IgE and IgG4 responses to shrimp allergen tropomyosin and its epitopes in patients from coastal areas of northern China

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Abstract. Sensitization to allergens and their peptides varies among patients due to geographical or ethnic differences. The present study aimed to investigate immunoglobulin (IgE) and IgG4 responses to tropomyosin and its peptides in shrimp allergic patients from northern China. A total of 92 subjects were studied, including 35 shrimp allergic patients, 29 patients with house dust mite (HDM) and/or cockroach allergic patients and 28 healthy volunteers. Serum IgE and IgG4 antibodies to recombinant shrimp tropomyosin (rPen a 1) and its peptides were measured by means of a light-initiated chemiluminescent assay. A total of 9 major sequential epitopes of Pen a 1 reported in the literature were synthesized. Of 35 shrimp allergic patients, 25 (71.4%) had positive Pen a 1-specific IgE (sIgE) antibodies and 22 (62.9%) contained measurable rPen a 1-specific IgG4 (sIgG4) antibodies. A strong IgG4 response accompanied the presence of IgE to Pen a 1. None of the patients with HDM and/or cockroach allergy demonstrated IgE reactivity to rPen a 1. The reaction frequency of IgE binding epitope was 20‑48%, while that of IgG4 binding epitope was 63.6‑90.9%. The IgE and IgG4 recognition patterns of the tropomyosin peptides demonstrated high interpatient heterogeneity. Diversity of IgE binding epitopes was positively correlated with Pen a 1 sIgE levels. In the study population, tropomyosin was a major allergen recognized by the majority of shrimp allergic patients, which is consistent with previous reports. However, none of the 9 epitopes are major (reaction frequency >50%) IgE-binding regions, indicating the epitopes profile may be different in other regions.

Introduction

Shellfish, a major sensitizing component of seafood, is widely consumed worldwide due to its nutritional value. Therefore, the increase in immunoglobulin (IgE)-mediated seafood allergy reports is particularly related to shellfish (1). The prevalence rates of shellfish allergy range from 0.5 to 2.5% depending on geographic locations, dietary habits and age. Unlike most other food allergies, a shellfish allergy persists for life in up to 90% of patients and is often associated with severe systemic anaphylactic reactions. Seafood-associated shellfish includes crustaceans and mollusks. The majority of species that cause allergic reactions are the crustaceans, with shrimp being by far the most frequently involved (1-3).

Tropomyosin, a protein from muscle, was the first major allergen identified in shrimp (4,5). Sensitization to the major allergen tropomyosin has been found in 80% of shrimp-allergic patients (6,7). Tropomyosin is also reported as being a panallergen of numerous other species such as lobster, crab and mollusks (8). Due to evolutionary lineages, there is a 78‑98% amino acid homology of tropomyosin between crustaceans and mites, and 80‑97% between crustaceans and cockroaches (3). Consequently, the highly conserved amino acid sequence of tropomyosin is responsible for clinical cross-reactivity among shellfish species (9). Tropomyosin is also considered to be responsible for cross-reactivity between food and aeroallergens of animal origin such as dust mites or cockroaches (10,11).

Tropomyosin is important for the diagnosis of seafood allergy. A recombinant tropomyosin from Penaeus aztecus, Pen a 1, has been commercially available for the component-resolved diagnostics (CRD) of shrimp allergy and could provide a more accurate diagnosis (12,13). Clearly, research on food allergens can be useful for the treatment of food allergy (14,15). Mapping the IgE and IgG4 epitopes of tropomyosin may reveal relevant information about antigen structure, on the basis of which a safe hypoallergenic agent can be designed to treat shrimp allergy (16).
However, there are still some problems with the clinical use of tropomyosin, which requires further study. One problem is that previous studies demonstrated that sensitization to allergens and their peptides varies among patients due to geographical or ethnic differences. First, the tropomyosin sIgE frequencies vary. Asero et al. (17) found that less than half (41.6%) of the Italian adult patients with shrimp allergy reacted to tropomyosin (Pen a 1). The variability depends on the route and dose exposure to allergens and individuals of different ages from different ethnic backgrounds (18,19). CRD has revealed that these sensitization profiles might show geographical differences with clearly distinct clinical outcomes (20). Second, the major sequential IgE binding epitopes of tropomyosin (Pen a 1) have been identified using overlapping peptide mapping by SPoTs membrane-based immunoassays to elucidate sensitization profiles (21,22), but previously published results have demonstrated great heterogeneity in the number of epitopes and their locations for the same allergens (23,24). These differences are probably related to the technology used, the overlapping peptide length and the populations selected (25-27). Third, the role of IgG4 may be different in different species and the role of IgG4 in shrimp allergy is not fully understood. IgG4 epitopes have been reported to be associated with immunologic tolerance to milk and peanuts (28). On the other hand, IgG4 has also been considered to be associated with atopy and allergic sensitization (29).

Limited work has been conducted regarding the potential allergenicity and antigenicity of tropomyosin and its peptides in patients from coastal areas of northern China (30). The object of the present study was to determine the frequency of IgE and IgG4 antibodies reactivity to shrimp tropomyosin (Pen a 1) in the northern Chinese population. The present study also investigated the IgE and IgG4 specificity and diversity to sequential epitopes of Pen a 1 in Pen a 1-positive patients.

Materials and methods

Patients. A total of 92 subjects were consecutively recruited from Tianjin Port Hospital and Academy of Traditional Chinese Medicine Affiliated Hospital between January 2018 and November 2018. Patient characteristics are shown in Table I. Upon study entry, all participants underwent a detailed medical examination and clinical history review. Clinical allergy was diagnosed by an experienced allergologist using the following criteria: i) A convincing history of acute allergic reactions after contact (including urticaria, abdominal pain and wheezing) and ii) increased sIgE levels [cutoff: >0.35 kU/l, measured by fluorescence enzyme immunoassay (1mmunoCAP, Phadia AB)] as defined by the European Academy of Allergy and Clinical Immunology guidelines (31). The study protocol was approved by the Ethics Committees of Tianjin Medical University (grant no. TMUHMEC20170008) and written informed consent was obtained from the patients and volunteers prior to study entry.

Preparation of recombinant tropomyosin. The gene coding Pen a 1 protein sequences (GeneBank NO. DQ151457) was synthesized and cloned into the BamHI and HindIII sites of pET28a (+) expression vector using the DNA Ligation kit (cat. no. D6020A; Takara Bio, Inc.), according to the manufacturer's protocol. The resulting plasmid containing the Pen a 1 coding regions and a poly-histidine affinity purification tag (6XHis) at the N-terminus was subsequently transformed into E. coli BL 21 (DE3) competent cells (Tiangen Biotech Co., Ltd.) using the heat shock transformation method. Briefly, 5 μl pET28a-Pen a 1 plasmid was transformed into 100 μl E. coli BL21 cells (DE3) and incubated on ice for 30 min, prior to being heated in a water bath at 40°C for 60 sec, followed by an ice bath for 2 min. The transformants were streaked on LB agar plate supplemented with kanamycin (50 mg/ml). When cultures reached an optical density at 600 nm level of 0.4-0.6, the expression of Pen a 1 was induced by adding 1 mM isopropyl-B-D-thiogalactoside (Invitrogen; Thermo Fisher Scientific, Inc.) and then incubated for 3 h at 37°C and 220 rpm on an orbital shaker. E. coli cells were harvested by centrifugation at 4,000 x g for 5 min at 4°C, resuspended in phosphate-buffered saline (PBS, 0.01 M, pH 7.4), and sonicated at 60 kHz, with 10 sec pulse-on and 10 sec pulse-off for five cycles on ice. The recombinant protein was purified from the culture supernatant using Ni-NTA affinity column chromatography (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Briefly, prior to the purification, 6 ml binding buffer (50 mM PBS, 0.3 M NaCl) was used to prepare the 10 ml purification column. Then, 5 ml lysate was added into the column and incubated for 30-60 min at 4°C using gentle agitation to keep the resin suspended in the lysate solution. The column was washed three times with washing buffer (20 mM imidazole, 50 mM PBS, 0.3 M NaCl) to remove the proteins that were not bound to the resin. Finally, the target protein was eluted with elution buffer (250 mM imidazole, 50 mM PBS, 0.3 M NaCl); the column flow rate was ~1.0 ml/min and all of the obtained eluted fractions were collected for further analysis. Then the concentration of purified recombinant tropomyosin was determined using a bicinchoninic acid assay and further characterized by SDS-PAGE gel electrophoresis and mass spectrometry. The purity of the recombinant allergen was determined by ImageJ software version 1.8.0 (National Institutes of Health). SDS-PAGE was performed using the Bio-Rad Mini Protean II cell (Bio-Rad Laboratories, Inc.). 25 μg recombinant protein was boiled for 5 min in loading buffer before being separated using 12% polyacrylamide gels. Following the protein separation, the protein was visualized using Coomassie Brilliant Blue R-250 staining (Sigma-Aldrich; Merck KGaA).

Based on the results of the SDS-PAGE, the spot identified in the Coomassie G250-stained gel that matched the position of the theoretical molecular weight was excised and submitted for analysis by MS/MS at the proteomic service provided by Sangon Biotech Co., Ltd. Briefly, prior to MS/MS analysis, the purified recombinant fusion protein was digested in-gel into smaller peptides using trypsin (Beijing Solarbio Science & Technology Co., Ltd). A tandem matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF/TOF) mass spectrometer (Model 4800; Applied Biosystems; Thermo Fisher Scientific, Inc.) was used for peptide sequencing and the acquired data was further analyzed using ABI GPS Explorer and MASCOT software (http://www.matrixscience.com). Databases, including the NCBI database (http://www.ncbi.nlm.nih.gov), were searched to characterize the protein.
Preparation of biotinylated recombinant Pen a 1.

Sulfo-NHS-LC-Biotin was purchased from Thermo Fisher Scientific, Inc. Recombinant Pen a 1 was biotinylated according to manufacturer’s protocol. Briefly, the protein was dissolved in PBS. The appropriate volume of biotin reagent solution was added to the protein solution at a molar ratio of 20:1 and incubated on ice for 2 h. Calculations were performed based on the product instructions. To remove unreacted biotin, the solution was desalted or dialyzed against PBS buffer at 4˚C. Finally, the conjugate was stored at 4˚C until further use.

Preparation of Pen a 1 peptides.

A total of 65 linear epitopes of Penaeus aztecus tropomyosin (Pen a 1) were found in the Immune Epitope Database (http://www.iedb.org). All 9 major epitopes of Pen a 1 as reported in the literature (21,22) were chosen for the present study. A ‘major epitope’ of an allergen is defined as an IgE-Binding region recognized by >50% of allergic patients (21,32) whereas a ‘minor epitope’ was recognized by <50% of allergic patients.

The 9 peptides and their corresponding biotinylated peptides with a purity of >95% by high performance liquid chromatography, consisting of 15-27 amino acids in length and corresponding to the primary sequences of tropomyosin, were commercially synthesized (Sangon Biotech Co., Ltd.). Peptides were suspended in PBS at 1 mg/ml and stored at -70˚C until use. The sequences of synthetic peptides are shown in Table S1.

A light-initiated chemiluminescent assay.

The allergen specific IgE and IgG4 antibody levels are usually low in serum and not easy to detect by routine methods such as ELISA. Sandwich fluoro-immunochemical, lumino-immunochemical and radioimmunologic are common methods to increase the detection sensitivity (33).

The levels of sIgE and sIgG4 to rPen a 1 as well as its peptides were measured using a light-initiated chemiluminescent assay (LICA) carried out as previously described (34,35). LICA is based on the formation of nanoparticle pairs and luminescence oxygen channeling immunoassay technology (36,37). Studies have shown that this new method possesses excellent performance characteristics with high sensitivity, broad analytical range and rapid turnaround cycles and is suitable for immunodetection of allergen sIgE and sIgG4 (34,35). Two-step assay procedures were performed. Briefly, test serum samples (25 µl) diluted 1:20 in PBS containing 1% human serum albumin (Beijing Solarbio Science & Technology Co., Ltd.) were added to the 96-well plates. A 50 µl mix of anti-human IgE (cat. no. ab7382; Abcam) and/or IgG4 antibody (cat. no. ab238320; Abcam)-chemibeads (Beyond Biotech Co., Ltd.) (33,34), diluted 1:1,000, and biotinylated rPen a 1 and/or peptides, diluted 1:200 in PBS/HSA, was then added to each well and incubated for 2 h at room temperature.

Figure 1. SDS-PAGE analysis of recombinantly expressed and purified tropomyosin rPen a 1. (A) M: Molecular weight marker; Lane 1: Total cell lysate from un-induced bacteria; Lane 2: Total cell lysate from induced bacteria; Lane 3: Lysate supernatant; Lane 4: Lysate pellet. (B) M: Molecular weight marker; Lane 1: Total cell lysate from induced bacteria; Lane 2: Purified recombinant tropomyosin rPen a 1.

Table I. Demographic and clinical characterization of subjects.

| Patient characteristics | Shrimp allergic, n=35 | HDM/cockroach allergic, n=29 | Control, n=28 |
|-------------------------|-----------------------|-----------------------------|--------------|
| Sex, no. (%)            |                       |                             |              |
| Male                    | 119 (54.3%)           | 12 (41.4%)                  | 16 (57.1%)   |
| Female                  | 116 (45.7%)           | 17 (58.6%)                  | 12 (42.9%)   |
| Age, median with range (years) | 26 (2-67)       | 17 (5-45)                   | 32 (10-61)   |
| sIgE, median with range (kU/l) |                 |                             |              |
| Shrimp                  | 6.80 (0.36->100)       | <0.35                       | <0.35        |
| Dust mite               | 4.3 (<0.35->100)       | 10.21 (0.91->100)           | <0.35        |
| Cockroach               | 5.7 (<0.35->100)       | 2.64 (0.36-20.74)           | <0.35        |
| Allergic symptoms (most frequent only) |          |                             |              |
| Cutaneous               | 23 (65.7%)            | 2 (6.9%)                    | 0            |
| Digestive               | 16 (45.7%)            | 1 (3.4%)                    | 0            |
| Respiratory             | 7 (20.0%)             | 27 (93.1%)                  | 0            |
| Anaphylaxis             | 3 (8.6%)              | 0 (0%)                      | 0            |
| Other reported allergies| 19 (54.3%)            | 12 (41.4%)                  | 0            |

HDM, house dust mite; s, Pen 1-specific; Ig, immunoglobulin.
and/or asthma, to HdM and/or cockroaches and increased
frequency of a recent hypersensitivity reaction, including allergic rhinitis
their clinical reactivity, 35 individuals were diagnosed with a
shrimp-specific sIgE of >0.35 kUA/l. Furthermore, 29 subjects were
reactions immediately following shrimp ingestion and positive
searches.

| GenBank protein | Protein description                             | Theoretical molecular weight/D  | Score | Matching peptide number |
|-----------------|-----------------------------------------------|---------------------------------|-------|-------------------------|
| AAZ76743        | *Penaeus monodon*, tropomyosin                | 32830                           | 292   | 4                       |
| CDW59661        | *Trichuris trichiura*, elongation factor tu   | 24465                           | 171   | 2                       |
| KZC10872        | *Dufourea novaegangiicae* tropomyosin-1        | 35055                           | 108   | 2                       |

well and incubated at 37°C for 30 min with mild agitation. Subsequently, 150 µl streptavidin-coated sensibeads (Beyond Biotech, Co., Ltd.) were added. The reaction mixture was incubated at 37°C for 10 min in the dark. Finally, the chemiluminescence (CL) signal was measured on a LICA instrument (LICA series 500; Beijing Chemclin Biotech Co., Ltd.) and expressed as Relative Light Units. This immunoassay was run with samples in duplicate.

The healthy nonatopic sera were processed in parallel as negative controls. If the CL value of the tested sera was higher than the cut-off values, determined by adding two standard deviations to the mean CL value of the negative control, the reactivity was considered positive.

**Peptide inhibition experiment.** A peptide inhibition assay for IgE binding was performed as described above except that the serum pool from 3 patients was preincubated with several peptides unconjugated with biotin (the first, the third, the fifth, the seventh and the ninth peptide) at a concentration of 5 µg/ml at 37°C for 30 min with mild agitation. The same serum pool without peptides addition was analyzed in parallel and incubated under the same conditions as a control.

**Statistical analysis.** Mean values for each serologic parameter and log-transformed antibody levels (IgG binding to rPen a 1 and its peptides) were compared among patients and control subjects. Between the two groups, differences were compared with two-tailed Student’s t-test for parametric data and/or with the Mann-Whitney U test for non-parametric data. The frequency of IgG responses to Pen a 1 and its peptides between patients and control subjects were compared using a χ² test. Spearman’s correlation was used to evaluate the correlation. P<0.05 was considered to indicate a statistically significant difference. All statistical computations were performed using GraphPad Prism Version 7.0 (GraphPad Software, Inc.) and R Version 3.6.1 (R core Team; https://cran.r-project.org) software.

**Results.**

**Patient characteristics.** The demographic and clinical characteristics of the subjects are presented in Table I. According to their clinical reactivity, 35 individuals were diagnosed with a shrimp allergy based on a clear-cut clinical history of allergic reactions immediately following shrimp ingestion and positive shrimp-sIgE of >0.35 kUA/l. Furthermore, 29 subjects were allergic to HdM and/or cockroaches with a convincing history of a recent hypersensitivity reaction, including allergic rhinitis and/or asthma, to HdM and/or cockroaches and increased serum sIgE antibodies. As control group, 28 nonatopic healthy subjects (no convincing history of clinical reaction and negative serum sIgE level) were also included for providing negative serum. None of the patients received immunotherapy. Glucocorticoid or antihistamine drug such as loratadine or were used to alleviate symptoms.

**Expression and purification of rPen a 1.** The recombinant shrimp tropomyosin, rPen a 1, was produced in *Escherichia coli* and purified as described above (Fig. 1, Tables II and SII). With respect to purified rPen a 1, a unique band with the expected MW (~37 kDa) was observed in SDS-PAGE analysis. The 95-99% purity was obtained by further analyzing SDS-PAGE results using ImageJ software.

**Cross-reactivity study.** Cross-reactive responses to tropomyosin between crustaceans and HdM and/or cockroaches were frequently observed. However, in the present clinical and serological study, the HdM and/or cockroach allergic patients and controls did not show significant differences in rPen a 1 sIgE levels (P=0.196), which indicated that no IgE to rPen a 1 was detected among patients with HdM and/or cockroach allergy (Fig. 3).

**IgE and IgG₄ binding regions of rPen a 1.** Individual sera from 25 patients with positive rPen a 1 sIgE levels and 22 patients with positive IgG₄ levels together with 4 healthy volunteers were used to identify IgE and IgG₄ binding areas on Pen a 1. The frequency of positive IgE and IgG₄ binding to each peptide of Pen a 1 is shown in Fig. 4. All synthetic peptides were recognized by between 28-48% of the sera tested for IgE binding. None of the peptides were major epitopes in this population and 9 peptides were identified as IgG₄ epitopes because the majority of patients (>50%) had positive IgG₄ antibodies to all synthetic peptides. The most frequent IgG₄
recognized antigenic areas were the second and the eighth peptides (AA 85-105 and AA 259-273), both of which were recognized by 90.9% of the patients. In addition, IgE and IgG4 binding regions of Pen a 1 largely overlapped.

**Epitope profiles of individual patients.** As demonstrated in Fig. 5, the number of IgE and IgG4 positive peptides per subject and recognition patterns varied considerably among 25 shrimp-allergic, rPen a 1-reactive individuals. A total of 28% (7/25) of the sera demonstrated no peptide IgE binding reactivity, while 16% (4/25) sera exhibited positive IgE binding to all peptides. The mean number of IgE binding peptides recognized per individual was 3.2 (range, 0-9). Serum IgE levels to rPen a 1 was positively correlated with the number of IgE positive peptides (r=0.680, P<0.001). The number of sera which recognized IgG4...
binding peptides varied from 0 (0%) for 2 peptides to 8 (67%) for 9 peptides.

The specificity of IgE binding was assessed by a peptide inhibition assay. As shown in Fig. 6, peptides preincubated with the serum pool partially inhibited the IgE binding to the same peptide or neighboring peptide, indicating that the detected binding was due to epitope-specific IgE antibodies.

**Discussion**

The present study analyzed profiles of IgE and IgG4 against shrimp tropomyosin, Pen a 1 and its epitopes among patients from coastal areas of northern China using LICA. It was found that Pen a 1 is a major allergen recognized by the majority of shrimp allergic patients. The data from the present study demonstrated that 71.4% of shrimp-allergic patients in this population exhibited IgE reactivity to shrimp tropomyosin. The current study demonstrated that Pen a 1 was the major shrimp allergen, which was consistent with previous studies (6,7) and confirmed its clinical diagnostic value in patients from coastal areas of northern China. However, none of the 9 epitopes are major (reaction frequency >50%) IgE-binding regions, indicating that the epitope profile may be different for other regions.

In particular, 62.9% of allergic sera had increased IgG4 antibodies to Pen a 1 compared with the non-atopic healthy group. A strong IgG4 response accompanied the presence of IgE to Pen a 1. This was consistent with previous studies which revealed that levels of sIgG and IgG subclasses were significantly higher in individuals with allergic sensitization and could develop in parallel with or invariably preceding IgE responses (38,39). However, the role played by allergen sIgG and IgG4 antibodies in allergic reactions, unlike that of IgE, remains controversial. On one hand, allergen-related IgG4 antibodies have been proposed to be associated with the development of clinical tolerance due to their induction by allergen-specific immunotherapy (40,41). On the other hand, a previous study reported peanut-specific IgG4 is significantly higher in the avoidance group compared with the peanut-eating group in peanut-sensitized children and supposes that IgG4 may not indicate tolerance from sensitization (38). There is also a suggestion that allergen-related IgG antibodies are an epiphenomenon with no functional relevance (42). From
the point of view of the present study, it is the difference between natural- and therapy-induced tolerance that causes these contradictions. In therapy-induced tolerance, allergen-specific IgG₄ should be considered as a protective factor rather than the cause of sensitization (28). In natural tolerance, there may be two mechanisms: One is IgG₄-associated tolerance (43) and the other is IgG₄-independent tolerance (38).

In the present study, the IgE reactivity to rPen a 1 was not observed in HDM and/or cockroach allergic patients with no sensitization to shrimp. This meant tropomyosin did not appear to be the markers of cross-reactivity with other arthropods in this population, which appears to conflict with previous studies (8,10,11,44). One possible explanation could be the difference between the recombinant allergens and their native counterparts due to lack of posttranscriptional modifications (27). Conformational epitopes play an important role in allergenicity, particularly for globular inhaled allergens (45,46). However, this was unconvincing as Reese et al (6) found the recombinant tropomyosin (Pen a 1) behaved similarly to its native counterpart. Another possibility could be that tropomyosin was a minor allergen in HDM and/or cockroaches since Hu et al (47) reported the frequency of Der p 10 was only 10% in mite allergic patients in China, so tropomyosin may not be the major cause of cross-reactivity between shrimp and other arthropods. Similarly, Pascal et al (48) recently described that individuals allergic to HDM and/or cockroaches, with no sensitization to shrimp, recognized arginine kinase and hemocyanin. Immunoblotting using HDM extract should be included in future studies to confirm a presence of sIgE against HDM tropomyosin in HDM and/or cockroach allergic patients. Despite the above, the detection of specific IgE to allergenic components could provide higher specificity for allergy diagnosis (48); accurate allergy diagnosis without oral food challenge test remains a challenge (31,49) because it is heavily influenced by cross-reaction. When considering cross-reactions, it is important to distinguish whether a specific allergen is the cause of allergic symptoms (50). For instance, tropomyosin has been proved to be non-allergic in the majority of vertebrate foods although ~1/2 of the amino acid sequences between invertebrate and vertebrate tropomyosin are similar (3). That is, the biologic activity of cross-reactive allergens is often relatively low and analysis of recognition profiles of specific epitopes on allergen could better diagnose allergies (32).

The peptides of Pen a 1 were further evaluated by LICA to identify IgE and IgG₄ binding regions. It was worth noting the following points: First, the IgE and IgG4 epitope mapping of Pen a 1 are fundamental for designing safe hypoallergenic agents for the treatment of shrimp allergy. Effective immunotherapy for food allergy with IgE binding epitope modified hypoallergens has been reported (16,51); 28% (7/25) of patients were sensitized to tropomyosin but did not recognize any major epitope of Pen a 1. In addition, none of the 9 peptides were the major epitopes recognized by the north Chinese population. This indicated that there may be geographical or ethnic differences with regard to the epitopes profiles of tropomyosin. The major epitope of a certain allergen in one population may be a minor epitope in another population. This means an immunotherapy product designed for one region may have no effect in another. Individual allergen specific immunotherapy should be recommended as a plan. Secondly, it was found that the number of peptides recognized by IgE was positively correlated to level of allergen sIgE (r=0.680, P<0.001), which was similar to the result of a previous study (25). This indicated that the diversity of IgE binding epitopes is positively correlated with rPen a 1 IgE levels. However, according to the present study, there was no association between the number of IgE epitopes of Pen a 1 and shrimp-allergic symptoms. Other studies have demonstrated that identification of allergen epitopes is of great value in the diagnosis of food allergies (52-55). IgE-recognizing certain sequential immunodominant regions as well as broad IgE epitope diversity correlate with the severity of an allergic reaction (48,56). One possible explanation as to why the present study arrived at a different conclusion could be that the number of tropomyosin peptides synthesized was insufficient and the epitopes of other major allergens in shrimp were not tested. Another explanation could be that IgG₄-related tolerance may alleviate the symptoms. On the last point, the epitope recognition regions of IgE and IgG₄ largely overlapped, which was in agreement with previous studies (48,57). Coincident shared IgE and IgG₄ binding epitopes could be critical for the development of tolerance by blocking of the sIgE antibodies to the same allergen by sIgG₄ antibodies (58).

There were some limitations to the present study. For financial reasons, not all the 65 epitopes of Pen a 1 were analyzed. Although double blind, placebo-controlled food challenge is considered the gold standard for diagnosis of food allergy, the present study did not conduct provocative studies because it is an in vivo test involving a relatively complex procedure (59).

In conclusion, the present study used, for the first time to the best of our knowledge, LICA technology to determine the IgE and IgG₄ responses to Pen a 1 and its peptides among shrimp allergic patients from coastal areas of northern China. It was identified that 71.4% of patients with shrimp allergy were sensitized to Pen a 1 and 62.9% had increased IgG₄ antibodies to Pen a 1. Pen a 1 might not be responsible for cross-reactivity between shrimp and other arthropods. The results of the present study indicated that a strong IgG₄ response accompanied the presence of IgE to Pen a 1. It was also found that there may be geographical or ethnic differences with respect to the IgE and IgG₄ recognition patterns of the Pen a 1 peptides. The present study suggested that understanding the molecular basis of clinical reactivity will be useful for the accurate diagnose of shrimp allergy and to further implement better immunotherapy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
The authors declare that they have no competing interests.

Competing interests
not applicable.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committees of Tianjin Medical University (approval no. TMUHM2017008) and written informed consent was obtained from the patients and volunteers prior to study entry.

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Patient consent for publication

Not applicable.

Competing interests

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