



Association of HLA-DQ and IL13 gene variants with challenge-proven shrimp allergy in West Bengal, India

Arghya Laha¹ · Amlan Ghosh² · Saibal Moitra³ · Himani Biswas⁴ · Nimai Chandra Saha⁵ · Srijit Bhattacharya⁶ · Goutam Kumar Saha⁷ · Sanjoy Podder¹

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Abstract

Little is known about genetic factors and mechanisms underlying shrimp allergy. Genome-wide association studies identified HLA class-II and IL13 genes as highly plausible candidates for shrimp allergy. The present study was designed to investigate potential associations of HLA-DQ rs9275596, IL13 rs20541, and IL13 rs1800925 polymorphisms with challenge-proven shrimp allergy using the data from 532 people of West Bengal, India; selected on basis of positive skin prick test, elevated specific IgE and medical history. Risk genotypes, i.e., HLA-DQ rs9275596 CC, IL13 rs20541 AA, and IL13 rs1800925 TT, were found to be significantly associated with challenge positive shrimp allergy ($P = 0.04$, 0.01 , and 0.03 , respectively). Distribution of genotypes for HLA-DQ and IL13 polymorphisms in allergic and control subjects showed significant difference between younger (20–40 years) and older (> 40 years) age group ($P = 0.006$). Risk genotypes significantly associated with elevated shrimp-specific IgE. IL13 TA haplotype significantly associated with shrimp allergy and elevated specific IgE ($P = 0.02$). Synergistic effect of IL13 TA haplotype–HLA-DQ rs9275596 CC genotype interaction significantly elevated specific IgE ($P = 0.03$). The present study suggests that HLA-DQ and IL13 polymorphisms pose major risk for shrimp allergic patients in West Bengal, India and thus could be helpful for early target-specific therapeutic intervention in near future.

Keywords HLA-DQ · IL13 · Shrimp challenge · Shrimp-specific IgE · SNP

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✉ Sanjoy Podder
skpzoo2@rediffmail.com

- ¹ Allergology and Applied Entomology Research Laboratory, Department of Zoology, University of Burdwan, Bardhaman 713104, West Bengal, India
- ² Department of Life Sciences, Presidency University, Kolkata 700073, West Bengal, India
- ³ Allergy and Asthma Research Centre, Kolkata- 700029, West Bengal, India
- ⁴ Post Graduate Department of Zoology, Krishnagar Government College, Krishnagar 741101, West Bengal, India
- ⁵ Vice-Chancellor, University of Burdwan, Bardhaman 713104, West Bengal, India
- ⁶ Post Graduate Department of Physics, Barasat Government College, Kolkata 700124, West Bengal, India
- ⁷ Department of Zoology, University of Calcutta, Kolkata 700019, West Bengal, India

Introduction

Shrimp is one of the leading causes of food allergy worldwide, with a prevalence of 2.8–8% among all food allergies, and it is also a common cause of food-induced anaphylaxis (Tejedor-Alonso et al. 2015). The prevalence of shrimp allergy in general population ranges from 0.2 to over 5%, based on geographic region (Moonesinghe et al. 2016; Ruethers et al. 2018). The enhanced production and consumption of shrimp has led the path of increasing adverse reactions to shrimp in India particularly in West Bengal. Our previous study reported shrimp as a dominant food allergen among West Bengal people (Laha et al. 2020). Unlike other food allergies, shrimp allergy persists in adult stage in up to 90% of patients (Zotova et al. 2019). Difficulties in effective prevention and management of food allergies arise due to our partial knowledge of its causes and underlying biological mechanisms, while previous literatures support a role for genetic factors in food allergies (Hong et al. 2009; Tsai et al. 2009).

Genome-wide association studies (GWAS) have reported a number of loci associated with increased susceptibility to food allergies, including one on chromosome 6p21.32 that contains human leukocyte antigen (HLA) class II molecules HLA-DR and HLA-DQ (Hong et al. 2015; Khor et al. 2018). HLA gene associations were established for several food allergies like peanut (Madore et al. 2013; Hong et al. 2015; Martino et al. 2017), milk and egg (Dimitrov and Doytchinova 2016), shrimp, and peach (Khor et al. 2018). An epigenome-wide association study identified food allergy-specific methylations in HLA-DQB1 gene (Martino et al. 2014) which is mainly caused by allelic variation of rs9275596, an intergenic single nucleotide polymorphism (SNP) between HLA-DQB1 and HLA-DQA2 genes (Hong et al. 2015). However, the finding has not been validated on case control basis till date. On the other hand, IL13 located on chromosome 5q.31, often linked to asthma, total serum IgE, airway responsiveness and other asthma related symptoms (Liu et al. 2000), although a few studies reported association with food allergies (Liu et al. 2004; Ashley et al. 2017). Study of genetic polymorphisms across IL13 locus in European ancestry subjects found a block of SNPs in the coding region (Graves et al. 2000), among which rs20541 (+2044G/A) produces a gain of function amino acid substitution (Arg130Gln) variant associated with IgE class switching in B cells (Vladich et al. 2005; Rosenbaum et al. 2014). This SNP is in linkage disequilibrium (LD) with an important promoter polymorphism, rs1800925 (-1112 C/T) (Long et al. 2015) which increases IL13 production in Th2-polarized cells and has been associated with food sensitization (Liu et al. 2004).

In the present study, we have assessed the potential associations of HLA-DQ rs9275596, IL13 rs20541, and IL13 rs1800925 polymorphisms with challenge-proven shrimp allergy among a study population from West Bengal, India, in a case-control manner. To the best of our knowledge, the present study is the first candidate gene approach for the association of HLA-DQ rs9275596 polymorphism with any kind of food allergy. Furthermore, we sought to investigate any possible role of the above polymorphisms on specific IgE level of the allergic patients. Our study also attempted to determine whether there are gene–gene interactions between the genetic variants in HLA-DQ and IL13 genes that influence shrimp allergy or related phenotypes in West Bengal people.

Methods

Study population

The present study recruited 532 individuals between ages 20 and 70 years, both male and female, seeking treatment at

Allergy and Asthma Research Center, West Bengal, India, during January 2019 to August 2019. Individuals not providing their consent of participation ($n = 10$), suffering from other illness ($n = 16$), and pregnant/lactating women ($n = 3$) were excluded from the study.

Data and blood collection

Epidemiological details such as age, gender, age of onset, inhabitation, and allergic information were taken through computer-assisted personal interview with the help of a standard questionnaire. Three hundred seventy-eight participants were diagnosed of having shrimp allergy and considered as cases. A total of 125 age and gender matched participants who neither had diagnosed for any food allergy served as healthy controls, recruited from same demographic area and ethnic group as that of the patients. Both patients and control subjects were categorized in accordance with their sex as well as their age, i.e., 20–40 years and above 40 years. Peripheral blood samples were drawn from all the participants, centrifuged for 15 min at 1300 rpm to separate serum and finally stored at -20°C until analysis.

Skin prick test

Skin prick test (SPT) was performed on all 503 participants using standard shrimp allergen extract (Credisol, Mumbai, India). Histamine phosphate at 10 mg/ml and 0.9% sterile saline were used as positive and negative controls, respectively. After 15 min, wheal diameter (average of longest diameter and diameter diagonal to it) of $\geq 3\text{mm}$ in comparison to the positive control was considered as evidence of sensitization.

Estimation of shrimp-specific IgE

Shrimp-specific IgE was quantified by ImmunoCAP Phadia 100 system (Thermo Fisher Scientific, USA) using serum aliquots. World Health Organization (WHO) standard for specific IgE with a range of 0.35–100 kU/L was taken for calibration and < 0.35 kU/L was designated as negative. Specific IgE levels were transformed to their log values before analysis for normalization.

Oral food challenge

Single blinded oral food challenge (OFC) was performed under supervision of a physician on 378 patients with suspected shrimp allergy on basis of medical history, results of SPT and/or shrimp-specific IgE. Freeze-dried shrimp powder was mixed with a masking vehicle (100 mg Infant formula + 250 mL water) to reduce the bias, and only the observer was aware of the food being tested. One hundred

five patients could not complete the OFC because they had refused to ingest the food. Before start of the test, some physical parameters like pulse rate, respiratory rate, oxygen saturation in blood (sPO₂), and peak expiratory flow rate (PEFR) of the patients were recorded. OFC were administered in 6 escalating doses every 30 min for a cumulative dose of 19.7 g food, with final dose of 10 g food (~ 2400 mg protein). The challenge was stopped and considered positive in case of objective symptoms (rhino-conjunctivitis, angioedema, urticaria, emesis, hoarseness, stridor, wheezing), if subjective symptoms (oral swelling/itching, nausea, abdominal pain or throat tightness) occurred at 3 subsequent doses or any marked change observed in pulse rate, respiratory rate and sPO₂ and 20% or more reduction in PEFR after completion of the test in comparison to those at the beginning.

SNP selection and haplotype formation

Three SNPs were selected for the present study: rs9275596 of HLA-DQ gene and rs20541 and rs1800925 of IL13 gene. SNP selection was based on information gathered from SNPpedia database regarding SNP validation, putative function, known association with food allergy and related phenotypes. IL13 haplotype blocks were constructed according to Gabriel et al. (2002) using Haploview software v.4.2.

DNA isolation and genotyping

Genomic DNA was isolated from blood samples of 378 patients and 125 control subjects using QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). Presence of polymorphisms was detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers (forward 5'-GGCCGTCACAGAAAGATCCTCTTCAGCTTTGCTG-3' and reverse 5'-TCAGCCAGAGACGAAAGTGT-3') flanking the region around rs9275596 SNP site within HLA-DQ gene were designed for this study using Primer3 software. The PCR product was of 211 bp, which was digested with TseI restriction enzyme (New England Biolabs). The primers amplifying IL13 rs1800925 SNP site were forward 5'-GGAATCCAGCATGCCTTGTGAGG-3' and reverse 5'-GTCGCCTTTTCCTGCTCTTCCCGC-3' and that of rs20541 SNP site were forward 5'-CTTCCGTGAGGACTGAATGAGACG GTC-3' and reverse 5'-GCAAATAATGATGCTTTTCGAA GTTTCAGGGA-3'. The PCR products were of 246 bp and 236 bp, which were digested with BstUI and NlaIV restriction enzymes (New England Biolabs), respectively (Yadav et al. 2012, Kazemi et al. 2019). Each PCR amplification was performed in a 20 µl of reaction mixture containing 50 ng of genomic DNA, 200 nM of each primer (Sigma), 200 µM of each deoxyribonucleotide triphosphate (dNTP) (Thermo Fisher Scientific, USA), and 2.5 units of Taq polymerase

(Applied Biological Materials, Canada). The cycling conditions were as follows: 95 °C for 5 min, followed by 30 cycles at 95 °C for 30 s, annealing temperature for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 7 min.

DNA sequencing

Sanger sequencing was performed using representative PCR products for each of the three polymorphic genotypes of selected polymorphisms to confirm the RFLP results.

Statistical analysis

Continuous data are expressed as mean ± standard error (SE) and categorical data by number with percentages. Patients and control subjects were compared by independent *t* test for continuous variables or Pearson chi-square test for categorical variables. Distribution of alleles and genotypes in shrimp challenge positive (allergic), challenge negative (sensitized) patients, and control subjects was analyzed using contingency chi-square or Fisher's exact test, where appropriate. Associations between polymorphisms and food allergy risk were estimated by odds ratio (OR) and 95% confidence intervals (CIs) under additive and recessive models. Crude OR was determined through univariate logistic regression with only genotypes/alleles taken into consideration. Adjusted OR was determined using multivariate logistic regression method with an adjustment for age and sex. Hardy-Weinberg equilibrium (HWE) was tested for the genotypes by goodness-of-fit chi-square test with one degree of freedom (*df*). Non-parametric Mann-Whitney *U* test was done to estimate any significant differences between polymorphic genotypes with respect to shrimp-specific IgE level. Gene-gene interactions between HLA-DQ and IL13 variants were tested by stratifying the study subjects according to the genotype of one gene and performed the analysis of other gene on different strata using Mantel-Haenszel test. All statistical analysis was calculated using GraphPad Prism ver. 7 (San Diego, CA). Results were considered significant at *P* < 0.05.

Results

Demographic and clinical features

The association study was performed among 378 shrimp allergic patients and 125 control individuals. Baseline demographic and clinical details are provided in Table 1. Majority of the patients had oral allergy syndrome followed by urticaria/atopic eczema and asthma. A larger part of the study population including patients and control are inhabitants of urban area. SPT wheal size and shrimp-specific IgE level of

Table 1 Demographic and clinical characteristics of patients and control subjects

Parameters	Patients (<i>n</i> = 378)	Control (<i>n</i> = 125)	<i>P</i> value
1. Age (years)			0.16
20–40 (median 31 years)	208 (55.03%)	59 (47.20%)	
> 40 (median 54 years)	170 (44.97%)	66 (52.80%)	
2. Sex			0.90
Male	186 (49.21%)	60 (48.00%)	
Female	192 (50.79%)	65 (52.00%)	
3. Symptoms			
Oral allergy syndrome	258 (68.25%)	Nil	
Urticaria/atopic eczema	97 (25.66%)	Nil	
Asthma	23 (6.08%)	Nil	
4. Family history			
Paternal (P)	101 (26.72%)	Nil	
Maternal (M)	78 (20.63%)	Nil	
P + M	44 (11.64%)	Nil	
Absent	199 (52.65%)	125 (100%)	
5. Age of onset			
Childhood onset (< 20 years)	325 (85.98%)	Nil	
Adult onset (> 20 years)	53 (14.02%)	Nil	
6. Weight (kg) ^a	62.53 ± 1.35 (59.84–65.22)	61.41 ± 1.23 (58.95–63.87)	0.54
7. Height (cm) ^a	160.21 ± 0.82 (158.57–161.85)	161.12 ± 0.91 (159.31–162.93)	0.46
8. Residence			0.35
Urban	249 (65.87%)	78 (62.40%)	
Semi-urban	102 (26.98%)	41 (32.80%)	
Rural	27 (7.14%)	6 (4.80%)	
9. SPT (mm) ^a	7.42 ± 0.30 (6.83–8.01)	1.46 ± 0.10 (1.27–1.65)	< 0.0001
10. Shrimp-specific IgE (kU/L) ^a	13.81 ± 2.07 (9.70–17.92)	0.18 ± 0.01 (0.15–0.19)	< 0.0001
11. Shrimp challenge positive (<i>n</i> = 113)			
Pulse rate (per min) ^a	121 ± 0.66 (120.05–122.67)	Nil	< 0.0001 ^b
Respiratory rate (per min) ^a	31 ± 0.22 (30.88–31.74)	Nil	< 0.0001 ^b
sPO ₂ (%) ^a	86.53 ± 0.10 (86.34–86.72)	Nil	< 0.0001 ^b
PEFR (male) (L/min) ^a	Predicted ^c 467 ± 3.89 (459.54–475.60)	Nil	-
	Actual 353 ± 3.27 (347.37–360.39)	Nil	< 0.0001 ^b
PEFR (female) (L/min) ^a	Predicted ^d 308 ± 3.87 (300.35–315.99)	Nil	-
	Actual 240 ± 3.04 (234.73–246.81)	Nil	< 0.0001 ^b
12. Shrimp-sensitive (<i>n</i> = 160)			
Pulse rate (per min) ^a	89 ± 0.61 (88.77–91.21)	Nil	
Respiratory rate (per min) ^a	14 ± 0.13 (13.85–14.37)	Nil	
sPO ₂ (%) ^a	97.52 ± 0.16 (97.20–97.84)	Nil	
PEFR (male) (L/min) ^a	Predicted ^c 467 ± 4.31 (459.04–476.46)	Nil	
	Actual 447 ± 3.41 (441.07–454.63)	Nil	
PEFR (female) (L/min) ^a	Predicted ^d 312 ± 3.98 (304.89–321.09)	Nil	
	Actual 298 ± 3.07 (292.38–304.60)	Nil	

Significant associations (*P* < 0.05) are shown in italicsPEFR peak expiratory flow rate, SPT skin prick test, sPO₂ oxygen saturation in blood^aRepresented as mean ± standard error (95% confidence interval)^b*P* value calculated for difference between shrimp challenge positive and sensitive group^cPredicted PEFR of males between 18 and 80 years (L/min) = − 1.807 × age in years + 3.206 × height in cm^dPredicted PEFR of females between 18 and 80 years (L/min) = − 1.454 × age in years + 2.368 × height in cm Taken from Kodgule et al. (2014)

the patients and control subjects were significantly different ($P < 0.0001$). OFC-positive patients were considered as allergic and OFC-negative patients considered as sensitive ones without having any clinical symptoms. A significant difference was observed between allergic and sensitive group with respect to different health parameters as depicted in Table 1.

Genotype and allelic distributions of selected SNPs in patients and controls

Genetic associations were examined among HLA-DQ rs9275596, IL13 rs20541, and IL13 rs1800925 polymorphisms in the three study groups (shrimp challenge positive, shrimp sensitive, and control) under both additive and recessive models (Table 2). The genotype frequencies under recessive model differ significantly between the study groups ($P = 0.01$, 0.03 , and 0.03 , respectively). Neither SNPs demonstrated significant deviation from HWE in the control population ($\chi^2 = 0.64$, 0.009 , and 0.96 , respectively, at $df = 1$) (Supplementary Table 1). In univariate analysis, the frequencies of the HLA-DQ rs9275596CC, IL13 rs20541AA, and IL13 rs1800925TT genotypes were significantly higher in shrimp challenge positive patients than in controls (OR = 2.88, 95% CI = 1.06–7.86, $P = 0.04$; OR = 3.60, 95% CI = 1.30–9.95, $P = 0.01$; OR = 5.48, 95% CI = 1.16–26.01, $P = 0.03$, respectively). The OR in challenge positive patients was significantly higher than sensitive ones ($P = 0.03$) indicating higher risk of shrimp allergy in challenge positive patients. In multivariate analysis, similar trend was followed after adjustment by age and sex.

Genotype and allele frequencies according to age

Distribution of genotypes for HLA-DQ and IL13 polymorphisms in challenge positive patients and control subjects showed a significant difference between younger (20–40 years) and older (> 40 years) age group ($P = 0.006$) (Supplementary Table 2). Genotypes HLA-DQ rs9275596CC, IL13 rs20541AA, and IL13 rs1800925TT were significantly associated with a higher risk of shrimp allergy in the younger patients (OR = 4.46, $P = 0.005$; OR = 5.51, $P = 0.002$; and OR = 6.41, $P = 0.02$, respectively) than the older ones (OR = 1.54, $P = 0.38$; OR = 2.47, $P = 0.09$; and OR = 3.85, $P = 0.11$ respectively).

LD and haplotype analysis of IL13 polymorphisms

IL13 rs1800925 and rs20541 SNPs were reported in high LD with each other ($r^2 = 0.41$; $D' = 0.81$) forming a distinct haplotype block spanning 3 kb. The haplotype frequencies (Supplementary Table 3) differed significantly ($\chi^2 = 15.34$, $df = 6$, $P = 0.02$) among the study groups.

Haplotype consisting of rs1800925T and rs20541A alleles showed higher frequency in challenge positive (OR = 2.11, 95% CI = 1.11–4.00) and sensitive patients (OR = 1.51, 95% CI = 0.81–2.79) in comparison to control. For the CA haplotype, we found a marked rare presence in challenge positive (1.5%) and sensitive patients (3.7%) compared with the controls (9.8%) and thus associated with a low risk of shrimp allergy or sensitization with an OR 0.08 ($P = 0.02$) and 0.37 ($P = 0.05$), respectively. All other haplotypes investigated showed a lack of statistically significant association with shrimp allergy. Moreover, the individuals with CG haplotypes at IL13 gene along with TC genotype at HLA rs9275596 locus found to be significantly associated with lower risk of challenge positive shrimp allergy (OR = 0.54, 95% CI = 0.31–0.96, $P = 0.04$) (Fig. 1).

Association of shrimp-specific IgE level with HLA-DQ and IL13 polymorphisms

HLA-DQ rs9275596CC, IL13 rs20541AA, and IL13 rs1800925TT genotypes were found to be associated with higher shrimp-specific IgE level (Fig. 2 (I–III)). In the challenge positive patients, rs9275596CC genotype had significantly higher IgE level than those with the TC or TT genotypes (CC vs. TC, $P < 0.00001$; CC vs. TT, $P < 0.00001$). Similarly, significant associations were also detected in IL13 rs20541 (AA vs. GA, $P < 0.00001$; AA vs. GG, $P < 0.00001$) and IL13 rs1800925 polymorphisms (TT vs. CT, $P = 0.0005$; TT vs. CC, $P = 0.0002$). The above associations were also significant in shrimp-sensitive group (rs9275596: CC vs. TC, $P < 0.00001$; CC vs. TT, $P < 0.00001$; rs20541: AA vs. GA, $P < 0.00001$; AA vs. GG, $P < 0.00001$; rs1800925: TT vs. CT, $P = 0.0003$; TT vs. CC, $P = 0.0002$). However, the increase of IgE level in challenge positive patients was superior to that in sensitive ones. On the other hand, IL13 TA haplotype was significantly associated with elevated shrimp-specific IgE level in allergic patients ($P = 0.02$). The IL13 TA haplotype–HLA-DQ rs9275596CC genotype interaction was also significantly associated with elevated specific IgE level in shrimp allergic patients (OR = 4.38, 95% CI = 1.14–16.72, $P = 0.03$) (Fig. 1).

Discussion

Food allergies have been increasing in prevalence in the last 2 to 3 decades all over the world (Sicherer and Sampson 2010; Gupta et al. 2013). However, the determination of non-disputable prevalence statistics remains elusive because there are many manifestations of food allergy with different severities depending upon geographic variations, exposure to diet, age, race, ethnicity and myriad other factors influencing prevalence. It has

Table 2 Genotype/allele distributions of rs9275596, rs1800925, and rs20541 polymorphisms

Gene	SNP ID	Model	Genotype/allele	Shrimp challenge positive (n = 113)	Shrimp sensitive (n = 160)	Control (n = 125)	χ^2 (P value)	OR (95% CI)	Adjusted OR [#]	P value	MAF
HLA-DQ	rs9275596	Additive	TT	59	87	63	12.24 (P = 0.02)	1	1	1	0.28
			TC	34	61	54		0.67 ^a (0.37–1.23)	0.83 ^b (0.47–1.49)	0.80 ^a 0.97 ^b	0.20 ^c 0.54 ^d
			CC	20	12	8		2.88 ^a (1.06–7.86)	1.26 ^b (0.41–3.88)	2.40 ^a 1.33 ^b	0.04 ^c 0.69 ^d
		Recessive	TT + TC	93	148	117	10.31 (P = 0.01)	1	1	1	-
			CC	20	12	8		3.44 ^a (1.30–9.07)	1.36 ^b (0.45–4.08)	2.91 ^a 1.48 ^b	0.01 ^c 0.58 ^d
			T allele	152	235	180	2.57 (P = 0.28)	1	1	1	-
		Additive	C allele*	74	85	70		1.27 ^a (0.69–2.32)	0.95 ^b (0.51–1.77)	1.03 ^a 0.94 ^b	0.44 ^c 0.87 ^d
			CC	70	101	85	7.43 (P = 0.11)	1	1	1	0.17
			CT	32	49	38		1.02 ^a (0.55–1.90)	1.11 ^b (0.61–2.05)	0.90 ^a 0.97 ^b	0.94 ^c 0.72 ^d
			TT	11	10	2		5.48 ^a (1.16–26.01)	3.24 ^b (0.63–16.64)	4.00 ^a 2.87 ^b	0.03 ^c 0.16 ^d
IL13	rs1800925	Recessive	CC + CT	102	150	123	7.32 (P = 0.03)	1	1	1	-
			TT	11	10	2		5.44 ^a (1.16–25.52)	3.13 ^b (0.62–15.89)	3.93 ^a 2.87 ^b	0.03 ^c 0.17 ^d
		Additive	C allele	172	251	208	3.86 (P = 0.14)	1	1	1	-
			T allele*	54	69	42		1.54 ^a (0.77–3.09)	1.30 ^b (0.64–2.64)	1.45 ^a 1.32 ^b	0.22 ^c 0.47 ^d
			GG	49	85	72	8.91 (P = 0.06)	1	1	1	0.24
			GA	46	59	46		1.49 ^a (0.83–2.71)	1.09 ^b (0.61–1.97)	1.24 ^a 0.93 ^b	0.19 ^c 0.76 ^d
			AA	18	16	7		3.60 ^a (1.30–9.95)	2.19 ^b (0.70–6.82)	3.33 ^a 1.67 ^b	0.01 ^c 0.18 ^d
			GG + GA	95	144	118	6.88 (P = 0.03)	1	1	1	-
			AA	18	16	7		2.98 ^a (1.12–7.98)	2.11 ^b (0.69–6.42)	2.47 ^a 1.84 ^b	0.03 ^c 0.19 ^d
			G allele	144	229	190	8.83 (P = 0.01)	1	1	1	-
			A allele*	82	91	60		1.78 ^a (0.96–3.29)	1.05 ^b (0.57–1.94)	1.34 ^a 1.13 ^b	0.06 ^c 0.88 ^d

Significant associations are shown in italics

CI confidence interval, MAF minor allele frequency in control subjects, OR odds ratio, SNP single nucleotide polymorphism

[#] Adjusted for age and sex

*Minor (risk) allele

^aOR of shrimp challenge positive patients against control (P value^c)^bOR of shrimp sensitive patients against control (P value^d)

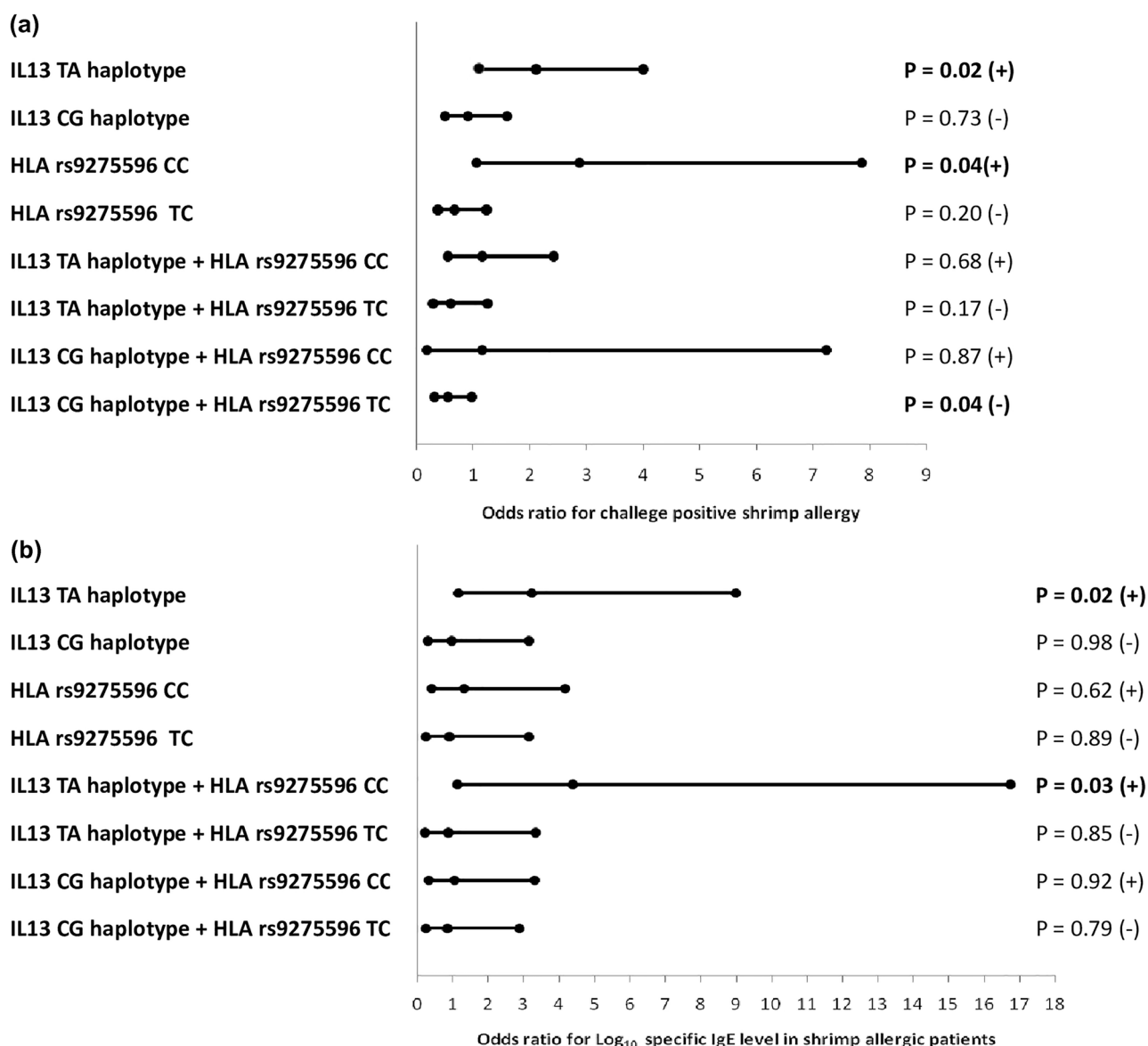


Fig. 1 HLA-DQ and IL13 genotypes. OR for **a** challenge-positive shrimp allergy and **b** log₁₀ specific IgE in allergic patients (+ = OR > 1; – = OR < 1). Significant associations ($P < 0.05$) are shown in bold

been established that self-reported food allergy rates are substantially higher than those confirmed by medically supervised OFCs (Muraro et al. 2014). Currently, OFC is considered as gold standard in any food allergy diagnosis (Sampson et al. 2012; Muraro et al. 2014). In India, most of the works has been done on food sensitization (Mandal et al. 2009; Dey et al. 2014; Laha et al. 2020) but a little is published regarding OFC proven food allergy (Kumar et al. 2010). In the present study, we have established the prevalence of shrimp allergy by single blinded OFC.

Shrimp plays a major role in causing anaphylaxis in adulthood (≥ 20 years) in most Asian countries like India, China, Japan, Hong Kong, Singapore, and Thailand (Smit et al. 2005; Thong et al. 2005; Techapornroong et al.

2010; Lertnawapan and Maek-a-nantawat 2011; Ebisawa et al. 2017; Laha et al. 2020). Genes related to Th2-type cytokines like IL13 which can alter IgE levels and the major histocompatibility complex (MHC) alleles (HLA-DR and DQ) which are related to immune responsiveness are thought to be responsible for shrimp allergy. The present study demonstrated the association of HLA-DQ rs9275596, IL13 rs20541, and IL13 rs1800925 polymorphisms with challenge-proven shrimp allergy among West Bengal population which have been barely available so far from India.

Association of HLA polymorphisms with peanut allergy in particular has been discussed in previous studies (Madore et al.

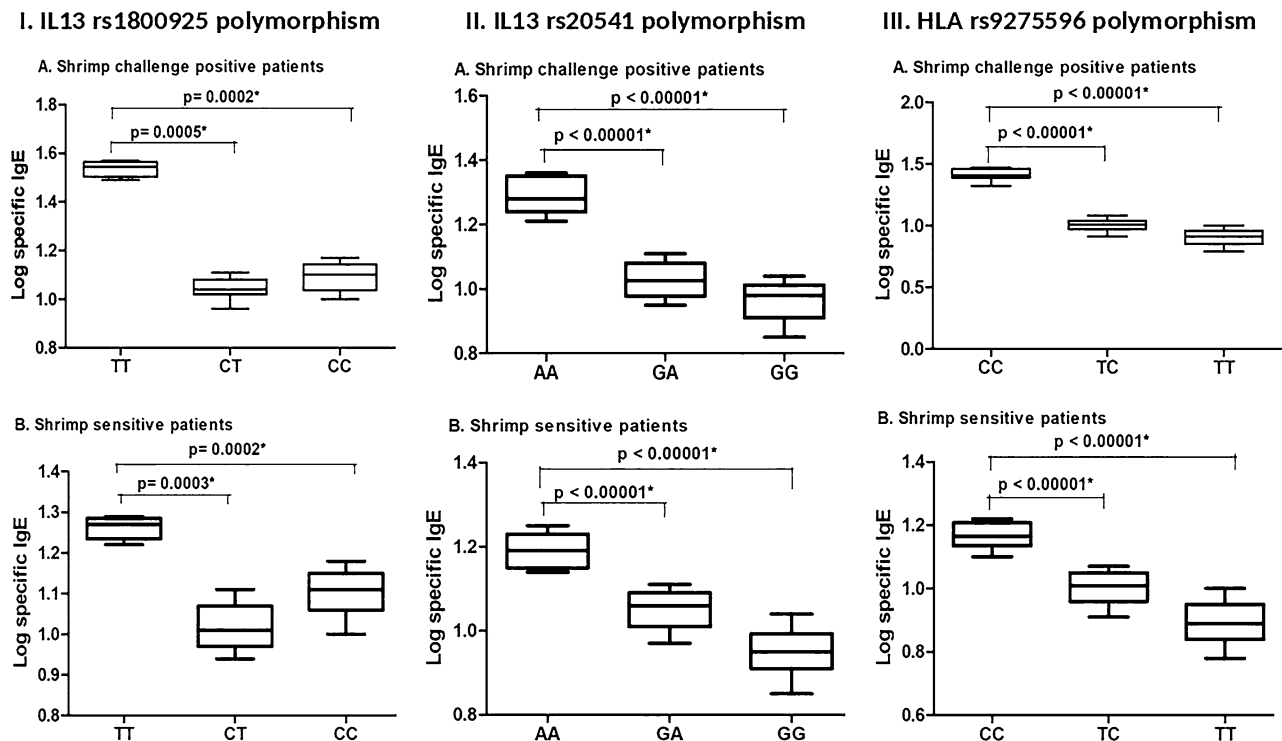


Fig. 2 Log₁₀ shrimp-specific IgE levels according to (I) IL13 rs1800925, (II) IL13 rs20541, and (III) HLA-DQ rs9275596 polymorphisms in (A) shrimp challenge positive and (B) shrimp sensitive patients

2013; Hong et al. 2015). There are scarcely available literatures reporting association between HLA polymorphism and shrimp allergy. A recent GWAS study on a Japanese population found strong association of HLA haplotype (HLA-DRB1*04:05-HLA-DQB1*04:01) with shrimp allergy although the study was based on self-reported food reactions rather than OFC (Khor et al. 2018). Our studied SNP rs9275596, located in HLA-DQ region, possess significant genetic risk for peanut allergy in subjects of European ancestry (Hong et al. 2015). But no such association established with shrimp allergy till date. In the present study, rs9275596 polymorphic CC genotype showed significantly higher association with challenge positive shrimp allergy (OR = 2.88, $P = 0.04$). Previous association studies identified DNA methylation in HLA-DQ region for IgE mediated food allergy (Martino et al. 2014) suggesting the fact that CC genotype may have a role in modifying DNA methylation levels in HLA-DQ region that could enhance the risk of shrimp allergy.

Literature studies provided evidence that IL13 pathway also play an important role in food allergy (Liu et al. 2004; Ashley et al. 2017). Two SNPs of the IL13 gene, rs20541 (+2044G→A) and rs1800925 (-1112C→T), have been associated with enhanced IL13 levels (Arima et al. 2002) and IL13 promoter functions (Cameron et al. 2006), respectively. IL13 rs20541 is a coding SNP in exon 4 causing non-conservative change of a positively charged arginine to a neutral glutamine at position 130 (Arg130Gln). The Arg130Gln substitution results

in increased expression of IL13 and promoting IgE production (Chen et al. 2004; Vladich et al. 2005). Similarly, in the present study, we also found individuals with rs20541AA genotype have significantly higher risk of shrimp allergy (OR = 3.60, $P = 0.01$) than those with GA or GG genotype. Another polymorphism is -1112C/T (rs1800925) which is located within the extended matrix of a STAT binding site in the IL13 promoter. Our present study showed that rs1800925TT genotype significantly associated with shrimp allergy (OR = 5.48, $P = 0.03$) which is in concordance with previous studies (Liu et al. 2004). Associations between the IL13 -1112T allele and allergic phenotypes, such as elevated serum IgE levels and positive SPT, have also been established (Graves et al. 2000).

Though the overall association between allergic inflammation and SNPs in IL13 has been replicated in many ethnically diverse populations (Hoffjan et al. 2003), the interpretation of the mechanisms underlying these associations become complex by the extended LD blocks discovered in chromosome 5q31 that can mask the effects of individual SNPs (Daly et al. 2001). In the present study, we found a strong LD ($D' = 0.81$) between rs1800925 and rs20541 polymorphisms. TA haplotype formed by the combination of these two SNPs was significantly associated with shrimp allergy ($P = 0.02$). Previous literature stated that the transcriptional up-regulation provided by rs1800925T may be relatively modest, but the enhancement of IL13 activity by rs20541A (R→Q), combined with the

concomitant increase in IL13 transcription associated with the rs1800925T allele may synergistically amplify IL13 dependent mechanisms (Vladich et al. 2005) which strongly support our present findings.

The present study revealed that HLA-DQ and IL13 polymorphisms, after stratifying according to age groups, found to be associated with an elevated risk of shrimp allergy in younger age (20–40 years). In addition, 85.98% of total patients' population reported to have persistent shrimp allergy from their childhood (< 20 years) which supports the previous studies done on shrimp allergy in India and other Asian countries (Ebisawa et al. 2017; Laha et al. 2020).

Further, in this study design, we divided our patients' population into shrimp challenge positive and sensitive ones (shrimp challenge negative) which provided us the opportunity to distinguish between the mechanisms of clinical shrimp allergy and shrimp sensitization, respectively. We found weak evidence that HLA-DQ and IL13 polymorphisms were associated with shrimp sensitization whereas the associations were more prominent with that observed in clinical shrimp allergy. These observations suggest that shrimp sensitization does not always correlate to clinical shrimp allergy. The probable reason may be the use of commercial extracts in our study during SPT to validate sensitization which may give a false positive result (Carnes et al. 2007). Majority of reactions against shrimp allergens are IgE-mediated with rapid onset (Woo and Bahna 2011). The present study showed that both allergic and sensitive individuals bearing polymorphic genotypes had significantly higher specific IgE level than those having wild-type genotypes. However, the elevation was slightly higher in allergic patients than sensitive ones.

Shrimp allergy is known to be mediated through the IgE mechanism, with the recognition by naive CD4+ T cells of allergenic peptides presented by HLA class II molecules known to be the trigger for the IgE-mediated hypersensitivity reaction via IL13 pathway (Ravkov et al. 2013). In the present study, the synergistic effect of IL13 TA haplotype–HLA-DQ rs9275596CC genotype interaction significantly elevated shrimp-specific IgE level ($P = 0.03$), which supports the above fact.

Conclusion

The present findings indicated that HLA-DQ rs9275596, IL13 rs20541, and IL13 rs1800925 polymorphisms are risk factors for shrimp allergic patients in West Bengal, India. Further candidate gene studies in different ethnic populations are now warranted to confirm and extend these findings. Understanding the genetic factors and molecular pathways that contribute to perturbation of Th₂ immune responses will be helpful in the future development of novel target-specific therapeutic approaches for the prediction, prevention, and treatment of shrimp allergy

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Author contributions All the authors have made substantial contributions. Arghya Laha contributed to data compilation and wrote first draft of the manuscript. Amlan Ghosh designed the genotyping protocol for HLA-DQ rs9275596 and helps in LD analysis. Saibal Moitra diagnosed the disease, provided the sample, and supervised the oral food challenge. Himani Biswas helped in data collection. Srijit Bhattacharya did the statistical analysis. Nimai Chandra Saha, Goutam Kumar Saha, and Sanjoy Podder conceived the study design. Sanjoy Podder supervised the work, critically revised the manuscript, and made the final draft. All authors read and approved the final manuscript.

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Data availability The datasets generated and/or analyzed during the current study are not publicly available due to consent requirements to protect subjects' privacy but are available from the corresponding author on reasonable request.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethics approval and consent to participate The study protocol was approved by the Clinical Research Ethics Committee, Allergy and Asthma Research Center, West Bengal, India (CREC-AARC Ref: 004/17). All the procedures performed in the present study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all included subjects and patient anonymity was preserved using methods approved by the Ethics Committee.

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Immunogenetics

Association of HLA-DQ & IL13 gene variants with challenge-proven shrimp allergy in West Bengal, India

Arghya Laha¹, Amlan Ghosh², Saibal Moitra³, Himani Biswas⁴, Nimai Chandra Saha⁵, Srijit Bhattacharya⁶, Goutam Kumar Saha⁷, Sanjoy Podder^{1*}

¹ Allergology and Applied Entomology Research Laboratory, Department of Zoology, University of Burdwan, Bardhaman-713104, West Bengal, India

² Department of Life Sciences, Presidency University, Kolkata- 700073, West Bengal, India

³ Allergy and Asthma Research Centre, Kolkata- 700029, West Bengal, India

⁴ Post Graduate Department of Zoology, Krishnagar Government College, Krishnagar- 741101, West Bengal, India

⁵ Vice-Chancellor, University of Burdwan, Bardhaman- 713104, West Bengal, India

⁶ Post Graduate Department of Physics, Barasat Government College, Kolkata-700124, West Bengal, India

⁷ Department of Zoology, University of Calcutta, Kolkata- 700019, West Bengal, India

***Corresponding Author:** Dr. Sanjoy Podder, Associate Professor, Department of Zoology, University of Burdwan, Bardhaman-713104, West Bengal, India, E-mail: skpzoo2@rediffmail.com.

Supplementary Table 1: Hardy-Weinberg equilibrium (HWE) values for pooled samples and for patient and control groups separately. (*P value significant)

SNP	Genotype	Total	Patient	Control
HLA-DQ rs9275596	TT	209	146	63
	TC	149	95	54
	CC	40	32	8
HWE		2.98	6.73	0.64
P value		0.08	0.009*	0.42
IL13 rs1800925	CC	256	171	85
	CT	119	81	38
	TT	23	21	2
HWE		3.24	6.14	0.96
P value		0.07	0.013*	0.33
IL13 rs20541	GG	206	134	72
	GA	151	105	46
	AA	41	34	7
HWE		2.79	3.40	0.009
P value		0.09	0.065	0.92

Supplementary Table 2: Genotype/allele distributions of rs9275596, rs1800925 and rs20541 polymorphisms stratified by age.

[illegible]

Supplementary Table 3: IL13 haplotype frequencies in patients and control subjects.

IL13 haplotypes [#]	Frequency			χ^2 (P value)	Adjusted OR ⁺ (95% CI)		P Value	
	Shrimp challenge positive (n=113)	Shrimp sensitive (n=160)	Control (n=125)					
TA	0.270	0.214	0.146	15.34 (P= 0.02)	2.11^a (1.11 – 4.00)	1.51 ^b (0.81 – 2.79)	0.02^c	0.19 ^d
CG	0.708	0.739	0.734		0.91 ^a (0.51 – 1.59)	1.05 ^b (0.62 – 1.78)	0.73 ^c	0.86 ^d
TG	0.007	0.010	0.022		0.36 ^a (0.04 – 3.54)	0.51 ^b (0.08 – 3.13)	0.38 ^c	0.47 ^d
CA	0.015	0.037	0.098		0.08^a (0.01 – 0.66)	0.37 ^b (0.13 – 1.01)	0.02^c	0.05 ^d

[#] The haplotype consists of rs1800925 and rs20541 polymorphisms respectively.

⁺ Adjusted for age and sex. Each haplotype is included in a separate model with the reference category consisting of individuals who did not have that specific haplotype.

^a OR of shrimp challenge positive patients against control (P value^c)

^b OR of shrimp sensitive patients against control (P value^d)

Significant associations (p < 0.05) are shown in bold type.