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Single nucleotide polymorphisms (SNPs) in key cytokines may modulate food allergy phenotypes

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Abstract

Single nucleotide polymorphisms (SNPs) can play a direct or indirect role in phenotypic expression in food allergy pathogenesis. Our goal was to quantitate the expression of SNPs in relevant cytokines that were expressed in food allergic patients. SNPs in cytokine genes IL-4 and IL-10 are known to be important in IgE generation and regulation. We examined IL-4 (C-590T), IL-4R α (1652A/G) and IL-10 (C-627A) SNPs using real-time PCR followed by restriction fragment length polymorphism (RFLP) analysis. Our results show that the AA, AG and GG genotypes for IL-4R α (1652A/G) polymorphisms were statistically different in radioallergosorbent test (RAST) positive versus negative patients, and although no statistically significant differences were observed between genotypes in the IL-4 (C-590T) and IL-10 (C-627A) SNPs, we observed a significant decrease in IL-4 (C-590T) gene expression and increase in IL-4R α (1652A/G) and IL-10 (C-627A) gene expression between RAST⁺ versus RAST⁻ patients, respectively. We also observed significant modulation in the protein expression of IL-4 and IL-10 in the serum samples of the RAST⁺ patients as compared to the RAST⁻ patients indicating that changes in SNP expression resulted in altered phenotypic response in these patients.

Keywords

Single nucleotide polymorphisms; Cytokines; Atopy; Food allergy; RAST⁺; IgE

Introduction

Food allergy is a major health issue prevalent not only in the United States (US) but worldwide. The Center for Disease Control (CDC) reports that approximately 3 million children (3.9 %) under 18 years of age were reported to have food allergies [1]. There are significantly more cases of food allergy in children under the age of 5 years compared to their older counterparts [2]. An equal number of male and female children were affected (3.8 and 4.1 %, respectively) [3]. The CDC also recorded an 18 % increase in the prevalence of food allergies from 1997 to 2007 in children under age 18 years (<http://www.cdc.gov/healthyyouth/foodallergies/>).

Food allergy can be defined as an adverse immune response to a food protein. Immunopathogenesis of food allergy is a complicated subject that can involve multiple arms of the immune system [4]. One aspect of the immune system that is well studied in food allergy is the humoral response (specifically IgE) to food allergens [5]. Clinical manifestations to IgE-mediated reactions include generalized urticaria, angioedema, asthma exacerbation, nausea, vomiting, diarrhea, hypotension and anaphylaxis. Diagnosis of food allergy is made based on history, physical examination and diagnostic tests [6]. The common diagnostic tests, used for a suspected IgE-mediated reaction, are percutaneous skin testing and IgE-mediated detection of food allergen in serum [7]. Skin testing for foods has an excellent negative predictive value of greater than 95 %; however, its positive predictive value is less than 50 %. Serum analysis for IgE-mediated food allergenicity has high sensitivity and specificity [5, 7–10].

In children (less than 18 years of age), symptoms of food allergies manifest in infancy, peaking at 1 year of age and then can decline by age 3 [11]. The most common allergenic foods in children are cow's milk (2.5 %), egg (1.3 %), peanut (0.8 %), wheat (0.4 %), soy (0.4 %), tree nuts (0.2 %), fish (0.1 %) and shellfish (0.1 %). Eleven percent of egg allergic and 19 % of milk allergic patients resolve their allergy by age 4; while 80 % of these patients respectively resolve these allergies by 16 years of age. Allergies to tree nuts, peanuts and shellfish are usually permanent while 20 % of peanut allergic children have resolution of symptoms by age 5 [12–15]. In adults, the most common allergenic foods are fruits and vegetables (5 %), shellfish (2 %), peanut (0.6 %), tree nuts (0.5 %) and fish (0.4 %) [13, 16].

The pathogenesis of food allergy is quite complicated and involves multiple risk factors. Some of the risk factors involving food themselves include the type of food processing, the route of food exposure (gastrointestinal (GI) versus cutaneous versus respiratory), antigen dose and nature, rate of absorption of the proteins, etc. Some of the risk factors involving the food allergy include genetics, age, family history (specifically an atopic history), GI disease and defects in the immune system. Linkage and association studies of atopy and allergy have already identified a number of genes, which are important in the specificity and recognition of allergens and therefore represent good candidates for susceptibility factors. Genetic markers, called single nucleotide polymorphisms (SNPs), are associated with the development of a certain phenotype. Several studies provide strong evidence that host genetic susceptibility and environmental factors determine the complex regulation of IgE-mediated food allergies [17–22]. However, only a handful of studies have explored the association between SNPs and the development and prognosis of food allergies. Adverse immunologic reactions to food may be a consequence of both IgE- and non-IgE-mediated mechanisms. Non-IgE-mediated allergic responses tend to involve a T cell-mediated delayed hypersensitivity reaction, and the cytokines released may act as determinants of the immune response [23]. Our goal was to examine the genetic polymorphisms in 3 key cytokine genes namely (interleukin (IL)-4), IL-4 (C-590T), (interleukin-4 receptor-alpha), IL-4Rα (1652A/G) and (interleukin (IL)-10), IL-10 (C-627A) in radioallergosorbent test (RAST) positive patients, since these cytokines are involved in IgE generation and Th2-mediated response.

Materials and methods

Patient selection

Patients with mild to severe food allergies were enrolled in the study after informed consent. In case of minors; parental consent was obtained. The study was approved by the Health Sciences Institutional Review Board (HSIRB). Approximately, 3-ml blood is drawn from the subjects and is used for a RAST (radioallergosorbent test) as a routine clinical procedure. RAST is a blood test used to detect specific IgE antibodies to suspected or known allergens,

in this case specific to food allergens. The IgE antibody is associated with Type I allergic response. Based on the results of the RAST test, the patients were classified into RAST positive (RAST⁺) or RAST negative (RAST⁻). A total of $n = 12$ RAST⁻ and $n = 19$ RAST⁺ patients were recruited for the study. Table 1 provides demographic and clinical information for the food RAST⁺ and RAST⁻ subjects. Comparative analysis was done for genotypes in IL-4, IL-4Rα and IL-10 between RAST⁺ and RAST⁻ patient groups using real-time PCR gene expression studies using DNA extracted from the blood sample.

DNA isolation and quantification

DNA was obtained from blood after the isolation of serum for the RAST test. DNA was extracted using the TRIzol[®] reagent (Life Technologies; Grand Island, NY) [24] according to the manufacturer's instructions and then stored at -80°C until further use. The amount of DNA was quantified using the NanoDrop (Thermo Scientific; Wilmington, DE).

Real-time quantitative PCR (Q-PCR)

The primers used for DNA amplification for IL-4 (C-590T), IL-4Rα (1652A/G) and IL-10 (C-627A) SNP gene expression analysis were 5'-CACTAAACTTGGGA GAACATGGT-3' (Forward primer) and 5'-TGCTTTGCA TAGAAGGGA-3' (Reverse primer) for IL-4 C-590T; 5'-C CCCACCAGTGGCTACC-3' (Forward primer) and 5'-GC CTTGTAACCAGCCTCTCCT-3' (Reverse primer) for IL-4Rα 1652A/G; and 5'-GAAACATGTGCCTGAGAA TCC-3' (Forward primer) and 5'-TTAGGCAGTCACCT-TAGGTCTC-3' (Reverse primer) for IL-10 -627C/A, respectively, as previously used by Negoro et al. [22]. Real-time Q-PCR was used to quantitate IL-4 (C-590T), IL-4Rα (1652A/G) and IL-10 (C-627A) SNP expression. Relative abundance of each SNP is obtained using the SNP-specific primers and the Brilliant[®] SYBR[®] green QPCR master mix from Stratagene (Stratagene Inc., La Jolla, CA; Cat # 600548–51). The real-time PCR conditions for amplification of these genes were as follows: denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30 s. This was followed by a final extension at 72°C for 6 min. Relative gene expression of the SNPs in each sample is calculated using the comparative C_T method where all data are controlled for quantity of DNA input by performing measurements on an endogenous reference gene, β-actin. Results are expressed as Transcript accumulation Index (TAI) [25]. The comparative C_T method or the $\Delta\Delta C_T$ method is used for relative quantitation of gene expression [26]. This method enables relative quantitation of template by quantitating expression levels of the gene of interest relative to an active reference or housekeeping gene or control (normalizer). It is imperative that the gene of interest or target gene and normalizer or control gene have similar dynamic ranges. The calculations for the quantitation include, obtaining the difference (ΔC_T) between the C_T values of the target gene and the normalizer or control gene: $\Delta C_T = C_T(\text{target}) - C_T(\text{normalizer})$. This value is calculated for each sample to be quantitated. After normalization, samples of experimental control or in this case the control RAST⁻ negative subjects were chosen as the reference (baseline) for each comparison to be made. The comparative $\Delta\Delta C_T$ calculation involves finding the difference between each sample's ΔC_T and the baseline's ΔC_T . The last step in quantitation is to transform these values to absolute values, and the formula for this is comparative expression level = $2^{-\Delta\Delta C_T}$, also expressed as transcript accumulation index or TAI. This calculation assumes that the efficiency of the all the PCR is 100 %. Yet another important factor to achieve optimal relative expression results is appropriate normalization strategies. These are important to control for experimental error and to ensure identical cycling performance during real-time PCR. All the relative comparisons in our study were made based on equal or normalized amount of genomic DNA input for all PCR. Further, the endogenous gene used as a loading control (or internal standard) was β-actin since it is not only expressed in all cells but its relative expression levels across all samples were similar.

SNP endonuclease enzyme digestion for restriction fragment length polymorphism (RFLP) analysis

Identification of the SNP alleles in the PCR products was performed by incubation of the PCR product with a restriction enzyme chosen to cut one of the two alleles, followed by electrophoresis. The following restrictive endonucleases were used for the respective genes. Ava II was used for IL-4 (C-590T), Msp I for IL-4R alpha (1652A/G) and Rsa I for IL-10 (-627C/A). Respective digestive enzymes were added and incubated at 37 °C for 2 h. An undigested control DNA was used with deionized water substituted for the restriction enzyme. Endonuclease digested DNA and their respective undigested control PCR products reaction mixtures were separated on a 2.5 % agarose gel by electrophoresis at 70 volts for 2 h. The size (bp) of the PCR amplified fragments were 256, 105 and 198 bp and for the IL-4 (C-590T), IL-4Rα (1652A/G) and IL-10 (C-627A) SNPs, respectively. See Table 2 for the percentage gene frequency distribution for these SNPs in the RAST⁺ and RAST⁻ patient cohorts. These fragment sizes were in concordance with those reported by Negoro et al. [22].

Elisa

IL-4 and IL-10 levels in serum were measured using commercially available ELISA kits (Cat # ELH-IL-4-001 and Cat # ELH-IL-10-001, respectively) from Ray Bio® Inc. (Norcross, GA).

Statistical analysis

Differences in gene and protein expression of cytokine SNPs between RAST⁺ versus RAST⁻ patients were statistically analyzed using Student *t*-test. *p* value <0.05 was considered statistically significant.

Results

Patient characteristics

There were 31 patients enrolled in the current study. Twelve patients had negative RAST results for food allergens while 19 had positive RAST results. The average ages for both groups were equivocal (between 7 and 8 years). There were more male patients in both groups than females. In terms of ethnicities, more white patients were present in the RAST⁻ group where as majority of RAST⁺ group consisted of black patients. Table 1 provides demographics of the patients enrolled in the study.

IL-4 (C-590T) genotype analysis

Genotypic analysis of IL-4 (C-590T) was done using the restriction enzyme Ava II which on endonuclease digestion of the gene segment in the selected IL-4 gene position that contains a C substitution for the wild-type T nucleotide results in three possible genotypes: TT, CT and CC (Fig. 1a). The chromosome containing the T base produced one 256 bp band while the chromosome containing the C base produced two band of 233 and 23 bp, respectively. The TT genotype was not recognized by Ava II produced only one band. The CT genotype produced three bands of 256, 233 and 23 bp, while the CC genotype produced two bands (the smallest segment was not detectable by gel electrophoresis).

Comparisons were made between the TT, CT and CC genotypes of the RAST⁺ and RAST⁻ patients (Fig. 1b). Our results show that there is a higher expression of the TT genotype in the RAST⁺ patients compared to the RAST⁻ patients but it was not statistically significant. There were no differences in the expression of the CT or CC expression between the two groups.

IL-4Rα(1652A/G) genotype analysis

Genotypic analysis of IL-4Rα (1652A/G) was done using the restriction enzyme Msp I which on endonuclease digestion of the gene segment in the selected IL-4 gene position that contains a G substitution for the wild-type A nucleotide results in three possible genotypes: AA, AG and GG (Fig. 2a). Of note, there were no detectable GG genotypes in any of the tested groups. The A genotype produced a band of 105 bp while the digested G genotype produces two fragments of 89 and 16 bp, respectively. The AA genotype was not recognized by Msp I and only produced one band; while the AG genotype was produced three bands (the smallest fragment was not detectable on gel electrophoresis).

Comparisons were made between the AA, AG and GG genotypes of the RAST⁺ and RAST⁻ patients (Fig. 2b). There are statistically significant differences in expression of AA and AG between the two groups. The RAST⁺ group had a higher expression of AG while the RAST⁻ group had a higher expression of AA. Again, neither group expressed the GG genotype.

IL-10 (-627C/A) genotype analysis

Genotypic analysis of IL-10 (-627C/A) was done using the restriction enzyme Rsa I which on endonuclease digestion of the gene segment in the selected IL-10 gene position that contains an A substitution for the wild-type C nucleotide results in three possible genotypes: CC, AC and AA (Fig. 3a). The chromosome with the C base produced a fragment of 198 bp while the chromosome with the A base produced two fragments of 126 and 72 bp, respectively. The CC genotype was not recognized by Rsa I and produced one band. The AC genotype resulted in three bands while the AA genotype produced two bands. Comparisons were made between the CC, AC and AA genotypes of the RAST⁺ and RAST⁻ patients (Fig. 3b). There are no statistically significant differences in expression of CC, AC and AA between the two groups.

SNP analysis of specific food allergens

Within the RAST⁺ group, effort was taken to subcategorize patients into specific food allergic groups. Out of the 19 RAST⁺ patients, there were six patients that were RAST⁺ for peanuts and four patients that were RAST⁺ for tree nuts (see Table 1). Comparison were made between the expression of these cytokine SNPs examined in the study in patients with allergies to specific foods; however, no statistically significant differences in the genotypes with respect to these SNPs were observed between these patient groups.

IL-4 and IL-10 protein expression in serum samples of RAST⁺ versus RAST⁻ patients. We evaluated the IL-4 and IL-10 protein expression in the serum samples of these RAST⁺ versus RAST⁻ patients using a commercially available ELISA kit. Our results show a significant decrease in IL-4 production in RAST⁺ (13.83 ± 2.54 versus 37.8 ± 4.2 pg/ml; $p = 0.05$) as compared to RAST⁻ patients. Comparison of IL-10 production in RAST⁺ (26.3 ± 12.1 versus 0.5 ± 0.12 pg/ml; $p = 0.01$) and RAST⁻ patients indicated a significant increase in IL-10 production in RAST⁺ patients (Fig. 4a).

Relative gene expression of IL-4 (C-590T), IL-4Rα (1652A/G) and IL-10 (-627A) in RAST⁺ versus RAST⁻ patients. SNP expression analysis of IL-4 (C-590T), IL-4Rα (1652A/G) and IL-10 (-627A) was done in DNA extracted from blood of RAST⁺ and RAST⁻ subjects. Our results show a significant decrease in IL-4 (C-590T) (TAI = 0.63 ± 0.09 versus 1.0 ± 0.10 pg/ml; $p = 0.05$); and a significant increase in IL-4Rα (1652A/G) (TAI = 1.51 ± 0.14 versus 1.0 ± 0.10 pg/ml; $p = 0.03$) and IL-10 (-627A) (TAI = 1.72 ± 0.11 versus 1.0 ± 0.10 pg/ml; $p = 0.01$) gene expression in RAST⁺ as compared to RAST⁻ patients, respectively (Fig. 4b). These gene and protein expression results indicate that changes in the

SNP gene expression will be translated into modulation of the levels of the cytokines thereby altering phenotypic responses in these patients with various food allergies.

Discussion

SNPs can play a direct or indirect role in phenotypic expression that may play an important role in food allergy pathogenesis [27]. Our goal was to quantitate the expression of SNPs that were only expressed in food allergic patients and to evaluate if those SNPs could be related to severity of food allergy. We choose SNPs in key cytokine genes namely IL4 and IL-10 that are known to be important in IgE generation and regulation since the mechanisms that underlie the initiation and development of an allergic response involves IL-4 which directly induces a robust Th2 response [17–22].

The modulation of allergic responses by eliminating IgE-binding epitopes using desensitization methods is the basis of current allergen immunotherapy. It is possible that the correct balance between these Th1/Th2 cytokines may be essential to avoid food allergy. The influence of food allergens on the profile of inflammatory markers in RAST⁺ and RAST[−] subjects, and their relevance in the control of allergic inflammatory processes has not been systematically evaluated. Of all the atopic disease, food allergy is of clinical importance secondary to the high risk of anaphylaxis. Therefore, early detection and possible analysis of severity of food allergic reactions would be of great importance to the patient, family members and physicians.

Conflicting reports appear in literature regarding specific association of SNPs of candidate genes to food allergy [18, 22, 28–33]. In our study, of the 3 SNPs examined, we found that the AA, AG and GG genotypes for the IL-4R α (1652A/G) polymorphisms were statistically different in RAST⁺ versus RAST[−] patients; however, no statistically significant differences were observed in the (TT, CT, CC genotypes for IL-4 (C-590T) and CC, AC, AA genotypes for IL-10 (C-627A) (Figs. 1, 2, 3). We believe that significant differences in these genotypes will become more evident with multiple comparisons between these groups when the number of samples in each group are increased. We did, however, observed a significant difference in the gene expression levels of these SNPs [IL-4 (C-590T), IL-4R α (1652A/G) and IL-10 (C-627A)] between RAST positive patients versus RAST negative patients. Variants in the IL-4R α gene and polymorphisms in the IL-4 gene on chromosome 5q have been previously reported to be associated with atopic phenotypes and the IL-4R gene encodes the α chain of the IL-4 and IL-13 receptors [34]. IL-4 and IL-13 are pleiotropic T helper cell type 2 cytokines which are associated with atopic phenotypes and play a major role in the regulation of IgE, which is a marker of atopic disease [34, 35]. Polymorphisms in the IL-4R α gene are associated with functional alterations of the IL-4 receptor [36, 37]; therefore, it is likely that variation in the IL-4R α gene influences atopy through an IL-4-mediated pathway. The significant modulation in the gene and protein expression of IL-4 and IL-10 in the serum samples of the RAST⁺ patients as compared to the RAST[−] patients indicates that changes in SNP expression results in altered phenotypic response in these patients (Fig. 4a, b). Based on the results of the current study, we hope to expand into future studies that continue to evaluate the association between genetic polymorphisms and food allergies using additional state of the art methodologies that include Sequenom arrays and proteomic analysis in a larger group of patients. Additional candidate genes such as the HLA markers, CD14, SPINK5, etc. that are also relevant to food allergy, will be analyzed. Further, it would also be quite interesting to evaluate genetic polymorphisms in patients who outgrow their particular food allergy.

Results of the current study show a definite association between the IL-4R α (1652A/G) polymorphism and altered atopy in food allergic patients, and we hope that this association

will help encourage early screening for this SNP, particularly in families that have a history of allergy-related diseases, thereby providing clinicians insight into better management of early sensitizations and symptoms of food allergies which are crucial to preventing subsequent allergic complications.

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Fig. 1.

Comparison of IL-4 (C-590T) genotypes in RAST⁺ versus RAST⁻ patients. **a** A representative gel image showing IL-4 (C-590T) genotype analysis comparing the TT, CT and CC genotypes of RAST⁺ and RAST⁻ patients. *Lane 1* Uncut DNA; *Lane 2* Cut DNA with TT genotype; *Lane 3* Uncut DNA; *Lane 4* Cut DNA with CT genotype; *Lane 5* Uncut DNA; *Lane 6* Cut DNA with CC genotype; *Lane 7* 100 bp marker. **b** Graphical representation of the results showing higher expression of the TT genotype in the RAST⁺ versus RAST⁻ patients. Data shown are mean $\pm$ SD from duplicate samples

Fig. 2.

Comparison of IL-4Ra (1652A/G) genotypes in RAST⁺ versus RAST⁻ patients. **a** A representative gel showing IL-4Ra (1652A/G) genotype analysis where comparisons were made between the AA, AG and GG genotypes of the RAST⁺ and RAST⁻ patients. *Lane 1* Uncut DNA; *Lane 2* Cut DNA with AG genotype; *Lane 3* Uncut DNA; *Lane 4* Cut DNA with AA genotype; *Lane 5* 100 bp marker. **b** Graphical representation of the results showing statistically significant differences in expression of AA and AG genotypes between RAST⁺ and RAST⁻ patients. Data shown are mean $\pm$ SD from duplicate samples

Fig. 3.

Comparison of IL-10 (-627C/A) genotypes in RAST⁺ versus RAST⁻ patients. **a** A representative gel showing IL-10 (-627C/A) genotype analysis where comparisons were made between the CC, AC and AA genotypes of the RAST⁺ and RAST⁻ patients. *Lane 1* Uncut DNA; *Lane 2* Cut DNA with AC genotype; *Lane 3* Uncut DNA; *Lane 4* Cut DNA with AA genotype; *Lane 5* Uncut DNA; *Lane 6* Cut DNA with CC genotype; *Lane 7* 100 bp marker. **b** Graphical representation of the results showing no statistically significant differences in expression of CC, AC and AA between the RAST positive and the RAST negative groups. Data shown are mean ± SD from duplicate samples

Fig. 4.

a Graphical representation of IL-4 and IL-10 protein expression in serum samples of RAST⁺ versus RAST⁻ patients as measured by ELISA. Our results show a significant decrease in IL-4 production and a significant increase in IL-10 production in RAST⁺ patients as compared to RAST⁻ patients. **b** IL-4 (C-590T), IL-4Rα (1652A/G) and IL-10 (-627A) gene expression in RAST⁺ versus RAST⁻ patients as quantitated by QPCR. SNP expression analysis of IL-4 (C-590T), IL-4Rα (1652A/G) and IL-10 (-627A) was done in DNA extracted from blood of RAST⁺ and RAST⁻ subjects. Both data are the mean $\pm$ SD of 3 separate experiments done in duplicate. Statistical significance was determined by Student's *t*-test

Table 1

Demographics of the Rast⁺ and Rast⁻ patients enrolled

Patient [#]	Age	Gender	Ethnicity	Clinical H/O	Skin test	RAST
RAST ⁺ group						
1	6	M	Black	Peanut: angioedema, SOB and itchy eyes	Not done	Peanut: [100
2	4	M	Black	Peanut: rash	Positive: peanut (5/20)	Peanut: 0.86
3	5	F	Black	Peanut: hives, soy: eczema flair	Positive: soy (5/10) and egg (3/7)	Peanut: 7.32, Soy: 5.15/tree nuts: 4.14–>100
4	3	M	Black	Peanut: hives	Positive: peanut (12/20)	Peanut: 72.8
5	7	M	Black	Sunflower seeds: periorbital swelling/diffuse pruritus, peach: hives	Not done: peach and sunflower seeds	Sunflower seed: 5.96; peach 39.90; peanut: 2.33 and tree nuts: 3–10
6	2	F	White	Peanut: eczema flare	Positive: peanut (15/40)	Peanut: 4.07
7	6	M	White	Tree nut: difficulty breathing	Not done	Tree nuts: 15– [100
8	27	M	Black	Peanut: hives	Not done	Apple: 2.26 and peanut 1.77
9	3	M	Black	Milk: urticaria	Not done	Milk: 2.44
10	1.5	M	White	Breastfeeding: SOB and wheezing, eczema	Positive: egg (5/12)	Peanut: 8.22
11	9	M	Not Specified	Walnut: angioedema and urticaria	Not done	Tree nuts: 0.35–17.50
12	16	M	Black	Crab: angioedema	Positive: crab (10/12), lobster (10/13) and clam (4/6)	Crab: 14.70, clam: 1.06 and lobster: 9
13	2	M	Interracial	Walnut: urticaria and egg: urticaria	Positive: egg (10/30)	Walnut: [100
14	4	M	Not Specified	Peanut: facial swelling and itching	Not done	Peanut: 1.77
15	4	M	Not Specified	Peanut: anaphylaxis	Not done	Peanut: 9.39
16	11	M	Black	Cashew: throat itching	Positive: cashew (9/9) and walnut (18/20)	Tree nuts: 0.51–9.24
17	16	F	Black	Peanut: itchy throat	Not done	Peanut: 20
18	1.5	F	Black	Egg: anaphylaxis	Not done	Egg: 62.80
19	9	F	White	Egg: urticaria	Negative	Egg: 2.78
RAST ⁻ group						
1	3	F	White	Peanut: urticaria and rash after peanut food challenge	Negative: peanut 2/4	Negative: peanut and tree nuts
2	15	F	Black	Tree nut: anaphylaxis (angioedema, SOB and abdominal pain)	Not done	Negative: peanut and tree nuts
3	1	M	White	Eczema workup	Positive: milk 12/20	Negative: egg, milk and wheat
4	2	M	Hispanic	Eczema workup	Negative: Egg 5/6	Negative: egg
5	3	M	Black	Fish: urticaria	Positive: mixed whitefish	Negative: tuna, salmon, cod

Patient#	Age	Gender	Ethnicity	Clinical H/O	Skin test	RAST
6	5	M	White	Workup for EE	Negative: peanut, corn and whole wheat	Negative: wheat and peanut
7	2	F	Black	Rash workup	Negative for milk and fish	Negative: milk tuna and salmon
8	8	F	White	Cocoa: headache, strawberry: rash	Positive: cocoa 5/45 negative: fresh strawberry	Negative: cocol and strawberry
9	26	M	White	None	Not done	Negative: egg, soy, salmon and shrimp
10	7	M	Not specified	Peanut: hives, egg: hives	Not done	Negative: egg, peanut and tree nuts
11	7	F	White	Tuna: hives	Negative: tuna 4/5 (negative)	Negative: tuna
12	9	M	Not specified	Tomato: facial edema	Not done	Negative: tomato

Table 2

Size of amplified fragment of interest and percentage gene frequency distribution

SNPs	Restriction enzyme	Size of amplified fragment	Sample group	Gene frequency distribution (%)		
IL-4 (C-590T)	Ava II	256	RAST ⁻	TT	CT	CC
			RAST ⁺	0	44	56
IL-4Ra (1652A/G)	Msp I	105	RAST ⁻	25	30	45
			RAST ⁺	AA	AG	GG
IL-10 (C-627A)	Rsa I	198	RAST ⁻	75	25	0
			RAST ⁺	10	90	0
			RAST ⁻	CC	AC	AA
			RAST ⁺	58	42	0
			RAST ⁻	60	35	5