

Association of the STAT6 rs3024974 (C/T) Polymorphism with IgE-Mediated Food Sensitization among West Bengal Population in India

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Keywords

Food allergy · Genetic polymorphism · STAT6

Abstract

Introduction: Genetic polymorphisms associated with IgE-mediated food sensitization have been a robust area of research for decades. A genome-wide search for susceptible loci regulating the IgE response (atopy) identified the candidate gene STAT6, which is important in the context of food allergic manifestations. **Objective:** The present study was designed to investigate the sensitization of West Bengal population against some common allergenic food items and to study the role of the STAT6 gene polymorphism in elevating food-specific IgE levels among sensitized individuals. **Methods:** Skin prick test was performed for 6 food items among 501 patients (126 children, 85 adolescents, and 290 adults) from West Bengal, India. Among them, 165 patients were selected for measurement of total IgE and food-specific

ic IgE levels along with 165 controls. Finally, the STAT6 (rs3024974 (C/T) polymorphism was genotyped in 139 cases and control subjects. **Results:** Shrimp was identified as a dominant food allergen in adolescents and adults, whereas milk sensitization was highest in children. Food-sensitized patients with onset during childhood had significantly higher total IgE levels compared to patients with onset during adulthood ($p < 0.00001$). The frequency of the rs3024974 CC genotype in both cases and control subjects (55.40 and 46.76%, respectively) was higher than that of CT or TT. Patients with childhood onset bearing the CC genotype had significantly higher specific IgE levels in comparison to those with adult onset ($p = 0.001$). **Conclusion:** Food sensitization has a genetic background and the rs3024974 polymorphism is associated with susceptibility and reaction severity in food-sensitized patients in West Bengal population in India.

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Introduction

Food allergy (FA) is a common medical problem and some estimates suggest that about 5% of adults and 8% of children in Western countries suffer from an immunoglobulin IgE-mediated hypersensitivity reaction to food [1]. The prevalence of FA in Asia is perceived to be low. In India, there is a paucity of data regarding the prevalence of FA. FA symptoms are characterized by anaphylaxis, urticaria, and angioedema, occurring immediately after consumption of the food, whereas reactions appearing several hours after ingestion of the responsible foods include fatigue, irritability, depression, insomnia, headache, itchy limbs, asthma, indigestion, diarrhea, bloating, and skin lesions [2]. However, very little is known regarding the frequency and characteristics of FA, sensitization, and its association with manifestations of atopy in India. From patient-based studies, it is known that the spectrum of food allergens between adults and children is different on the basis of different dietary habits [3]. Therefore, to estimate the severity and types of allergic reactions in patients, identification of the disease causing foods is crucial for the prevention and management of food-induced allergic reactions. It is believed that FA, like other allergic diseases such as asthma and atopic dermatitis, is caused by a complex interaction between environmental and genetic factors [4]. Hence, study of the genetic predisposition would be useful in prior risk assessments and it would lead to more effective prevention and treatment. Among the susceptible genes associated with FA, the signal transducer and activator of transcription 6 (STAT6) gene plays an important role [5], although a few STAT6 single-nucleotide polymorphisms (SNP) have been reported to be associated with FA [6, 7]. The STAT6 intron 18 SNP rs3024974 was found to be associated with elevated total serum IgE levels in a study of asthmatic patients from a Caucasian population [8].

In the present study, we assessed sensitization against 2 non-vegetarian (egg white and shrimp) and 4 vegetarian (milk, peanut, banana, and eggplant) items through skin prick test (SPT) among the study population, who visited the Allergy and Asthma Research Center, West Bengal, India, during April 2017 to March 2018 with complaints of different FA symptoms. In order to establish their genetic association, we genotyped rs3024974 SNP based on a case-control study. Furthermore, we sought to investigate the correlation between polymorphic genotypes with specific IgE levels among the patient population.

Materials and Methods

Study Participants

Here we report on a case-control study that was performed between April 2017 and March 2018 at the Allergy and Asthma Research Center, Kolkata, India. The participants were stratified into the following 3 age groups: children (age 5–12 years), adolescents (age 13–19 years), and adults (age 20–50 years). As part of the study protocol, a computer-assisted standardized interview was performed. The subjects were asked whether they had allergic reactions to food and, if so, the type of reaction was recorded in detail for the 6 food allergens. A questionnaire was used to summarize the patients' age, sex, habitat, family history (paternal or maternal), onset of disease, food habits, and clinical features like oral (oral allergy syndrome defined as itching or swelling of the lips and throat), gastrointestinal (diarrhea or pain), skin (rash, wheals, itchiness, or redness), systemic (shortness of breath and asthma) or multiple symptoms. The history and doctor's diagnosis of atopic diseases (asthma or atopic eczema) as well as urticaria was also recorded. In addition, 165 control subjects with no past or present history of FA and willingness to participate were also included. Patients who did not provide consent for participation or those who suffered from other illnesses were excluded from this study.

Skin Prick Test

SPT was performed on the flexor side of the forearm of 501 patients using extracts (Credisol, Mumbai, India) of the following 6 food allergens: egg white, milk, peanut, shrimp, banana, and eggplant. The allergens were selected depending on the food habits and previous reports of FA within the people of West Bengal, India [9]. Saline and histamine were used as negative and positive controls, respectively, and the results were read after 15 min. The tests were scored according to the mean wheal diameter and considered positive when the wheal diameter exceeded 3 mm [10]. Results showing a positive reaction to saline or no reaction at all (including histamine) were excluded from further analysis. On the basis of the positive control, the results were further divided into the following 3 categories: mild (wheal diameter between 3 and 5 mm), moderate (wheal diameter 5–7 mm), and severe (wheal diameter >7 mm).

Estimation of Total Serum IgE

A total of 165 patients who showed moderate to severe SPT reactions and 165 controls (both male and female) were selected for the measurement of total IgE in serum via the enzyme immunoassay method using pathozyne immunoglobulin (Glaxo Smithkline Pharmaceuticals Ltd., Mumbai, India). Before the assay, calibration was done as per the World Health Organization (WHO) standard for IgE in different ages, i.e., 1–5 years, <60 IU/mL; 6–9 years, <90 IU/mL; 10–16 years, <200 IU/mL; and 16+ years, <100 IU/mL [11].

Quantification of Food-Specific IgE

The presence of specific IgE against the 6 selected foods in the serum of 165 patients was measured using the ImmunoCAP Phadia 100 system (Thermo Fisher Scientific, USA). 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 [12].

Table 1. Demographic and clinical features of the patients and controls

Features	Patients	Controls	Mean ± SD	95% CI
<i>Age</i>			33±1	
Children (5–12 years)	126 (25.15)	34 (20.61)		
Adolescents (13–19 years)	85 (16.97)	19 (11.52)		
Adults (20–50 years)	290 (57.88)	112 (67.87)		
<i>Gender</i>				
Male				
Children	73 (25.89)	23 (26.74)		
Adolescents	47 (16.67)	12 (13.95)		
Adults	162 (57.45)	51 (59.30)		
Female				
Children	53 (24.20)	11 (13.92)		
Adolescents	38 (17.35)	7 (8.86)		
Adults	128 (58.45)	61 (77.22)		
<i>Symptoms</i>				
Urticaria/atopic eczema		–		
Children	85 (26.81)			
Adolescents	72 (22.71)			
Adults	160 (50.47)			
Gastrointestinal		–		
Children	76 (33.63)			
Adolescents	56 (24.78)			
Adults	94 (41.59)			
Oral allergy syndrome		–		
Children	45 (28.85)			
Adolescents	34 (21.79)			
Adults	77 (49.36)			
Asthma		–		
Children	15 (17.64)			
Adolescents	13 (15.29)			
Adults	57 (67.05)			
<i>Family history</i>				
Paternal		–		
Children	54 (44.63)			
Adolescents	49 (40.50)			
Adults	18 (14.88)			
Maternal		–		
Children	32 (36.36)			
Adolescents	25 (28.41)			
Adults	31 (35.23)			
Paternal and maternal		–		
Children	25 (55.56)			
Adolescents	13 (28.89)			
Adults	7 (15.56)			
Absent		–		
Children	40 (13.70)			
Adolescents	11 (3.77)			
Adults	241 (82.53)			
<i>Age at onset</i>				
Childhood (<20 years)	216 (43.11)	–		
Adult (>20 years)	285 (56.89)	–		
Weight, kg			63.2±12.81	61.1–65.3
Height, cm			165.2±12.99	163.0–167.4

Table 1 (continued)

Features	Patients	Controls	Mean $\pm$ SD	95% CI
<i>Residence</i>				
Urban				
Children	69 (24.38)	14 (15.73)		
Adolescents	47 (16.61)	12 (13.48)		
Adults	167 (59.01)	63 (70.79)		
Semiurban				
Children	50 (26.04)	15 (25.00)		
Adolescents	32 (16.67)	7 (11.67)		
Adults	110 (57.29)	38 (63.33)		
Rural				
Children	7 (26.92)	5 (31.25)		
Adolescents	6 (23.08)	0 (0)		
Adults	13 (50.00)	11 (68.75)		
<i>Food habits</i>				
Vegetarian				
Children	25 (25.77)	1 (5.56)		
Adolescents	15 (15.46)	3 (16.67)		
Adults	57 (58.76)	14 (77.78)		
Nonvegetarian				
Children	101 (25.00)	33 (22.45)		
Adolescents	70 (17.33)	16 (10.88)		
Adults	233 (57.67)	98 (66.67)		
<i>Positive SPT (wheal diameter), mm</i>				
Egg white	370 (73.85)	–	6.25 $\pm$ 1.47	5.83–6.67
Milk	319 (63.67)	–	5.83 $\pm$ 1.41	5.43–6.23
Peanut	136 (27.15)	–	4.76 $\pm$ 1.18	4.42–5.10
Shrimp	395 (78.84)	–	6.79 $\pm$ 2.10	6.19–7.39
Banana	272 (54.29)	–	5.52 $\pm$ 1.37	5.13–5.91
Eggplant	348 (69.46)	–	6.03 $\pm$ 1.82	5.51–6.55
Total IgE, IU/mL			1,348.5 $\pm$ 116.5	1,328.97–1368.03
<i>Specific IgE (kU/L) (n = 165)</i>				
Egg white	100 (71.94)		5.37 $\pm$ 11.67	2.58–8.16
Milk	81 (58.27)		4.38 $\pm$ 7.69	2.53–6.23
Peanut	26 (18.71)		4.2 $\pm$ 10.17	1.77–6.63
Shrimp	112 (80.58)		6.22 $\pm$ 14.36	2.79–9.65
Banana	63 (45.32)		5.7 $\pm$ 12.53	2.7–8.7
Eggplant	90 (64.75)		5.39 $\pm$ 11.10	2.73–8.05

The total number of patients is 501. The total number of controls is 165. Values are presented as numbers (%) unless otherwise stated.

Genomic DNA Isolation and Genotyping of the STAT6 rs3024974 Polymorphism

The experimental set-up was done with 139 patients who had specific IgE against any one of the above mentioned foods and 139 controls. Genomic DNA was extracted from EDTA-preserved peripheral blood samples using proteinase-K digestion followed by phenol-chloroform method [13]. The flanking region around the rs3024974 SNP site within intron 18 of STAT6 gene was amplified by polymerase chain reaction (PCR) using primers (forward 5'-GAACAGATGGGTAAGGATGG-3' and reverse 5'-TCTGGGGTAGGAAGTGGTTG-3'). A total of 20 μ l of reac-

tion mixture was prepared containing 50 ng of genomic DNA, 200 nM of each primer (Sigma), 200 μ M of each deoxyribonucleotide triphosphate (Thermo Fisher Scientific), 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, 57 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 7 min. The PCR product was of 198bp, which was digested with PstI restriction enzyme (New England Biolabs). The restriction site for PstI is 5'-CTGCAG-3', which is only found in PCR products bearing the rs3024974 C allele. Hence, for CC homozygotes, PstI digestion yielded 2 fragments of 115 and 83 bp,

whereas for TT homozygotes PCR product remain undigested. All 3 bands (198, 115, and 83 bp) were observed in the case of CT heterozygotes.

DNA Sequencing

Sanger sequencing was performed using representative PCR products for each of the 3 genotypes (CC, CT, and TT) to confirm the RFLP results. Both forward and reverse strands in PCR products were amplified using an ABI PRISM™ BD Terminator cycle sequencing kit (Applied Biosystems) and electrophoresed in a 3100-Avant genetic analyzer (Applied Biosystems). Polymorphisms were identified by aligning the forward and reverse complement sequences with respective reference sequences (ensemble.org) using Clustal W multiple alignment software (European Bioinformatics Institute).

Statistical Analysis

Quantitative results are presented as means ($\pm$ SE) and categorical results as numbers (%). A one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was performed to compare the intensity of SPT reactions against different food allergens. The SPT sensitivity and mean total IgE level among different age groups and the association of the STAT6 polymorphism with specific IgE levels were analyzed using the Kruskal-Wallis test. A nonparametric Mann-Whitney U test was used to estimate any significant differences between patients and control subjects with respect to total IgE levels. A contingency χ^2 test was used to determine whether there was any significant difference in genotype and allele frequencies between patients and controls. The genotype and allele frequencies of the patients and controls were assessed for Hardy-Weinberg equilibrium by a χ^2 test. Bonferroni and Benjamin-Hochberg (BH) corrections (considering a 5% false discovery rate) were performed to find the age- and sex-adjusted p values of the Kruskal-Wallis test regarding the variation of specific IgE with genotype. All statistical analyses were done using Prism version 7 (Graph Pad Prism, San Diego, CA, USA).

Results

Patient Characteristics

The demographic and clinical features of 501 patients and 165 controls are shown in Table 1 along with mean and 95% CI. The majority of the participants were adults in both the patients and the control groups (57.88 and 67.87%, respectively). Male participants (56.29 and 52.12% of the patients and controls, respectively) were found in a higher proportion than females. The most prevalent symptom was urticaria/atopic eczema, observed in 63.27% of the patients, followed by gastrointestinal symptoms (45.11%), oral allergy syndrome (31.14%), and asthma (16.97%). Analysis of the family history of the above symptoms revealed that 58.28% of the patients did not inherit the disease from their parents, 24.15% had a paternal history, and 17.56% had a maternal history, whereas 8.98% reported having both. On the other hand,

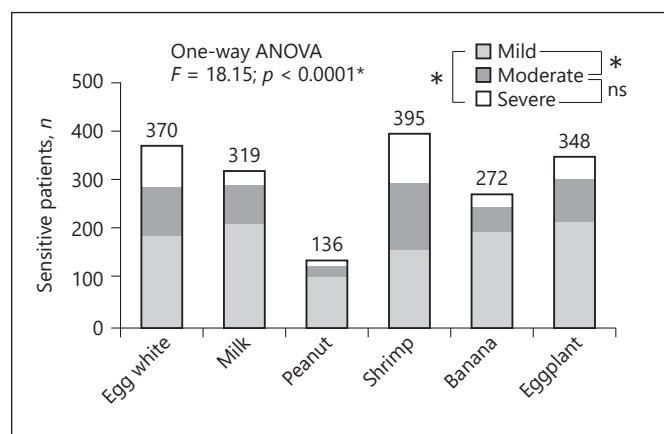


Fig. 1. SPT responses against the 6 food allergens tested. Each bar is divided into 3 parts, i.e., mild, moderate, and severe, according to the intensity of the SPT reactions. The total number of sensitive patients for each food is indicated above the bars. p values were calculated by one-way ANOVA followed by Bonferroni's multiple comparisons test for pairwise comparisons between mild, moderate, and severe patients. * Significant p value.

most of the patients (56.89%) became allergic in adulthood, i.e., after 20 years of age, in comparison 43.11% of patients were sufferers since childhood, i.e., before 20 years of age. A larger part of study population resided in urban areas (56.49 and 53.94% of patients and controls respectively), and the majority of them were non-vegetarian (80.64% of the patients and 89.09% of the controls).

Sensitization against Six Foods by SPT

Of a total of 501 patients, 84.83% were attributed to skin reactions. The highest prevalence of allergic sensitization was obtained with shrimp, followed by egg white, eggplant, milk, banana, and peanut (Fig. 1). Interestingly, 27% of the patients exhibited sensitivity to shrimp only. The mean SPT values against each food allergen are given in Table 1. According to the intensity of the SPT response, a significant variation persisted between the numbers of patients showing mild and moderate/severe sensitivities ($F = 18.15$, $p < 0.0001$), whereas the difference between moderate and severe reactions were found to be non-significant (Fig. 1).

The patients' sensitivity to different food allergens, stratified by age, is given in Table 2. Both the Shapiro-Wilk test and the Q-Q plot of the residuals indicated skewed characteristics of the data. Thus, a nonparametric Kruskal-Wallis test was done and it showed the existence of a significant difference between the 3 age groups ($H = 12.32$, $p = 0.002$). A pairwise Mann-Whitney U test also showed that both child versus adult ($p = 0.005$) and ado-

Table 2. Patient sensitivity according to age group

	Egg white	Milk	Peanut	Shrimp	Banana	Eggplant	Kruskal-Wallis test (<i>p</i> value)
Children (<i>n</i> = 136)	77 (56.62)	105 (77.21)	13 (9.56)	80 (58.82)	32 (23.53)	73 (53.68)	<i>H</i> = 12.32 (<i>n</i> = 18) (<i>p</i> = 0.002*)
Adolescents (<i>n</i> = 90)	39 (43.33)	38 (42.22)	10 (11.11)	43 (47.78)	27 (30.00)	35 (38.89)	
Adults (<i>n</i> = 275)	254 (92.36)	176 (64.00)	113 (41.09)	272 (98.91)	213 (77.45)	240 (87.27)	
Mann-Whitney U test	Children and adolescents: <i>Z</i> = 1.36, <i>p</i> = 0.17 Children and adults: <i>Z</i> = -2.80, <i>p</i> = 0.005* Adolescents and adults: <i>Z</i> = -2.80, <i>p</i> = 0.005*						

Values are presented as numbers (%) unless otherwise stated. The Kruskal-Wallis test was used for *p* value calculation. Pairwise significance levels between different age groups were evaluated using the Mann-Whitney U test. * Significant *p* value.

Table 3. STAT6 rs3024974 (C/T) genotype and allele frequencies**a** Patients vs. controls

Genotype/allele	Patients	Controls	χ^2	OR (95% CI)	Age-adjusted OR	Sex-adjusted OR
CC	77 (55.40)	65 (46.76)	2.21 (<i>p</i> = 0.331)	1.62 (0.69–3.76)	1.60	1.62
CT	51 (36.69)	59 (42.45)		1.18 (0.50–2.80)	1.17	1.18
TT	11 (7.91)	15 (10.79)		1	1	1
CC+CT	128 (92.09)	124 (89.21)	1.96 (<i>p</i> = 0.161)	1.32 (0.92–1.91)	1.32	1.32
C allele	205 (73.74)	189 (67.99)		1	1	1
T allele	73 (26.26)	89 (32.01)		1	1	1

b According to age of onset

Genotype/allele	Childhood onset (<i>n</i> = 64)	Adult onset (<i>n</i> = 75)	χ^2	OR (95% CI)
CC	40 (62.50)	37 (49.33)	2.67 (<i>p</i> = 0.264)	1.30 (0.36–4.61)
CT	19 (29.69)	32 (42.67)		0.71 (0.19–2.66)
TT	5 (7.81)	6 (8.00)		1
CC+CT	59 (92.19)	69 (92.00)	1.26 (<i>p</i> = 0.261)	1.42 (0.82–2.44)
C allele	99 (77.34)	106 (70.67)		1
T allele	29 (22.66)	44 (29.33)		1

Values are presented as numbers (%) unless otherwise stated. *p* values were calculated using a contingency χ^2 test. *p* < 0.05 is considered statistically significant.

lescent versus adult (*p* = 0.005) sensitivities were significant.

A history of FA on the basis of symptoms is given in online supplementary Table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000504575). Reactions were most frequently observed after consumption of the responsible food. Detailed information with respect to symptoms showed that the majority of the shrimp-sensitive patients had urticaria/atopic eczema (65.62%). Milk was reported as the predominant

trigger of gastrointestinal complaints in the patients (62.83%), whereas oral allergy syndrome has been found to be prevalent in eggplant-sensitive patients (58.97%).

*Estimation of IgE and Its Association with Age**Total IgE*

The median total IgE of 165 patients was 313.8 IU/mL, whereas in the controls it was 95.75 IU/mL (online suppl. Fig. 1A), and the difference was found to be statistically

significant ($p < 0.00001$). Among the patients, the results of the Kruskal-Wallis test revealed that there was a significant difference in total IgE among different age groups ($p < 0.00001$). The total IgE level gradually decreased with age (online suppl. Fig. 1B), with children having the highest level of total IgE (median 1,000.0 IU/mL), followed by adolescents (median 580.5 IU/mL) and adults (median 269.5 IU/mL). We also found that patients with a childhood onset of food sensitization (median 940.9 IU/mL) had significantly higher IgE levels than those with onset during adulthood (median 272.0 IU/mL) ($p < 0.00001$; online suppl. Fig. 1C).

Specific IgE

Specific IgE was estimated in sera of patients showing marked positive SPT ($n = 165$) to food extracts. Among the 165 serum samples tested, 139 (84.24%) had demonstrated positive ImmunoCAP results against at least 1 of the 6 food allergens. The majority of the patients (80.58%) had shrimp-specific IgE followed by egg white (71.94%), eggplant (64.75%), milk (58.27%), banana (45.32%), and peanut (18.71%) (online suppl. Table 2A). Moreover, among the different age groups, the incidence of a positive response to the tested food allergens was significantly higher in adults (online suppl. Table 2B) in comparison to children ($p = 0.01$) or adolescents ($p = 0.005$), except for milk allergen, where the maximum response (80%) was shown by children. Besides, stronger positive reactions to different allergens were observed in patients suffering since childhood in comparison to those with adulthood onset (online suppl. Table 2C), but the difference was statistically non-significant (Mann-Whitney U test, $p = 0.94$). The normality of the data was checked using a Q-Q plot and nonparametric tests were done as the data was found to be skewed. The mean specific IgE level in patients against different food allergens are shown in Table 1, and in the controls the mean value was below 0.35kU/L.

STAT6 rs3024974 Polymorphism

Allele and Genotype Analysis

The allele and genotype distributions of rs3024974 in our study population are shown in Table 3. The genotype frequencies in the patient and control populations did not show any significant departure from Hardy-Weinberg equilibrium ($\chi^2 = 0.38$ and 0.09, respectively, at d.f. = 1). We observed that the CC genotypes occurred more frequently than either the CT or the TT genotype. However, the CC genotype was found in patients in a higher proportion, whereas the TT genotype was found in the con-

Table 4. STAT6 rs3024974 association with specific IgE levels in patients.

rs3024974	Geno- type	n (%)	Specific IgE, kU/L						Kruskal-Wallis test	Raw <i>p</i> values for different age groups (Kruskal-Wallis test)	Age- and sex- adjusted <i>p</i> value after Bonferroni correction	Age- and sex-adjusted BH reference <i>p</i> value (false discovery rate 5%)
			egg white	milk	peanut	shrimp	banana	eggplant				
CC	77 (55.40)	51 (36.69)	9.34±2.13 (5.02–13.66)	10.89±2.47 (5.76–16.02)	6.70±1.87 (2.92–10.48)	12.42±2.82 (6.63–18.21)	8.44±1.91 (4.60–12.28)	9.18±2.02 (5.12–13.24)	Children = 0.002 Adolescents = 0.002 Adults = 0.029	Children = 0.006* Adolescents = 0.006* Adults = 0.087	Children = 0.017* Adolescents = 0.033* Adults = 0.05*	
			6.47±1.56 (3.34–9.60)	7.72±1.53 (4.62–10.82)	4.92±1.41 (2.10–7.74)	9.46±2.16 (5.10–13.82)	5.68±1.40 (2.88–8.48)	5.64±1.45 (2.73–8.55)				
			2.90±1.12 (0.62–5.18)	5.70±1.21 (3.27–8.13)	2.50±1.16 (0.17–4.83)	5.67±1.40 (2.88–8.46)	2.50±2.06 (0.22–4.78)	3.00±1.40 (0.19–5.81)				
TT	11 (7.91)								<i>H</i> = 11.24 (<i>n</i> = 18) (<i>p</i> = 0.004*)			
Mann-Whitney U test			CC and CT: <i>Z</i> = 2.002, <i>p</i> = 0.04*; CC and TT: <i>Z</i> = 2.802, <i>p</i> = 0.005*; CT and TT: <i>Z</i> = 2.001, <i>p</i> = 0.04*							Males = 0.007 Females = 0.002	Males = 0.021* Females = 0.006*	Males = 0.05* Females = 0.025*

Values are presented as means ± SE (95% CI) unless otherwise stated. Mann-Whitney U test is performed to check the pair-wise significance level among the genotypes. Raw and age- and sex-adjusted *p* values were evaluated using the Kruskal-Wallis test. * Significant *p* value.

Values are presented as means ± SE (95% CI) unless otherwise stated. Mann-Whitney U test is performed to check the pair-wise significance level among the genotypes. Raw and age- and sex-adjusted p values were evaluated using the Kruskal-Wallis test. * Significant p value.

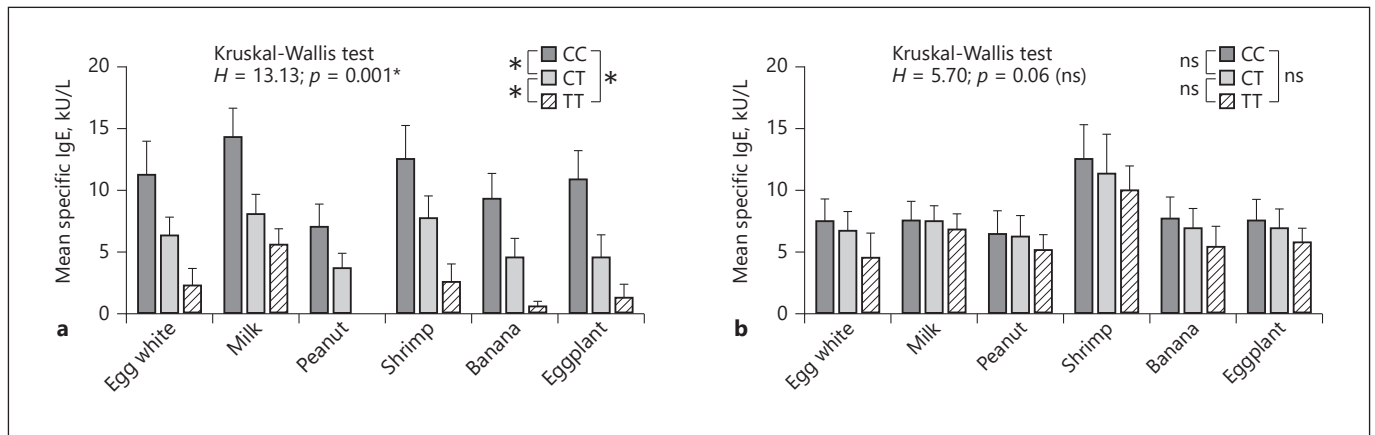


Fig. 2. Specific IgE levels and their association with 3 rs3024974 genotypes. **a** Association with childhood onset. **b** Association with adult onset. The p value was calculated using the Kruskal-Wallis test followed by the Mann-Whitney U test for pairwise comparisons between the CC, CT, and TT genotypes. * Significant p value.

trols, but the difference in genotype or allele frequencies for the rs3024974 polymorphism was non-significant ($p = 0.331$ and 0.161 , respectively) between food-sensitized patients and controls. Patients bearing the CC genotype were more prone to different food sensitization with an OR of 1.62 in comparison to those having the TT genotype. The OR defines the measurement of the association that compares the ratio of the odds of one event in the presence of another event to the odds of the first event in the absence of the second event [14]. The OR remained approximately the same after age and sex adjustments. When patients' frequencies were further categorized according to the age of onset, the difference in genotype or allele frequencies remained insignificant ($p = 0.264$ and 0.261 , respectively). The frequency of the CC genotype was higher in patients with childhood onset than that of the TT genotype (OR = 1.30). The representative chromatogram for all 3 genotypes is shown in online supplementary Figure 2.

Specific IgE Level of rs3024974 Genotypes and Its Association with Age

The mean levels of specific IgE in patients' serum were compared for association with SNP genotypes using the Kruskal-Wallis test (Table 4). A significantly higher specific IgE level was observed in patients with the CC genotype compared to those with the CT or TT genotype ($H = 11.24$, $p = 0.004$). Age and sex were identified as the possible confounders. The raw p values were found to be 0.002, 0.002, and 0.029 for the child, adolescent, and adult age groups, respectively, and the raw p values for sex were

0.007 and 0.002 for the male and female patients, respectively. Bonferroni-corrected age-adjusted p values indicated a similar trend, with adults showing non-significant p values. Interestingly, this fact is substantiated in Fig. 2a and b, which shows a significant ($H = 13.13$, $p = 0.001$) association of the CC genotype with elevated specific IgE levels in patients with a childhood onset of FA (Fig. 2a), whereas in the case of adult onset (Fig. 2b) it was non-significant ($H = 5.70$, $p = 0.06$).

However, Bonferroni corrections could not guarantee a prudent result by predicting the false discovery rate. Therefore, in addition, the BH approach [15] for adjusting p values has been taken here. Assuming a false discovery rate of 5%, age- and sex-adjusted p values were found to be significant for all of the age and sex groups. Table 4 gives the reference BH p values. The raw p values were lower than the BH reference p values and thus can be termed as significant.

Discussion

FA is an adverse immunological reaction resulting from the consumption of certain foods; it induces excessive IgE production and is also responsible for a wide range of anaphylactic reactions [16]. Allergy-causing foods vary according to the lifestyle, food habits, and genetic background of the inhabitants of a particular area [17]. The present study was based on FA, sensitization, and associations with the STAT6 gene polymorphism among West Bengal population, which have been barely

available so far from India. A major strength of this study lies in its specific population-based setting.

In the present study design, subjects were selected from a representative study base by sensitization status and reported symptoms. Here we demonstrated the sensitization against some major foods among patients of West Bengal, India. In the current study it was found that more than 78% of the participants had been sensitized to shrimp. In recent years there has been a steady growth in the production and consumption of seafood, especially shellfish, in India, particularly in West Bengal. This is similar to the ongoing trend in other Asian countries, accentuating the importance of this work [18]. Another food chosen here is egg, which is one of the most important allergens globally. Allergy to egg has been estimated to affect 0.5–2.5% of young children [19–21], with a recent estimate of up to 8.9% of infants reacting to raw egg in one study from Australia [22]. In egg allergy, avoidance is difficult and allergic reactions from accidental ingestion are highly common [23], suggesting the possibility of an increase in the number of egg-allergic adults. In the present study it was found that more than 73% of the participants had allergic sensitivity to egg white, and most of them were adults (92.36%). It has been reported that ovomucoid is the dominant allergen in egg white [24] and its allergenicity depends mostly on its resistance to heat and digestive enzymes [25]. In recent years, allergic reactions to eggplant (commonly known as brinjal) have been reported mainly from India [26]. In accordance, the present study revealed that the maximum sensitivity was shown by eggplant (69.46%) among vegetarian items. Milk is considered to be a staple food for children and an essential supplement in the diet of adults, since it has a balanced composition of nutrients with optimal digestibility, resulting in a product with a high biological value. One of the main milk proteins is casein, which constitutes about 80% of all milk proteins, causing a greater sensitivity in individuals [27, 28]. Cow's milk protein allergy is the most common FA in early childhood, affecting 2–5% of the child population aged less than three years [29]. The present study showed that 63.67% of the patients were sensitive to milk and among them more than 77% were children. Banana is another important source of nutrients; the fruit is both grown and consumed in high amounts, especially in the eastern region of our country. However, only a few studies have been conducted to investigate the diagnostic approach and clinical presentation of banana allergy in people living in this region [9, 17]. The estimated prevalence of peanut allergy in developed coun-

tries has been found to be between 0.6 and 1.0% [30–32]. It tends to present early in life and, in highly sensitized people, trace quantities can induce an allergic reaction [33–35]. Despite the growing peanut allergy epidemic in Western populations, the prevalence of peanut allergy in Asia is relatively low [36]. A substantial percentage of food-sensitive subjects in our study reacted to peanut (27.15%), an allergen that accounts for even more reactions in countries where peanut consumption is more common.

In the present study there was a significant difference in sensitivity among the different age groups. Adults were more sensitive than adolescents and children except for milk allergy where children were the worst sufferers. Clinical reactions against milk are usually detected in early childhood after breastfeeding is terminated and cow milk is introduced into the infant's diet [37]. In adulthood, about 85% of sensitization and resulting FA are caused by peanuts, crustaceans, and certain fruits and vegetables [38], which is in concurrence with our findings.

The present study showed a significantly higher level of total IgE in patients in comparison to controls. Moreover, we found a significant difference in total IgE distribution by age group and the level of IgE decreased with age, which is in accordance with our previously published work on aeroallergy [39]. The present study also depicted an inverse correlation between IgE levels and age of onset of food sensitization. Warren et al. [40] reported that, other than IgE mediation, increasing age, a history of multiple food allergies, and female sex are generally associated with the development of allergic symptoms in adulthood. Accordingly, our study population included a considerable number of female patients (43.71%) and the majority had sensitization against more than 1 of the above food items.

The present study depicts positive responses on specific IgE tests against the 6 food allergens. The agreement between SPT and specific IgE was reported to be moderate when tested without a clinical correlation [41]. In contrast, our data demonstrated a stronger agreement between SPT and specific IgE results for all of the allergens. Moreover, all of the study participants had more or less history of allergic symptoms as revealed by the questionnaire. Hence, the occurrence of contradictory SPT and specific IgE results is unlikely due to asymptomatic sensitization.

The risk of allergy development in children of healthy parents ranges 5 to 15%. When one parent is allergic it increases to 40% and if both parents are sick it reaches

60–80% [3]. Table 1 shows that 41.72% of our study population inherited food sensitization from their family, which supports the role of genetic predisposition in food allergen sensitization. In the present study, we reported a significant association of rs3024974 SNP in the intron 18 region of the STAT6 gene with elevated food-specific IgE levels in West Bengal population in India. The present study demonstrated the predominance of polymorphic CC genotypes in the population. However, no significant difference in genotypic frequency was observed between patients and controls in this study. So, it may be said that the polymorphism in the STAT6 gene might not be involved in the development of food-allergic manifestations. The present study also revealed that 7.91% of food-sensitized individuals had the rs3024974 polymorphic T/T variant, which further confirmed STAT6 as the gene (or one of a set of genes) driving Th2 differentiation and IgE production.

When we consider that the majority of food allergies usually develop during childhood, there appear to be some differences in pathogenesis (especially genetic background) between allergies with a childhood onset and those with an adulthood onset. The present study showed that the STAT6 rs3024974 CC genotype was significantly associated with elevated specific IgE levels in patients with a childhood onset of sensitization, although such a relationship was absent in adult-onset food sensitization.

The present findings suggest that rs3024974 SNP of the STAT6 gene seems to be a prominent marker for predicting sensitization against different foods among patients with childhood onset and thus could be helpful for early therapeutic intervention in the near future.

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Statement of Ethics

The study protocol was approved by the Clinical Research Ethics Committee, Allergy and Asthma Research Center, West Bengal (CREC-AARC Ref. 004/17). Written informed consent was obtained from the selected patients.

Disclosure Statement

The authors have no conflict of interests to declare.

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Author contributions

All of the authors made substantial contributions to this study. Arghya Laha conducted the experiments, contributed to data compilation, and wrote the first draft of this paper. Amlan Ghosh designed the genotyping protocol, analyzed the respective data, and critically revised this paper. Saibal Moitra diagnosed the disease and provided the sample. Goutam Kumar Saha, Ishita Saha, Srijit Bhattacharya, and Sanjoy Podder conceived the study design and performed statistical analysis. Sanjoy Podder supervised this work, critically revised this paper, and wrote the final draft.

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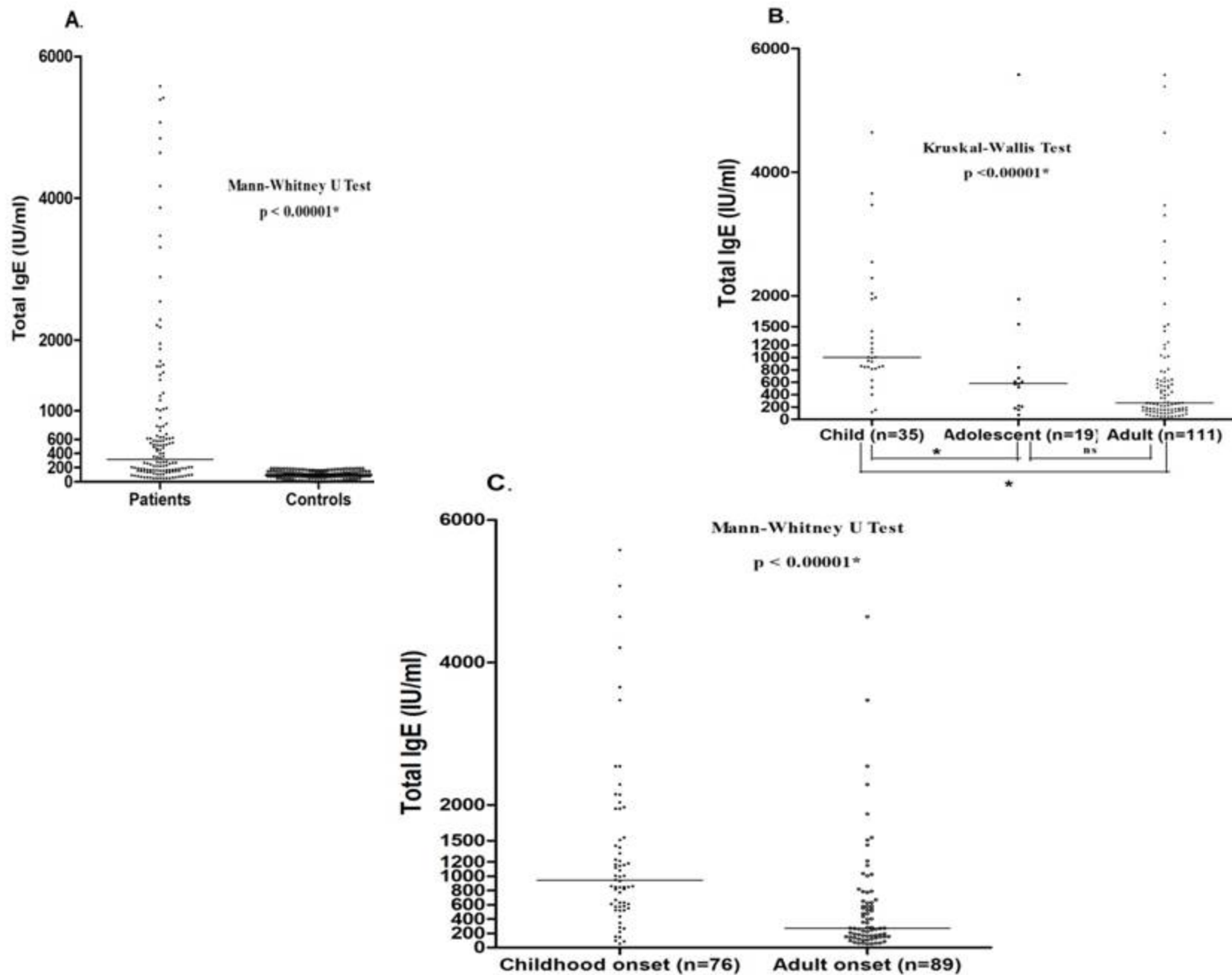
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Online Suppl. Fig. 1. Analysis of total IgE. **A.** Patients vs. Control (Mann-Whitney U Test)
B. Child vs. adolescent vs. adult (Kruskal-Wallis Test followed by Mann-Whitney U Test for pair-wise comparison) **C.** According to age of onset i.e. childhood onset (<20 years) vs. adult onset (>20 years) (Mann-Whitney U Test). (*p value significant).

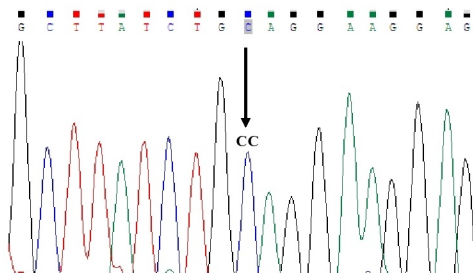
Online Suppl. Fig. 2. Representative chromatogram showing polymorphism. Arrows indicate polymorphic site. a) CC homozygote b) CT heterozygote c) TT homozygote.

Supplementary Fig. 1

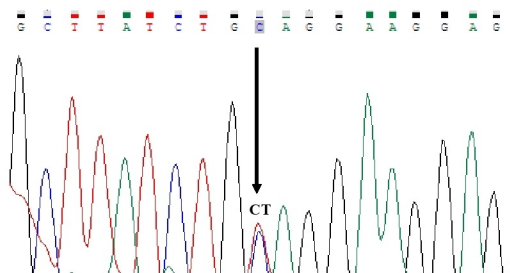


Supplementary fig. 2

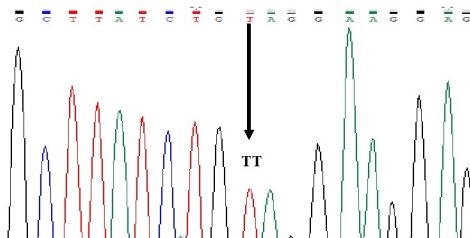
a.



b.



c.



Supplementary Table 1: Symptom-wise categorization of different food sensitive patients.

	Eggwhite	Milk	Peanut	Shrimp	Banana	Eggplant
Urticaria/ Atopic eczema (n=317)	193 (60.88%)	133 (41.96%)	68 (21.45%)	208 (65.62%)	155 (48.90%)	150 (47.32%)
Gastrointestinal (n=226)	113 (50.00%)	142 (62.83%)	48 (21.24%)	114 (50.44%)	73 (32.30%)	95 (42.04%)
Oral Allergy Syndrome (n=156)	59 (37.82%)	70 (44.87%)	24 (15.38%)	81 (51.92%)	73 (46.79%)	92 (58.97%)
Asthma (n=85)	24 (28.24%)	20 (23.53%)	9 (10.59%)	28 (32.94%)	13 (15.29%)	22 (25.88%)

Supplementary Table 2: Analysis of specific IgE in patients (n=139) against selected allergens. Kruskal-Wallis Test is performed for p value calculation according to age group. Pair-wise variation between age groups are evaluated by Mann-Whitney U Test (*p value significant).

A. Total no. of positive reactions						
	Eggwhite	Milk	Peanut	Shrimp	Banana	Eggplant
	100 (71.94%)	81 (58.27%)	26 (18.71%)	112 (80.58%)	63 (45.32%)	90 (64.75%)
B. Positive reactions in different age groups						
Child (n=30)	19 (63.33%)	24 (80.00%)	1 (3.33%)	23 (76.67%)	7 (23.33%)	16 (53.33%)
Adolescent (n=16)	11 (68.75%)	10 (62.50%)	2 (12.50%)	12 (75.00%)	7 (43.75%)	9 (56.25%)
Adult (n=93)	70 (75.27%)	47 (53.54%)	23 (24.73%)	77 (82.79 %)	49 (52.69%)	65 (69.89%)
H= 11.24, p= 0.004* (Kruskal-Wallis Test)						
Child & Adolescent Z=1.12; p= 0.26 (Mann-Whitney U Test)						
Child & Adult Z= -2.56; p= 0.01* (Mann-Whitney U Test)						
Adolescent & Adult Z= -2.80; p= 0.005* (Mann-Whitney U Test)						
C. Positive reactions according to age of onset						
Childhood onset (n=64)	47 (73.44%)	49 (76.56%)	13 (20.31%)	55 (85.94%)	37 (57.81%)	43 (67.19%)
Adult onset (n=75)	53 (70.67%)	32 (42.67%)	13 (17.33%)	57 (76.00%)	26 (34.67%)	47 (62.67%)
Z= 0.08; p= 0.94 (Mann-Whitney U Test)						