics of Anaphylaxis in 907 Chinese Patients Referred to a Tertiary Allergy Center: A Retrospective Study of 1,952 Episodes. *Allergy Asthma Immunol Res* 8: 353-361.
4. Wolbing F, Fischer J, Koberle M, Kaesler S, Biedermann T (2013) About the role and underlying mechanisms of cofactors in anaphylaxis. *Allergy* 68: 1085-1092.
5. Barg W, Wolanczyk-Medrała A, Obojski A, Wytrychowski K, Panaszek B, et al. (2008) Food-dependent exercise-induced anaphylaxis: possible impact of increased basophil histamine releasability in hyperosmolar conditions. *J Investig Allergol Clin Immunol* 18: 312-315.
6. Sigdel K, Duan L, Wang Y, Hu W, Wang N, et al. (2016) Serum Cytokines Th1,

Th2, and Th17 Expression Profiling in Active Lupus Nephritis-IV: From a Southern Chinese Han Population. *Mediators of inflammation* 2016: 4927530.

7. Lédée N, Petitbarat M, Chevrier L, Vitoux D, Vezmar K, et al. (2016) The Uterine Immune Profile May Help Women With Repeated Unexplained Embryo Implantation Failure After In Vitro Fertilization. *American journal of reproductive immunology* (New York, NY : 1989) 75: 388-401.
8. Kurt E, Aksu K, Dokumacioglu A, Alatas O (2012) Bronchial hyperresponsiveness in seasonal allergic rhinitis patients is associated with increased IL-18 during natural pollen exposure. *Cytokine* 60: 100-103.
9. Imaoka H, Takenaka S-i, Kawayama T, Oda H, Kaku Y, et al. (2013) Increased serum levels of soluble IL-18 receptor complex in patients with allergic asthma. *Allergology International* 62: 513-515.
10. Gohar M, Atta A, Nasr M, Hussein D (2017) Serum Thymus and Activation Regulated Chemokine (TARC), IL- 18 and IL-18 Gene Polymorphism as Associative Factors with Atopic Dermatitis. *The Egyptian journal of immunology* 24: 9-22.

11. Cheng D, Hao Y, Zhou W, Ma Y (2014) The relationship between interleukin-18 polymorphisms and allergic disease: a meta-analysis. *Biomed Res Int* 2014: 290687.
12. Yin J, Wen LP (2010) Wheat-dependant Exercise-induced Anaphylaxis Clinical and Laboratory Findings in 15 Cases. *Chinese Journal of Allergy & Clinical Immunology*.
13. Jacquenet S, Morisset M, Battais F, Denery-Papini S, Croizier A, et al. (2009) Interest of ImmunoCAP system to recombinant omega-5 gliadin for the diagnosis of exercise-induced wheat allergy. *Int Arch Allergy Immunol* 149: 74-80.
14. S P, SS C, PC S (2003) Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics (Oxford, England)* 19: 149-150.
15. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-265.
16. Sengler C, Lau S, Wahn U, Nickel R (2002) Interactions between genes and

environmental factors in asthma and atopy: new developments. *Respir Res* 3:

7.

17. Sebelova S, Izakovicova-Holla L, Stejskalova A, Schuller M, Znojil V, et al.

(2007) Interleukin-18 and its three gene polymorphisms relating to allergic rhinitis. *J Hum Genet* 52: 152-158.

18. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H (2001) Interleukin-18

regulates both Th1 and Th2 responses. *Annu Rev Immunol* 19: 423-474.

19. Tungtrongchitr A, Jumpasri J, Sookrung N, Visitsunthorn N, Tantilipikorn P, et al.

(2017) Alteration of -656(G/T) and -607(C/A) polymorphisms in interleukin-18 (IL-18) gene in house dust mite-sensitive allergic rhinitis patients in Thailand. *Genetics and molecular research : GMR* 16: undefined.

20. Cheng D, Hao Y, Zhou W, Ma Y (2014) The relationship between interleukin-18

polymorphisms and allergic disease: A meta-analysis. *BioMed research international* 2014.

21. Ibrahim GH, ElTabbakh MT, Gomaa AH, Mohamed EA (2012) Interleukin-18

gene polymorphisms in Egyptian patients with allergic diseases. *American*

journal of rhinology & allergy 26: 385-389.

22. Ma Y, Zhang B, Tang R-K, Liu Y, Peng G-G (2012) Interleukin-18 promoter polymorphism and asthma risk: a meta-analysis. *Molecular biology reports* 39: 1371-1376.

23. Yang B, Feng W, Li Y, Shi Y, Cai B, et al. (2017) Interleukin 18 -607 A/C Gene Polymorphism is Associated With Susceptibility to IgA Nephropathy in a Chinese Han Population. *Applied immunohistochemistry & molecular morphology* : AIMM 25: 725-730.

24. Harada M, Obara K, Hirota T, Yoshimoto T, Hitomi Y, et al. (2009) A functional polymorphism in IL-18 is associated with severity of bronchial asthma. *Am J Respir Crit Care Med* 180: 1048-1055.

25. Esmailbeig M, Ghaderi A (2017) Interleukin-18: a regulator of cancer and autoimmune diseases. *European cytokine network* 28: 127-140.

26. Kruse S, Kuehr J, Moseler M, Kopp MV, Kurz T, et al. (2003) Polymorphisms in the IL 18 gene are associated with specific sensitization to common allergens and allergic rhinitis. *J Allergy Clin Immunol* 111: 117-122.

27. Xu M, Yuan F, Wang S, Xu H, Li C, et al. (2017) Association of interleukin-18 and asthma. *Inflammation* 40: 324-327.
28. Kim E, Lee JE, Namkung JH, Park JH, Kim S, et al. (2007) Association of the single-nucleotide polymorphism and haplotype of the interleukin 18 gene with atopic dermatitis in Koreans. *Clin Exp Allergy* 37: 865-871.
29. Izakovicova Holla L, Hrdlickova B, Schuller M, Buckova D, Kindlova D, et al. (2010) Haplotype analysis of the interleukin-18 gene in Czech patients with allergic disorders. *Hum Immunol* 71: 592-597.

Figure legends

Figure 1. Linkage disequilibrium (LD) plot and haplotype block construction of the three SNPs at *IL18* gene. The relative chromosomal positions of the three SNP were marked on the straight lines. The D' value between pairs of SNPs was expressed as a percentage and shown within the respective square.

Figure 2. Association of rs1946518 genotypes with total IgE and sIgE levels in the WDEIA group. (a) Comparison of the log-transformed total IgE values among the different genotypes (b) Comparison of the levels of sIgE to wheat among the different genotypes (c) Comparison of the levels of serum sIgE to gluten among the different genotypes (d) Comparison of the levels of serum sIgE to ω -5-gliadin among the different genotypes.

Figure 3. IL18 serum levels in WDEIA and normal controls detected by ELISA. IL18 serum levels in WDEIA patients were significantly higher than that in normal controls (A). In WDEIA group, serum IL18 level in GT group was higher than that in TT and GG groups (B).

Conflict of interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Highlights

1. SNP genotyping was conducted for 130 patients with Wheat-dependent exercise-induced anaphylaxis (WDEIA) and 600 healthy individuals.
2. The rs1946518 polymorphism at the IL-18 gene locus was associated with the increased risk for WDEIA.
3. Carriage of the rs1946518 variant G allele might be a risk factor for the elevated serum total IgE levels.
4. The haplotype AGG formed by rs360729, rs360717, and rs1946518 alleles was associated with a high risk of WDEIA.

Table 1 Characteristics of the WDEIA and control groups

WDEIA	Controls	<i>P</i>
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	(n=130)	(n=600)	
Gender, n (%)			
Male	73 (56.15%)	319 (53.17%)	0.54
Female	57 (43.85%)	281 (46.83%)	
Age (years)			
Mean±SD	36.34±15.75	39.53±12.56	0.11
Totle IgE (kU _A /L)	209 (115.50, 597.5)	33.80(26.30, 49.15)	
log-transformed	2.376±0.47	1.53±0.22	<0.0001
total IgE			
Wheat sIgE (kU _A /L)	0.56 (0.12, 1.51)	0 (0,0)	
Gluten sIgE (kU _A /L)	1.87 (0.75, 4.84)	0 (0,0)	
ω-5-Gliadin sIgE	5.70 (1.91, 11.70)	0 (0,0)	
(kU _A /L)			

Table 2 Comparison of the minor allele frequencies of the three SNPs in IL-18 gene between WDEIA and control groups

SNPs	Alleles	P_{HW}^a	MAF		χ^2
			WDEIA, n (%)	Controls, n (%)	
rs360729	A>T	0.86	35 (13%)	164 (14%)	0.007
rs360717	G>A	1.00	36 (14%)	159 (13%)	0.066
rs194651	T>G	0.46	145 (56%)	538 (45%)	10.12

^a P value from Hardy–Weinberg equilibrium test among controls

^b $P < 0.050.0167$ (Bonferroni corrected p-value)

Table 3 Association between IL-18 SNPs and risk of WDEIA under four different genotypes co-dominant and log-additive models

Genotypes	WDEI		Co-dominant		Log-Additive		OR
	A	Controls	OR (95% CI)	P	OR (95% CI)	P	
	(N=130)	(N=600)					
rs360729							
A/A	98 (75.4%)	446 (74.3%)	1.00	0.83	0.98 (0.66-1.46)	0.93	
T/A	29 (22.3%)	144 (24%)	0.92 (0.58-1.45)				
T/T	3 (2.3%)	10 (1.7%)	1.37 (0.37-5.05)				
<u>T/A + T/T vs A/A</u>							<u>0.95</u>
<u>T/T vs A/A + T/A</u>							
rs360717							
G/G	97 (74.6%)	451 (75.2%)	1.00	0.89	1.05 (0.71-1.55)	0.80	
A/G	30 (23.1%)	139 (23.2%)	1.00 (0.64-1.58)				
A/A	3 (2.3%)	10 (1.7%)	1.39 (0.38-5.16)				
<u>A/G + A/A vs G/G</u>							<u>1.03</u>
<u>A/A vs G/G + A/G</u>							
rs1946518							
T/T	28 (21.5%)	177 (29.6%)	1.00	0.0031 ^a	1.56 (1.18-2.04)	0.0014 ^a	
G/T	59 (45.4%)	306 (51.1%)	1.22 (0.75-1.98)				
G/G	43 (33.1%)	116 (19.4%)	2.34 (1.38-3.98)				
<u>G/T + G/G vs T/T</u>							<u>1.53</u>
<u>G/G vs T/T + G/T</u>							

OR (95% CI) and P values were adjusted by age and gender

^a P < 0.0167 (Bonferroni corrected p-value)⁵

Table 4 Haplotypes analysis using the three SNPs (rs360729-rs360717-rs1946518) between WDEIA and control groups

Haploty pe ^a	Case freq.	Controls freq.	<i>P</i>	OR (95% <i>CI</i>)
Block 1				
AGG	0.554	0.447	0.003 ^b	1.511 (1.153-1.980)
AGT	0.308	0.411	0.001 ^b	0.624 (0.468-0.832)
TAT	0.135	0.128	0.808	1.050 (0.708-1.558)
Global test of association	—	—	0.004 ^b	—

^a Haplotypes with frequency less than 1 % were not listed

^b *P* < 0.0167 (Bonferroni corrected p-value) ~~0.05~~

Supplementary table 1 General characteristics of SNPs in IL-18 genes and primers for multiple PCR

SNPs	Al leles	Position (bp)	Function	Primers	Sequence
rs360 729	A>T	112146198	Intron-variant	Upstream primer	ACGTTGGA
				Downstream primer	ACGTTGGA
rs360	G>A	112164002	UTR-variant-5-prime	Upstream primer	ACGTTGGA

717

				Downstream primer	ACGTTGGA
rs194	T>G	112164735	Upstream-variant-2KB	Upstream primer	ACGTTGGA
6518				Downstream primer	ACGTTG
					CG

Abbreviations list

WDEIA, wheat-dependent exercise-induced anaphylaxis;
FDEIA, food-dependent exercise-induced anaphylaxis;
AR, allergic rhinitis;
AA, allergic asthma;
AD, atopic dermatitis;
sIgE, antigen-specific IgE;
SNPs, single nucleotide polymorphisms;
CHB, Han Chinese in Beijing population;
MAF, minimum allele frequency;
PCR, polymerase chain reaction;
MALDI-TOF-MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry;
HWE, Hardy-Weinberg equilibrium;
LD, linkage disequilibrium