

Tropomyosin sensitization in house dust mite allergic patients

Sven Becker · Moritz Gröger · Martin Canis ·
Elisabeth Pfrogner · Matthias F. Kramer

Received: 20 July 2011 / Accepted: 28 October 2011
© Springer-Verlag 2011

Abstract The growing popularity and frequency of consumption of seafood is accompanied by an increasing number of adverse reactions reported in literature. Allergic reactions to seafood can generate a variety of symptoms ranging from a mild oral allergy syndrome to keen anaphylactic reactions. Tropomyosin, the major shellfish allergen is regarded to be responsible for clinical cross-reactivity to inhaled house dust mites. The aim of the study was to investigate the prevalence of sensitization to tropomyosin in house dust mite allergic patients in southern Bavaria and to compare the results with allergic symptoms. Sera of house dust mite allergic patients (positive skin prick test, allergen-specific IgE and intranasal provocation) were screened for IgE antibodies to tropomyosin (*Der p 10*). Patients were contacted by phone to evaluate allergic symptoms when consuming seafood. IgE antibodies to house dust mite tropomyosin (*Der p 10*) could be found in 4 out of 93 sera (4.3%). Two of these four patients (50%) showed itching and swelling of oral mucosa accompanied by bronchial obstruction after consumption of shrimp. Two patients had no problems when eating seafood. None of the seronegative patients complained about any health problems during or after consumption of seafood. In conclusion, cross-reactivity to tropomyosin in house dust mite allergic patients in southern Bavaria, Germany is rarer than suspected. Beside the direct

allergic reactions, a further part of reactions to seafood must therefore be ascribed to other mechanisms such as intoxication or intolerance to, e.g. additives in the food product.

Keywords House dust mite · Seafood · Shellfish · Allergy · Tropomyosin · Der p 10 · Cross-reacting antibodies

Introduction

The growing consumption of seafood in regions far away from the coast as well as the increasing pollution of the oceans with toxic load of its inhabitants lead to an increasing number of sometimes adverse health problems among consumers [1]. Beside allergic reactions to a variety of different allergens in seafood, a relevant cause for allergic reactions especially to crustacean and mollusks constitutes the molecular and clinical cross-reactivity to house dust mites or cockroaches [2].

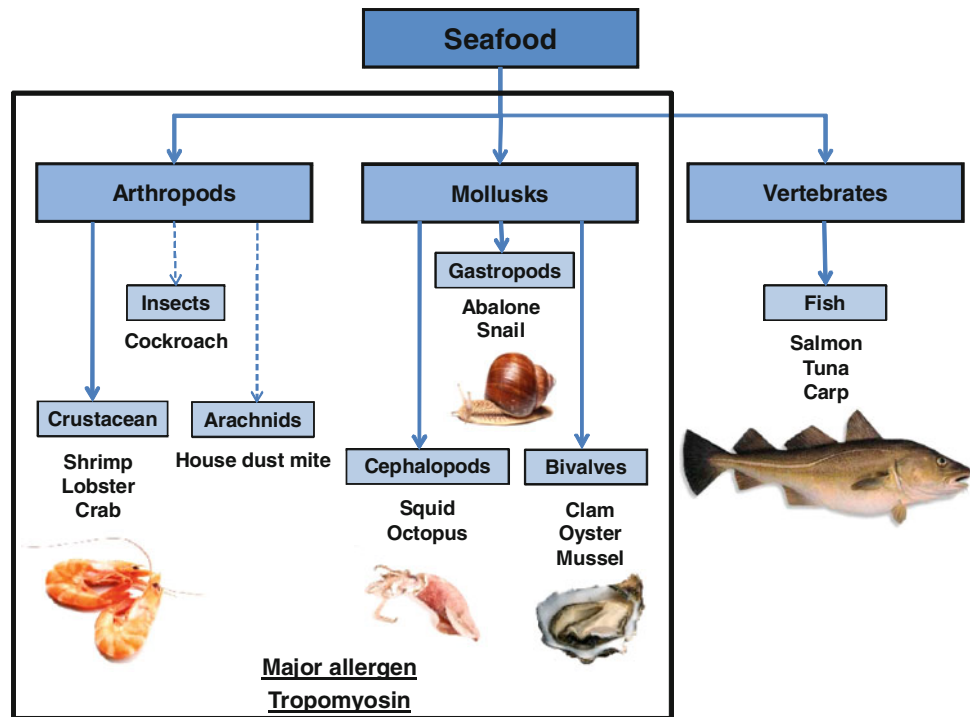
Seafood can be divided into three different groups (see Fig. 1). To take the biological taxonomy as a basis, there are arthropods, mollusks and vertebrates on the phylum-level. Crustacean such as shrimp, crab, prawn or lobster belong to the arthropod phylum as well as the arachnids (house dust mite, *Dermatophagoides pteronyssinus* and *D. farinae*) and the insects. The mollusks can also be divided into three different groups: gastropods (abalone and snail), bivalves (clam, oyster and mussels) and cephalopods (squid and octopus). The last group of seafood is fish (salmon, carp and tuna) which belong to the biological group of vertebrate. Fish contain a variety of different allergens and normally do not show cross-reactions in house dust mite allergic patients [1, 3, 4].

Tropomyosin is regarded to be responsible for the cross-reactivity between house dust mites and other invertebrates

S. Becker (✉) · M. Gröger · E. Pfrogner · M. F. Kramer
Department of Otorhinolaryngology-Head and Neck Surgery,
Ludwig Maximilian University, Marchioninistrasse 15,
81377 Munich, Germany
e-mail: sven.becker@med.uni-muenchen.de

M. Canis
Department of Otorhinolaryngology-Head and Neck Surgery,
University of Göttingen, Robert-Koch-Strasse 40,
37075 Göttingen, Germany

Fig. 1 Overview of seafood and connections to other tropomyosin carrying species. Connections to groups of importance for diet are shown in *straight lines*. Links to other species are shown in *dotted lines*



and consists of a phylogenetically conserved protein with different isoforms. Cross-reactivity describes the incident, when IgE antibodies originally created against the tropomyosin of house dust mites (*Der p 10*) also respond to tropomyosin of other species such as shrimp. Tropomyosin is present in muscle and non-muscle cells of vertebrates and invertebrates and shows a high heat stability [5]. The concordance of the amino acid sequence of tropomyosin from different arthropods and mollusks is very high, e.g. 81% between shrimp and house dust mites or 82% between shrimp and cockroaches. Ayuso et al. [6] demonstrated that the major shrimp epitope (tropomyosin, Pen a 1) shows even higher homology with corresponding regions in tropomyosin of house dust mites and cockroaches of more than 90%. This accordance in amino acid sequence can explain the clinically relevant cross-reactivity between different species of crustacean, between crustacean and mollusks and also between crustacean, mollusks and other invertebrates such as house dust mites, snails and insects [2, 7, 8].

Sensitization rates to tropomyosin (*Der p 10*) in house dust mite allergic patients vary in the literature. Weghofer et al. [9] found sensitization rates of 9% in France, 10% in Italy and 18% in Austria and Sweden. Comparable results for Austria of 15% could be shown by Resch et al. [10]. Westritschnig et al. analyzed sensitization profiles of African people in Zimbabwe and compared it with data from central Europe. These authors revealed sensitization rates to tropomyosin (*Der p 10*) of 55% in Zimbabwe in comparison with 10% in Austria [11]. The discrepancy in sensitization rates to *Der p 10* was ascribed to the different

sensitization rates to other tropomyosin carrying invertebrates such as cockroaches, crab or shrimp [12, 13]. Data from Australia showed slightly higher sensitization rates of 28% in comparison with Europe [14]. Although there are some data on the prevalence of specific IgE antibodies to *Der p 10*, there is nearly no data on the clinical relevance in connection with the intake of seafood.

Therefore, the aim of the study was to retrospectively investigate how many house dust mite allergic patients are sensitized against the cross-reacting allergen tropomyosin (*Der p 10*) in the catchment area of our university hospital in southern Bavaria, Germany. In the second step, we performed a telephone survey to analyze if sensitization to *Der p 10* was also clinically relevant.

Methods

Patient data were selected from the allergy database of the Department of Otorhinolaryngology-Head and Neck Surgery of the Ludwig-Maximilians-University in Munich, where all relevant diagnostic results of the patients are stored. The study was approved by the local ethics committee and the local data protection commissioner. All patients gave informed consent. The database was scanned for consecutive patients with a proven perennial allergy to house dust mites. This included: (1) a positive skin prick test for D pter. (skin prick test wheal >3 mm was considered as positive). The skin prick test (ALK-Abelló, Wedel, Germany) was read after 20 min. (2) CAP class ≥ 1 (≥ 0.35 kU/l,

UniCAP-FEIA, Phadia Diagnostics, Freiburg, Germany) for house dust mites (*Dermatophagoides pteronyssinus*). (3) Positive intranasal provocation with a standard provocation solution (D pter, ALK-Abelló, Wedel, Germany) due to guideline specifications [15] (decrease in rhinomanometry >40% at 150 Pa on the allergen challenged side or symptom-score >3, or decrease in rhinomanometry >20% at 150 Pa on the allergen challenged side and symptom-score >2). (4) Serum of the patient must be available at our allergy serum bank. Nasal symptoms were semi-quantitatively assessed in five categories: (1) obstruction, (2) rhinorrhoea, (3) sneezing, (4) itching, and (5) dryness. Each symptom could be answered between 0 and 3 (0 no impairment, 1 mild impairment, 2 moderate impairment, and 3 severe impairment).

Fluorescence enzyme immunoassay (FEIA) for tropomyosin (Der p 10)

Sera of patients which fulfilled the above-mentioned criteria were defrosted and specific IgE antibodies to *Der p 10* were measured using the FEIA method (UniCAP-FEIA, Phadia Diagnostics, Freiburg, Germany) with a commercial test kit (Phadia Diagnostics, Freiburg, Germany). Results were acquired in CAP classes as well as in concentration (kU/l).

Allergic symptoms

All patients were contacted by phone between January and March 2011. Patients were asked about any allergic symptoms due to the intake of seafood. Symptoms were assessed in seven categories: (1) itchiness of oral mucosa, (2) swelling of oral mucosa, (3) skin reactions, (4) shortness of breath, (5) circulatory problems, (6) abdominal pain, and (7) flatulence/diarrhea. Each category should be answered between 0 and 3 (0 no impairment, 1 mild impairment, 2 moderate impairment, and 3 severe impairment).

Statistical analysis

Statistical analysis was performed with SigmaStat (Jandel Corp., San Rafael, CA, USA) on a Lenovo ThinkPad X200s. For descriptive statistics we used mean values with standard deviation (SD). For the comparison of groups the Mann–Whitney rank sum test was used.

Results

The database query with the above-mentioned inclusion criteria resulted in 93 patients with a proven allergy to house dust mites (see Table 1). Gender distribution showed

Table 1 Patient demographics and study results

	Number of patients	Percent
All patients	93	100
Mean age, years (range)	31 (8–66)	
Gender		
Male	56	60
Female	37	40
Mono-sensitized to house dust mites	31	33
Poly-sensitized to further allergens	62	67
<i>Der p 10</i> positive	4	4
Telephone survey	67	72
<i>Der p 10</i> positive contacted	4	100
Symptoms	2	50
No symptoms	2	50
<i>Der p 10</i> negative contacted	63	71
Symptoms	0	0
No symptoms	63	100

37 (39.8%) female and 56 (60.2%) male patients with a mean age of 31.0 years and a range between 7.6 and 66.4 years. Thirty-one patients showed single sensitization to house dust mites, 62 patients showed additional sensitization to further perennial and seasonal allergens.

Results for the allergen-specific IgE concentrations and clinical nasal symptoms at the time of the initial examination can be seen in Table 2. The mean concentration of IgE antibodies to D pter was $8.85 \text{ kU/l} \pm 13.22 \text{ SD}$ in *Der p 10* positive patients in comparison to $11.76 \text{ kU/l} \pm 17.32 \text{ SD}$ in *Der p 10* negative patients. There was no statistically significant difference in IgE-antibody levels to D pter. between *Der p 10* positive or negative patients. The analysis of clinical nasal symptoms also showed no significant differences in the two groups.

FEIA results for tropomyosin *Der p 10* resulted in only 4 patients (4.3%) with CAP class ≥ 1 . There were a 17-year-old boy (skin prick test: 4, CAP class 2 $\triangleq 1.28 \text{ kU/l}$ for Der p 1, CAP class 1 $\triangleq 0.37 \text{ kU/l}$ for *Der p 10*), a 27-year-old woman (skin prick test: 4, CAP class 3 $\triangleq 4.79 \text{ kU/l}$ for Der p 1, CAP class 1 $\triangleq 0.62 \text{ kU/l}$ for *Der p 10*) and another 31-year-old woman (skin prick test: 4, CAP class 2 $\triangleq 0.81 \text{ kU/l}$ for Der p 1, CAP class 1 $\triangleq 0.47 \text{ kU/l}$ for *Der p 10*) with CAP class 1 for *Der p 10*. One 40-year-old man showed CAP class 2 for *Der p 10* (skin prick test: 2, CAP class 4 $\triangleq 28.50 \text{ kU/l}$ for D pter, CAP class 2 $\triangleq 2.08 \text{ kU/l}$ for *Der p 10*).

Altogether 67 of 93 patients (72%) could be contacted in the first 3 months of 2011 (see Fig. 2). From the 89 patients who were tested negative for *Der p 10*, we were able to contact 63 patients (71%) by phone. Twenty-six patients could not be contacted because of wrong phone-numbers

Fig. 2 Results of the telephone survey which was carried out between January and March 2011

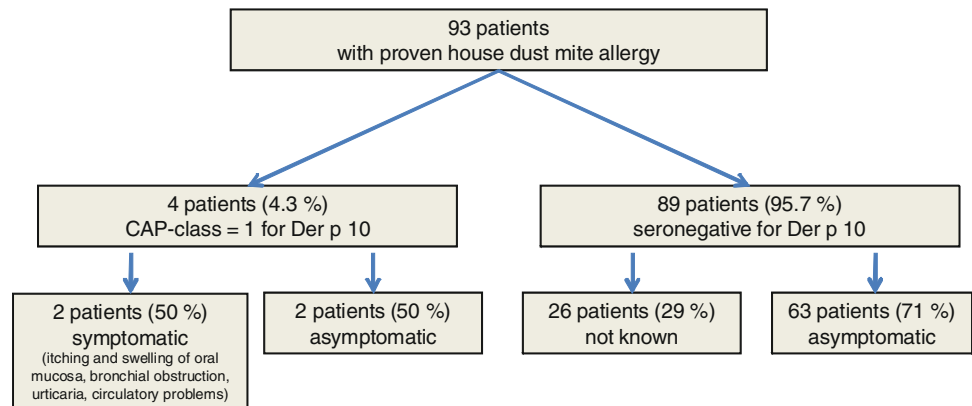


Table 2 Specific IgE concentrations to *D. pteronyssinus* and nasal symptoms at the time of initial examination

	<i>Der p 10</i> positive (n = 4)	<i>Der p 10</i> negative (n = 89)	P values
Specific IgE to <i>D. pteronyssinus</i> (kU/ml)	8.85 ± 13.22	11.76 ± 17.32	0.564
Nasal symptoms			
Obstruction	1.74 ± 1.15	2.00 ± 1.15	0.694
Rhinorrhoea	1.34 ± 1.18	1.75 ± 0.96	0.502
Sneezing	1.38 ± 1.04	1.00 ± 0.82	0.536
Itching	1.11 ± 0.93	1.25 ± 0.96	0.763
Dryness	1.06 ± 1.15	0.75 ± 0.96	0.703

Results are given as mean ± standard deviation. For the comparison between the groups the Mann–Whitney rank sum test was used. Nasal symptoms were scored between 0 and 3 (0 no impairment, 1 mild impairment, 2 moderate impairment, and 3 severe impairment)

given or removal. Calling was stopped after the fifth unsuccessful call within 1 week. No patients of the 63 seronegative complained about any of problems when eating seafood. Results for the seven symptoms: (1) itchiness of oral mucosa, (2) swelling of oral mucosa, (3) skin reactions, (4) shortness of breath, (5) circulatory problems, (6) abdominal pain, and (7) flatulence/diarrhea were 0, respectively. Ten of these 63 patients did not eat shellfish due to personal preferences.

All four patients who were tested CAP class ≥ 1 for *Der p 10* could be consulted via phone. Two patients (50%) reported of mainly mild to moderate recurrent health problems when eating shrimp (see Table 3). The other two patients had no problems when eating seafood.

Discussion

Molecular and clinical cross-reactivity between shellfish and house dust mites could be shown by different working groups so far [2, 7, 16–18]. This is the first study which ever evaluated the prevalence rate for a sensitization to

Table 3 Clinical symptoms, evaluated by telephone survey, of the four *Der p 10* positive patients when eating shellfish

	Patient 1	Patient 2	Patient 3	Patient 4
Itchiness of oral mucosa	2	1	0	0
Swelling of oral mucosa	2	1	0	0
Skin reactions	1	3	0	0
Shortness of breath	2	2	0	0
Circulatory problems	0	1	0	0
Abdominal pain	0	0	0	0
Flatulence/diarrhea	0	0	0	0

Symptoms were scored between 0 and 3 (0 no impairment, 1 mild impairment, 2 moderate impairment, and 3 severe impairment)

tropomyosin (*Der p 10*) in house dust mite allergic patients and compared it with clinical symptoms after seafood intake. The results of the present study demonstrate a far less sensitization rate to tropomyosin (*Der p 10*) in house dust mite allergic patients around Munich than suspected from the literature. Fifty percent of sensitized patients showed clinical symptoms when eating shellfish. The strength of the current study is that we were looking for not only serological parameters but also clinical symptoms. All included patients were diagnosed for a house dust mite allergy by state-of-the-art diagnostic procedures (clinical history, skin prick test, allergen specific IgE evaluation, and intranasal provocation).

Sensitization rates to *Der p 10* between 10 and 18% in Austria up to 55% in Zimbabwe were reported in the literature [9–11]. As Austria directly borders to the southern Bavarian part of Germany and eating habits with mainly meat and less seafood diet is common, we expected a comparable sensitization to tropomyosin of above 10%. Our results show a far less sensitization rate of 4.3% to *Der p 10* in the region around Munich. The reason for the low sensitization rate in our patients could be the fact that we are an otorhinolaryngological clinic, were mainly patients with nasal symptoms were treated. It is possible that patients with primarily postprandial reactions seek advice from

other departments and therefore sensitization rates in their patients could be higher. Depicted literature concerning *Der p 10* sensitization rates was mainly published from departments of immunology or pathophysiology pooling data from different other departments together (dermatology, otorhinolaryngology, pulmonology, and pediatrics). More detailed information regarding the detailed distribution to the different departments was not extractable from these publications. The high sensitization rates to *Der p 10* which were seen in Zimbabwe were ascribed to cross-reactions to other invertebrates such as cockroach, shrimp or crab. For example, sensitization rates for crab of up to 22% were detected in the African population with the multiallergen test [11].

Seafood allergy is a significant health problem in industrialized countries. 2.3% of the general population in the United States are affected by recurrent and sometimes severe reactions after seafood intake [19]. Against the background of our study, cross-reactivity to shellfish tropomyosin in house dust mite allergic patients seems to be one reason for those reactions. Although tropomyosin is regarded to be the major shellfish allergen, novel allergens have been identified and characterized in crustaceans which could serve as a new class of invertebrate pan allergen. Analysis of the protein sequence showed high similarity to arginine kinase [20, 21]. In addition, a sarcoplasmatic calcium-binding protein and a myosin light chain of 20 kDa have been identified [22–24]. Similar homologues of these allergens can be found among different invertebrates; whether they are clinically relevant regarding cross-reactivity between different groups of invertebrates is not evaluated so far.

Other reasons for mild to severe reactions to shellfish can be due to other components in the food product [1]. These reactions can be immunological and non-immunological to a variety of triggers such as marine toxins, biogenic amines, and ingredients for the preparation of the product such as flavors, preservatives, spices, and chemical additives [25, 26]. The colonization of seafood with other creatures such as bacteria [27], parasites (e.g. *Anisakis*) [28, 29] or viruses such as hepatitis A [30] can be a further reason for the increasing number of reactions.

Although patients were asked for detailed information concerning their problems when eating seafood, reactions could also be aroused by other mechanisms as discussed above. Double-blind placebo-controlled food challenges are necessary to prove a definite allergy to, e.g. shrimp [31]. However, we think that our results reflect the situation in a realistic way. We were able to contact more than 70% of the patients which is in good accordance with other studies using phone surveys [31–33].

The four *Der p 10* positive patients had no specific immunotherapy against house dust mites so far. It would be interesting to see if the symptomatic patients would feel an

improvement of their symptoms after immunotherapy when eating shrimp again. Improvement of oral allergy symptoms can be seen in birch pollen allergic patients under immunotherapy [34]. Birch pollen allergic patients often show oral symptoms when eating stone fruits or nuts [35]. If specific immunotherapy should be used to treat oral allergy symptoms is under controversial discussion [34, 36–38]. No study evaluated the benefit of a specific immunotherapy to house dust mites in patients suffering from cross-reactive allergy symptoms after ingestion of shellfish.

To clarify the importance of cross-reactivity in house dust mite allergic patients, further epidemiological studies with more patients are necessary. Ideally, these trials should include double-blind placebo-controlled food challenges to be sure that reactions were not triggered by other mechanisms.

Conflict of interest There is no conflict of interest from any of the authors.

References

1. Lopata AL, O'Hehir RE, Lehrer SB (2010) Shellfish allergy. *Clin Exp Allergy* 40(6):850–858
2. Witterman AM, Akkerdaas JH, van Leeuwen J, van der Zee JS, Aalberse RC (1994) Identification of a cross-reactive allergen (presumably tropomyosin) in shrimp, mite and insects. *Int Arch Allergy Immunol* 105(1):56–61
3. Lopata AL, Lehrer SB (2009) New insights into seafood allergy. *Curr Opin Allergy Clin Immunol* 9(3):270–277
4. van Ree R, Antonicelli L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi F (1996) Possible induction of food allergy during mite immunotherapy. *Allergy* 51(2):108–113
5. Iparraguirre A, Rodriguez-Perez R, Juste S, Ledesma A, Moneo I, Caballero ML (2009) Selective allergy to lobster in a case of primary sensitization to house dust mites. *J Invest Allergol Clin Immunol* 19(5):409–413
6. Ayuso R, Reese G, Leong-Kee S, Plante M, Lehrer SB (2002) Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. *Int Arch Allergy Immunol* 129(1):38–48
7. Thomas WR, Smith WA, Hales BJ, Mills KL, O'Brien RM (2002) Characterization and immunobiology of house dust mite allergens. *Int Arch Allergy Immunol* 129(1):1–18
8. Arlian LG, Morgan MS, Vyszynski-Moher DL, Sharra D (2009) Cross-reactivity between storage and dust mites and between mites and shrimp. *Exp Appl Acarol* 47(2):159–172
9. Weghofer M, Thomas WR, Kronqvist M et al (2008) Variability of IgE reactivity profiles among European mite allergic patients. *Eur J Clin Invest* 38(12):959–965
10. Resch Y, Weghofer M, Seiberler S et al (2011) Molecular characterization of *Der p 10*: a diagnostic marker for broad sensitization in house dust mite allergy. *Clin Exp Allergy* 41(10):1468–1477
11. Westritschnig K, Sibanda E, Thomas W et al (2003) Analysis of the sensitization profile towards allergens in central Africa. *Clin Exp Allergy* 33(1):22–27
12. Fernandez-Caldas E, Puerta L, Caraballo L, Lockey RF (2004) Mite allergens. *Clin Allergy Immunol* 18:251–270
13. Fernandez-Caldas E, Puerta L, Caraballo L, Lockey RF (2008) Mite allergens. *Clin Allergy Immunol* 21:161–182

14. Hales BJ, Martin AC, Pearce LJ et al (2006) IgE and IgG anti-house dust mite specificities in allergic disease. *J Allergy Clin Immunol* 118(2):361–367
15. Riechelmann H, Bachert C, Goldschmidt O et al (2003) Application of the nasal provocation test on diseases of the upper airways. Position paper of the German Society for Allergology and Clinical Immunology (ENT Section) in cooperation with the Working Team for Clinical Immunology. *Laryngorhinootologie* 82(3):183–188
16. Thomas WR, Smith WA, Hales BJ (2004) The allergenic specificities of the house dust mite. *Chang Gung Med J* 27(8):563–569
17. Asturias JA, Arilla MC, Gomez-Bayon N, Martinez A, Martinez J, Palacios R (1998) Sequencing and high level expression in *Escherichia coli* of the tropomyosin allergen (Der p 10) from *Dermatophagoides pteronyssinus*. *Biochim Biophys Acta* 1397(1):27–30
18. Purohit A, Shao J, Degreaf JM et al (2007) Role of tropomyosin as a cross-reacting allergen in sensitization to cockroach in patients from Martinique (French Caribbean island) with a respiratory allergy to mite and a food allergy to crab and shrimp. *Eur Ann Allergy Clin Immunol* 39(3):85–88
19. Sicherer SH, Munoz-Furlong A, Sampson HA (2004) Prevalence of seafood allergy in the United States determined by a random telephone survey. *J Allergy Clin Immunol* 114(1):159–165
20. Garcia-Orozco KD, Aispuro-Hernandez E, Yepiz-Plascencia G, Calderon-de-la-Barca AM, Sotelo-Mundo RR (2007) Molecular characterization of arginine kinase, an allergen from the shrimp *Litopenaeus vannamei*. *Int Arch Allergy Immunol* 144(1):23–28
21. Yu CJ, Lin YF, Chiang BL, Chow LP (2003) Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. *J Immunol* 170(1):445–453
22. Ayuso R, Grishina G, Bardina L et al (2008) Myosin light chain is a novel shrimp allergen, Lit v 3. *J Allergy Clin Immunol* 122(4):795–802
23. Ayuso R, Grishina G, Ibanez MD et al (2009) Sarcoplasmic calcium-binding protein is an EF-hand-type protein identified as a new shrimp allergen. *J Allergy Clin Immunol* 124(1):114–120
24. Shiomi K, Sato Y, Hamamoto S, Mita H, Shimakura K (2008) Sarcoplasmic calcium-binding protein: identification as a new allergen of the black tiger shrimp *Penaeus monodon*. *Int Arch Allergy Immunol* 146(2):91–98
25. McCann D, Barrett A, Cooper A et al (2007) Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. *Lancet* 370(9598):1560–1567
26. Scholl I, Jensen-Jarolim E (2004) Allergenic potency of spices: hot, medium hot, or very hot. *Int Arch Allergy Immunol* 135(3):247–261
27. Martinez O, Rodriguez-Calleja JM, Santos JA, Otero A, Garcia-Lopez ML (2009) Foodborne and indicator bacteria in farmed molluscan shellfish before and after depuration. *J Food Prot* 72(7):1443–1449
28. Audicana MT, Kennedy MW (2008) Anisakis simplex: from obscure infectious worm to inducer of immune hypersensitivity. *Clin Microbiol Rev* 21(2):360–379
29. Nieuwenhuizen N, Lopata AL, Jeebhay MF, Herbert DR, Robins TG, Brombacher F (2006) Exposure to the fish parasite Anisakis causes allergic airway hyperreactivity and dermatitis. *J Allergy Clin Immunol* 117(5):1098–1105
30. Pinto RM, Costafreda MI, Bosch A (2009) Risk assessment in shellfish-borne outbreaks of hepatitis A. *Appl Environ Microbiol* 75(23):7350–7355
31. Yum HY, Yang HJ, Kim KW et al (2011) Oral food challenges in children. *Korean J Pediatr* 54(1):6–10
32. Trupin L, Balmes JR, Chen H et al (2010) An integrated model of environmental factors in adult asthma lung function and disease severity: a cross-sectional study. *Environ Health* 9:24
33. Vandentorren S, Bois C, Pirus C, Sarter H, Salines G, Leridon H (2009) Rationales, design and recruitment for the Elfe longitudinal study. *BMC Pediatr* 9:58
34. Mari A, Ballmer-Weber BK, Vieths S (2005) The oral allergy syndrome: improved diagnostic and treatment methods. *Curr Opin Allergy Clin Immunol* 5(3):267–273
35. Holm J, Ferreras M, Ipsen H et al (2011) Epitope grafting: re-creating a conformational bet V 1 antibody epitope on the surface of the homologous apple allergen MAL D 1. *J Biol Chem* 286(20):17569–17578
36. Asero R (1998) Effects of birch pollen-specific immunotherapy on apple allergy in birch pollen-hypersensitive patients. *Clin Exp Allergy* 28(11):1368–1373
37. Asero R (2004) Effects of birch pollen SIT on apple allergy: a matter of dosage? *Allergy* 59(12):1269–1271
38. Bucher X, Pichler WJ, Dahinden CA, Helbling A (2004) Effect of tree pollen specific, subcutaneous immunotherapy on the oral allergy syndrome to apple and hazelnut. *Allergy* 59(12):1272–1276