

Identification of pyruvate kinase 2 as a possible crab allergen and analysis of allergenic proteins in crabs consumed in Taiwan

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ARTICLE INFO

Keywords:

Allergen
Crustacean
ELISA
Food allergy
Pyruvate kinase 2
Tropomyosin

ABSTRACT

In Taiwan, crab is one of the main causes for food allergy. Several proteins are recognized as crustacean allergens, and tropomyosin is known to be the major one. However, sensitization patterns of Taiwanese patients to crustacean allergens remain unclear. Therefore, we analyzed the specific-IgE binding ability of crucifix crab (*Charybdis feriatus*) allergens by western blot using patients' sera. In particular, we found a 56 kDa protein in crucifix crab reacted with specific-IgEs in patients' sera, and we further identified the protein as a novel crab allergen pyruvate kinase 2. Additionally, little is known about tropomyosin contents in crabs consumed in Taiwan. Thus, we also quantified the levels of tropomyosin by using enzyme-linked immunosorbent assay (ELISA) among raw and cooked crab species. Our results showed tropomyosin levels varied depending on crab species. In summary, these findings improve the understanding of crustacean allergens and contribute to the clinical diagnosis of crustacean allergies.

1. Introduction

Food allergy is an important public health issue worldwide. About 2–10% of world populations suffer from food allergies (Chan, Chen, Huang, & Wu, 2014). The incidence of food allergies in Taiwanese people was 6.9% (Wu et al., 2012). Food allergy is associated with specific diseases, including congestive heart failure, peripheral vascular disease, chronic pulmonary disease, rheumatologic disease and renal disease in Taiwanese population (Yu, Tu, Hong, & Lee, 2017). Epidemiological study reported crustaceans, such as crab and shrimp were the most common causes of food allergy in Taiwan (Chan et al., 2014).

Tropomyosin is the first allergen that had been identified in crustacean (Shanti, Martin, Nagpal, Metcalfe, & Rao, 1993), and accounts for 80% of crustacean allergies (Jeoung et al., 1997). It is a two-stranded α -helical coiled-coil protein composed of myofibrillar subunits with molecular weight of 34–38 kDa (Huang et al., 2010; Leung et al.,

1998). Furthermore, literature reported tropomyosin is a heat-stable protein (Liu et al., 2010; Usui et al., 2013). Thus, heat processing may not be able to disrupt the tropomyosin completely. Although studies suggest food processing, such as radiation, ultrasound, enzyme hydrolysis and high pressure heating may disrupt tropomyosin effectively (Li, Lin, Cao, & Jamil, 2007; Li, Linhong, & Jamil, 2006; Long, Yang, Wang, Hu, & Chen, 2015; Yu et al., 2011), traditional heating remains the most common way of cooking crab and shrimp in the typical house hold. Thus, heated crabs and shrimps could potentially cause allergy to consumers.

Besides tropomyosin, several studies found other allergic proteins using western blot with allergic patients' sera. They included arginine kinase, myosin light chain, troponin C, hemocyanin, glyceraldehyde-3-phosphate dehydrogenase, enolase, triose phosphate isomerase, myosin heavy chain, smooth endoplasmic reticulum Ca^{2+} ATPase and α -actin (Ayuso et al., 2008; Bauermeister et al., 2011; Khanaruksombat,

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Srisomsap, Chokchaichamnankit, Punyarit, & Phiriyangkul, 2014; Rahman, Kamath, Gagne, Lopata, & Helleur, 2013; Rahman, Kamath, Lopata, Robinson, & Helleur, 2011; Shiomi, Sato, Hamamoto, Mita, & Shimakura, 2008; Yu, Lin, Chiang, & Chow, 2003). Due to variable differences in allergic responses among consumers worldwide, the clinical significance of each allergen is unclear. For example, tropomyosin has been reported to be the most important allergen for patients from the US and Asia, however, Bauermeister et al. demonstrated non-tropomyosin allergens showed higher sensitization potential to European patients using ImmunoCAP analysis (Bauermeister et al., 2011; Daul, Slattery, Reese, & Lehrer, 1994). Thus, it is important to further understand various crustacean allergens for allergy control.

Since allergic reactions can be life-threatening, it is important to develop a reliable analytical method to detect food allergens. Currently, enzyme-linked immunosorbent assay (ELISA) is the most frequently used method for routine detection of allergens in food. This method has the advantages of direct detection of protein, ease of use, and high sensitivity. Several studies demonstrated that ELISA techniques can accurately quantify tropomyosin levels in crustacean (Fuller, Goodwin, & Morris, 2006; Schubert-Ullrich et al., 2009; Seiki et al., 2007; Werner, Faeste, & Egaas, 2007; Zhang, Lu, Ushio, & Shiomi, 2014).

Because sensitization patterns of various crustacean allergens in Taiwanese patients are not well-characterized, we used crucifix crab (*Charybdis feriatius*), which is a popular species consumed in Taiwan, as a sample for western blot. We analyzed the allergic response between allergens in crucifix crab (*Charybdis feriatius*) and specific-IgEs in patients' sera in search for a novel allergen. In addition, to investigate the effect of heating on tropomyosin, we quantified the tropomyosin levels in both raw and cooked crabs of nine species in the study.

2. Materials and methods

2.1. Patients' sera

7 patients (male/female = 4/3; 22–48 years old) with crustacean allergy were recruited, and their sera were collected in Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan. Written informed consent was obtained from each patient. The project were approved by Institutional Review Board (104-1569B) in Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan. Serum from one healthy subject without a history of crustacean allergy was used as a negative control. All sera were stored at -80°C until use.

2.2. Samples

Fresh samples of three-spot swimming crab (*Portunus sanguinolentus*), horse crab (*Portunus trituberculatus*), red swimming crab (*Portunus haanii*), crucifix crab (*Charybdis feriatius*), ridged swimming crab (*Charybdis natator*), swimming crab (*Charybdis amboinensis*), sand crab (*Ovalipes punctatus*) and common box crab (*Calappa Lophos*) were obtained from local markets in New Taipei City, Taiwan. Giant mud crab (*Scylla serrata*) was purchased from local market in Pingtung, Taiwan.

2.3. Sample extraction

Extraction method by Sahabudin et al. were used with minor modifications (Sahabudin et al., 2011). Meat from the crab claw was taken out and homogenized. 5 g of meat sample was mixed with 25 ml of cold PBS buffer (pH 7.4, 0.137 M NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 , 1.8 mM KH_2PO_4), and homogenized for 1 min. Then the sample was centrifuged at 7000g for 30 min at 4°C . Next, the filtrate was centrifuged at 15,000g for 15 min at 4°C , and the supernatant was taken out as protein extracts. Protein extracts were stored at -20°C . Cooked crabs were steamed in laboratory for 5 min using raw crabs, following the same protein extraction method. In addition, different parts of

tissues in three-spot swimming crab were taken out to quantify the tropomyosin levels.

2.4. Western blot analysis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate proteins in crab extracts. Then, the separated proteins were transferred to polyvinylidene fluoride (PVDF) membrane, blocked with 5% skim milk powder in PBS-T buffer for 1 h, and subsequently incubated with diluted patients' sera overnight. After washing three times with PBS-T buffer, mouse monoclonal B3102E8 Anti-human IgE Fc (HRP) (Abcam, USA) at 1:40,000 dilution were used as secondary antibody, and incubated for 1 h. After final washing, Clarity™ Western ECL Substrate (Bio-Rad, USA) was used as chemiluminescent substrate. Finally, membrane was photographed using chemiluminescence imaging system G:BOX Chemi XX8 (Syngene, UK).

2.5. Protein digestion and mass spectrometry for allergen identification

Protein digestion and mass spectrometry were carried out as described previously (Lee et al., 2018). In-gel digestion of protein bands excised from polyacrylamide gel was performed, followed by peptide extraction. The protein tryptic digests were fractionated using a nano-UPLC system (nanoACQUITY UPLC, Waters, Milford, MA, USA) coupled to an ion trap mass spectrometer (LTQ Orbitrap Discovery Hybrid FTMS, Thermo, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source. The mass spectral interpretation of protein was performed by uploading data to the Mascot (ver. 2.2.1, Matrix Science, London, UK) search engine.

2.6. ELISA procedure

Muscle proteins of crab were extracted first, and ELISA were performed based on manufacturer's instructions (Crustacean Tropomyosin ELISA Kit, Arigo, Taiwan).

3. Results

3.1. The reaction of crab proteins and specific-IgEs in patients' sera

Fig. 1 shows the sensitization patterns of 7 allergic patients against crucifix crab proteins. We observed proteins with 24, 27, 35, 38, 47, 75, 100 and 200 kDa in raw crab extracts bound to specific-IgEs in patients' sera (Fig. 1a). In contrast, proteins with 27, 35, 38, 75 and 100 kDa reduced their activities obviously in cooked crab extracts (Fig. 1b). Serum of control subject did not show any binding with crab proteins. Table 1 lists the reactivity of each protein. (Reactivity = number of sera that bound to this protein/total number of sera). These proteins were recognized as allergens, and bound to specific-IgEs in individual patient. The reactivity of these allergens in raw crab in the descending order were 100, 75, 56 and 35 kDa (100%) > 47 and 38 kDa (86%) > 27 kDa (71%) > 200 and 24 kDa (57%), while in cooked crab were 75 and 47 kDa (71%) > 56 kDa (57%) > 100 kDa (43%) > 200 and 35 kDa (29%) > 38 and 27 kDa (0%). The 56 kDa protein was not identified in previous studies, we further analyzed it by mass spectrometry.

3.2. Identification of the 56 kDa protein as pyruvate kinase 2 by mass spectrometry

The fragmentation spectra obtained by the UPLC-ESI-MS/MS analysis in gradient detection mode were compared with a non-redundant protein database using Mascot software. A summary of the protein identifications achieved is showed in Table 2. The Mascot search of the data yielded pyruvate kinase 2 as the first hit with the scores of 370, and the corresponding to 9 peptide matches. Proteins were initially

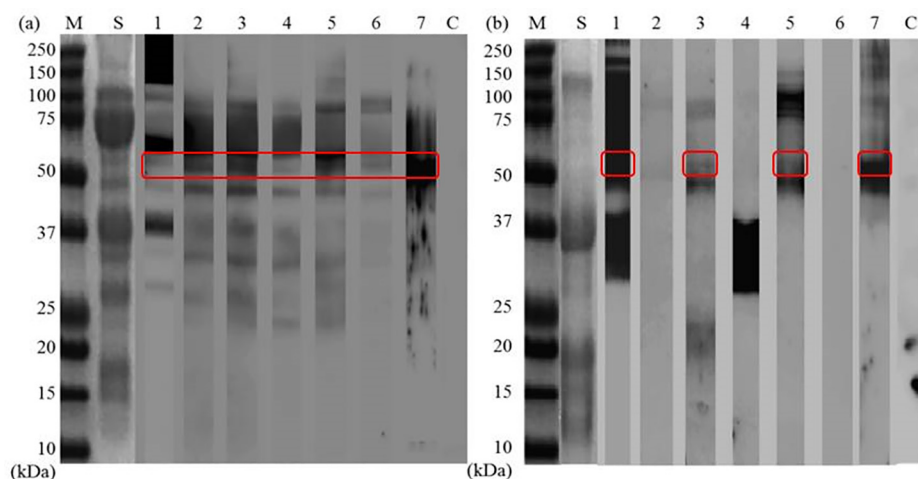


Fig. 1. Western blot analysis of specific IgE in patients' sera with (a) raw and (b) cooked crucifix crab. M: protein marker, S: SDS-PAGE of crab extract, 1–7: different patients' sera, C: serum of control subject. allergen with 56 kDa.

Table 1
Binding activity of proteins in crucifix crab with specific-IgE in patients' sera.

Allergen ^a	Molecular weight (kDa)	Raw crab		Cooked crab	
		Case ^b	Reactivity ^c (%)	Case ^b	Reactivity ^c (%)
MHC	200	4	57	2	29
SERCA	100	7	100	3	43
HC	75	7	100	5	71
Allergen identified in this study	56	7	100	4	57
EA	47	6	86	5	71
GAPDH, AK	38	6	86	0	0
TM	35	7	100	2	29
TIM	27	5	71	0	0
TnC	24	4	57	1	14
MLC	18	0	0	0	0

^a MLC: myosin light chain, TnC: troponin C, TIM: triose phosphate isomerase, TM: tropomyosin, AK: arginine kinase, GAPDH: glyceraldehyde 3-phosphate dehydrogenase, EA: enolase, HC: hemocyanin, SERCA: smooth endoplasmic reticulum Ca²⁺ ATPase, MHC: myosin heavy chain.

^b Number of patients' sera that bound to the band.

^c Reactivity = number of patients' sera that bound to the band/total number of patients' sera.

annotated by similarity searches using Swiss Institute of Bioinformatics (SIB).

3.3. Mass spectra of pyruvate kinase 2

Fig. 2 shows typical MS/MS spectra of the identified peptides. The MS/MS spectra represent the amino acid sequences of tryptic peptides, which were doubly and triply charged peptides. The amino acid sequences of the tryptic peptides are shown and peaks matched as b and y ions of the peptide sequence are labeled.

3.4. Levels of tropomyosin in muscle proteins of crabs

Table 3 shows the levels of tropomyosin in raw and cooked crabs. Levels of tropomyosin varied according to the crab species. In raw sample, sand crab and common box crab showed the two highest tropomyosin levels as 38.50 ± 1.23 mg/g and 31.90 ± 1.39 mg/g, respectively. While horse crab had only 0.30 ± 0.00 mg/g of tropomyosin levels.

4. Discussion

To best of our knowledge, this is the first time pyruvate kinase 2 has been identified as a crab allergen by mass spectrometry. Our previous study identified pyruvate kinase, an isozyme of pyruvate kinase 2 (Ma et al., 2015), as a novel shrimp allergen (Lee et al., 2018). This time we further demonstrated the importance of this allergen, which cause sensitization to patient with crustacean allergy. The amino sequence similarity of pyruvate kinase 2 and pyruvate kinase is shown in **Fig. 3**. The former consist of 522 amino acids, while the latter consist of 591

Table 2
Proteins identified and peptides matched sorted by residue number.

Accession No.	Protein name	Score	Sequence Coverage	MW	pI	Observed	Mr(expt)	Mr(calc)	Peptide
B6RHH4	Pyruvate kinase 2	370	15%	56,734	6.89	407.7551	813.4957	813.4960	R.GDLLLGVK.M
						527.7698	1053.5251	1053.5243	R.EAEAAIWHK.Q
						571.3091	1140.6037	1140.6026	R.GDLGIEIPAEK.V
						845.9732	1689.9319	1689.9301	K.GVNLPGVVPDLPVSEK.D
						910.0212	1818.0278	1818.0251	K.KGVNLPGVVPDLPVSEK.D
						910.5239	1819.0332	1819.0091	K.KGVNLPGVVPDLPVSEK.D + Deamidated (NQ)
						641.6645	1921.9717	1921.9675	K.VGKPVICATQMLES ~~M~~ VK.K + Carbamidomethyl (C); 2 Oxidation (M)
						641.9965	1922.9677	1922.9515	K.VGKPVICATQMLES ~~M~~ VK.K + Carbamidomethyl (C); Deamidated (NQ); 2 Oxidation (M)
						647.2963	1938.8670	1938.9035	R.SVEMLEKMMMEAGMNIAR.M

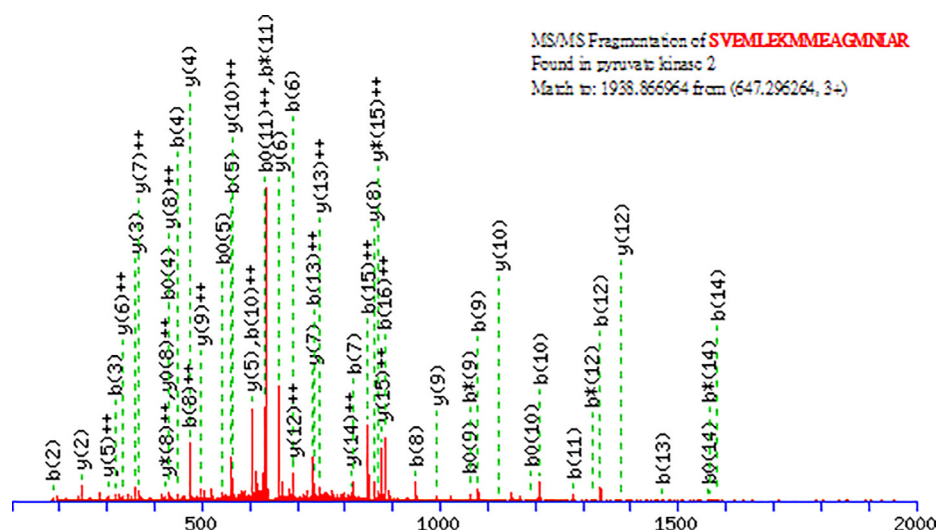


Fig. 2. Examples of significant match (product ion scan data against NCBI sequence database) derived from triply charged precursor ions, respectively. Peaks matched as b and y ions of the peptide sequence are labeled.

Table 3
Levels of tropomyosin in crabs consumed in Taiwan.

Common name	Scientific name	Tropomyosin levels (mg/g)	
		Raw	Cooked
Three-spot swimming crab	<i>Portunus sanguinolentus</i>	18.17 ± 1.46	8.70 ± 0.63
Horse crab	<i>Portunus triuberculatus</i>	0.30 ± 0.00	1.13 ± 0.00
Red swimming crab	<i>Portunus haanii</i>	26.69 ± 1.16	6.61 ± 0.26
Crucifix crab	<i>Charybdis feriatus</i>	14.48 ± 0.44	22.01 ± 3.52
Ridged swimming crab	<i>Charybdis natator</i>	17.19 ± 0.64	23.68 ± 3.37
Swimming crab	<i>Charybdis amboinensis</i>	27.31 ± 0.38	29.01 ± 1.74
Giant mud crab	<i>Scylla serrata</i>	23.19 ± 1.20	11.03 ± 2.95
Sand crab	<i>Ovalipes punctatus</i>	31.90 ± 1.39	31.40 ± 2.30
Common box crab	<i>Calappa Lophos</i>	38.50 ± 1.23	52.20 ± 5.08

amino acids with similar enzymatic function. Pyruvate kinase 2 catalyzes the conversion and the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP), yielding one molecule of pyruvate and one molecule of ATP in the final step of glycolysis and plays a role in regulating cell metabolism (Dashty, 2013; Ramos-Martinez, 2017), and was important in life cycle of crustacean (Lemos, Salomon, Gomes, Phan, & Buchholz, 2003; Salomon, Mayzaud, & Buchholz, 2000). Most Mascot results were manually confirmed (even the above criteria were passed) by visual assessment of the MS/MS spectra for their overall quality. In addition, the criterion for manual validation was required a readily observable series of least 4 y ions was used. The Mascot showed pyruvate kinase 2 as the first hit with the scores of 370, and corresponded to 9 peptide matches, suggesting the reliability of this data.

Our results demonstrated pyruvate kinase 2 triggered allergic responses in 7 patient sera (100%). Despite the steaming process, it still triggered allergic responses in 4 patient sera (57%). Therefore, the allergen showed high sensitization to Taiwanese patients. It is important

PK2 1	MSKVAPMQLGAADTHTQVDHMAALDIDSKPFSKRLSGIICTIGPVSRSVEMLEKMMMEAGMNIARMNFSHGTHEYHSETMM	80
PK 1	MSKVAPMQLGAADTHTQVDHMAALDIDSKPFSKRLSGIICTIGPVSRSVEMLEKMMMEAGMNIARMNFSHGTHEYHSETMM	80
PK2 81	NVRKAAQKYSKIGHSYPVAIALDTKGPEIRTLGLLEGGPSAEIELKEGATIKLTTDASYEKCSEDVLYLDYVNTIKVKV	160
PK 81	NVRKAAQKYSKIGHSYPVAIALDTKGPEIRTLGLLEGGPSAEIELKEGATIKLTTDASYEKCSEDVLYLDYVNTIKVKV	160
PK2 161	PGNRIFVDDGLISLIAKDVGSDSIDCEVKGGMGLSKKGVNLPVDPVLPVSEKDRGDLGLGVKMGVDIVFVSFIRDA	240
PK 161	PGNRIFVDDGLISLIAKDVGSDSIDCEVKGGMGLSKKGVNLPVDPVLPVSEKDRGDLGLGVKMGVDIVFVSFIRDA	240
PK2 241	GVREIRDVLGEKGNIKIISKIENHGCKNIDDIIEEGDGIMIARGDLGIEIPAQKVFVAQKQMIACNKVGPVICATQ	320
PK 241	GVREIRDVLGEKGNIKIISKIENHGCKNIDDIIEEGDGIMIARGDLGIEIPAQKVFVAQKQMIACNKVGPVICATQ	320
PK2 321	MLESVKKPRPTRAESVDVGNAILDGDACVMSGETAKGGYPLVCVRTMANIAREAEAAIWHKQLFTLSQQVHLPTDST	400
PK 321	MLESVKKPRPTRAESVDVGNAILDGDACVMSGETAKGGYPLVCVRTMANIAREAEAAIWHKQLFTLSQQVHLPTDST	400
PK2 401	HTTAIAAEVASFAMATAIIVITTTGRSAHLVSKYRPRCPVAVTRYQVARQCHLYRGIPIHYT---AERIEDWMND	476
PK 401	HTTAIAAEVASFAMATAIIVITPTGRSAHLVSKYRPRCPVAVTRYQVARQCHLYRGIPIHYTVPQNAERIEDWMND	480
PK2 477	VNARVDYAVQYQKECGFIKPGDPVVVVTGWQKAGFTNTMRVLIVP-----	522
PK 481	VNARVDYAVQYQKECGFIKPGDPVVVVTGWQKAGFTNTMRVLVVPSTGPTASIVKLGAHSSVKSGPVKSGPQASGEHQ	560
PK2	-----	
PK 561	TGKAQPDIPWPSFLAHMGPVYHLCAAIVANKI	591

Fig. 3. Amino acid sequences of pyruvate kinase 2 and pyruvate kinase. PK2: Pyruvate kinase 2 (Accession No. ABY66597.1). PK: Pyruvate kinase (Accession No. ABO21408.1). The work was performed by Constraint-based Multiple Alignment Tool (COBALT) from NCBI website.

to explore properties and characteristics of pyruvate kinase 2 in future studies and can be included as a crustacean allergen. Also, this study clarified the responses of various allergens in raw and cooked crabs in Taiwanese patients. For example, myosin light chain was recognized as an important allergen in European (Ayuso et al., 2008), but showed minimal allergic response in our study. Similarly, pyruvate kinase 2 was not sensitized to allergic patients in previous studies, but was sensitized in Taiwanese allergic patients in this study. This study illustrates that the value of using specific allergens in Taiwanese population to properly diagnose crustacean allergy. Due to the small sample number in this study, the results of this study will need to be further validated in larger populations, even by human allergic test, such as skin prick test.

In addition, we clearly showed the sensitization patterns of Taiwanese patients to other important allergens, which were published in previous study (Ayuso et al., 2008; Bauermeister et al., 2011; Khanaruksombat et al., 2014; Rahman et al., 2013; Rahman et al., 2011; Shiomi et al., 2008; Yu et al., 2003). Based on these studies, we speculate the proteins with 18, 24, 27, 35, 38, 47, 75, 100 and 200 kDa in crucifix crab corresponded to myosin light chain, troponin C, triose phosphate isomerase, tropomyosin, glyceraldehyde 3-phosphate dehydrogenase/arginine kinase, enolase, hemocyanin, smooth endoplasmic reticulum Ca^{2+} ATPase and myosin heavy chain, respectively.

Tropomyosin is the most common crab allergen, so we measured the tropomyosin levels in various Taiwanese crab, including raw and cooked samples. Our results showed both increased and decreased levels of tropomyosin after cooking depending on species. We speculate that decreased levels may be the result of masking of epitope from heating, leading to low allergic potential as suggested by previous studies (Liu et al., 2010). In contrast, we believe increased levels may be due to secondary structure change of the tropomyosin resulting in epitope exposure (Albrecht et al., 2009), and subsequent polyclonal antibody binding used in our ELISA kit. Interestingly, tropomyosin levels were measured in only trace amount as 0.30 ± 0.00 mg/g in horse crab. Several studies reported the difference of amino acid sequence in crustacean among species (Kamath, Rahman, Komoda, & Lopata, 2013; Leung et al., 2014; Werner et al., 2007), which may lead to miss binding to tropomyosin. This could be the reason why tropomyosin levels were low in this crab. Overall, future study should elucidate the structural differences of tropomyosin in various crab species.

Crabs have high quality protein, nutritional value, and savory taste, making them popular with consumers worldwide. Nine crabs used in this study, including three-spot swimming, horse crab, red swimming crab, crucifix crab, ridged swimming crab, swimming crab, giant mud crab, sand crab and common box crab are consumed in Taiwan widely. The tropomyosin was still detected after steaming, which is the most common way to cook crab in Taiwan. Previous studies demonstrated that trace amount of crustacean tropomyosin in cooked crustacean and processed food caused human allergy (Kamath et al., 2014; Rahman et al., 2013; Vang, Maehre, Jensen, & Olsen, 2013), suggesting trace amount of tropomyosin in cooked crab may still cause allergic reaction. In this study, tropomyosin levels were also measured in the claw, abdomen, gills and internal organs in three-spot swimming crab. As tropomyosin is a muscle protein, it is reasonable to see tropomyosin levels in claw and abdomen were higher than that in internal organs and gills by more than 9 fold (Table S1).

5. Conclusions

In summary, this is the first study to identify pyruvate kinase 2, a 56 kDa protein, as a crab allergen by proteomic mass spectrometry. This allergen strongly sensitized to allergic patients in our study, and should be included as potential crustacean allergen. We also showed specific sensitization pattern unique to Taiwanese patients. Together, these findings may improve the understanding of crustacean allergy in Taiwan.

Acknowledgments

This research work was supported by grants from the Taiwan Ministry of Science and Technology (MOST105-2320-B-020-001-, MOST105-2314-B-010-054-MY3) and Chang Gung Memorial Research Foundation (CORPG8F0023 and CORPG8F0021). We thank Instrument Development Center of National Cheng Kung University for mass spectrometry analyses.

Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2019.03.074>.

References

- Albrecht, M., Kuhne, Y., Ballmer-Weber, B. K., Becker, W. M., Holzhauser, T., Lauer, I., ... Vieths, S. (2009). Relevance of IgE binding to short peptides for the allergenic activity of food allergens. *Journal of Allergy and Clinical Immunology*, 124(2), 328–336.
- Ayuso, R., Grishina, G., Bardina, L., Carrillo, T., Blanco, C., Ibanez, M. D., ... Beyer, K. (2008). Myosin light chain is a novel shrimp allergen, Lit v 3. *Journal of Allergy and Clinical Immunology*, 122(4), 795–802.
- Bauermeister, K., Wangorsch, A., Garoffo, L. P., Reuter, A., Conti, A., Taylor, S. L., ... Reese, G. (2011). Generation of a comprehensive panel of crustacean allergens from the North Sea Shrimp *Crangon crangon*. *Molecular Immunology*, 48(15–16), 1983–1992.
- Chan, C. F., Chen, P. H., Huang, C. F., & Wu, T. C. (2014). Emergency department visits for food allergy in Taiwan: A retrospective study. *Pediatrics and Neonatology*, 55(4), 275–281.
- Dashty, M. (2013). A quick look at biochemistry: Carbohydrate metabolism. *Clinical Biochemistry*, 46(15), 1339–1352.
- Daul, C. B., Slattery, M., Reese, G., & Lehrer, S. B. (1994). Identification of the major brown shrimp (*Penaeus aztecus*) allergen as the muscle protein tropomyosin. *International Archives of Allergy and Immunology*, 105(1), 49–55.
- Fuller, H. R., Goodwin, P. R., & Morris, G. E. (2006). An enzyme-linked immunosorbent assay (ELISA) for the major crustacean allergen, tropomyosin, in food. *Food and Agricultural Immunology*, 17(1), 43–52.
- Huang, Y. Y., Liu, G. M., Cai, Q. F., Weng, W. Y., Maleki, S. J., Su, W. J., & Cao, M. J. (2010). Stability of major allergen tropomyosin and other food proteins of mud crab (*Scylla serrata*) by *in vitro* gastrointestinal digestion. *Food and Chemical Toxicology*, 48(5), 1196–1201.
- Jeoung, B. J., Reese, G., Hauck, P., Oliver, J. B., Daul, C. B., & Lehrer, S. B. (1997). Quantification of the major brown shrimp allergen Pen a 1 (tropomyosin) by a monoclonal antibody-based sandwich ELISA. *Journal of Allergy and Clinical Immunology*, 100(2), 229–234.
- Kamath, S. D., Rahman, A. M. A., Komoda, T., & Lopata, A. L. (2013). Impact of heat processing on the detection of the major shellfish allergen tropomyosin in crustaceans and molluscs using specific monoclonal antibodies. *Food Chemistry*, 141(4), 4031–4039.
- Kamath, S. D., Thomassen, M. R., Saptarshi, S. R., Nguyen, H. M. X., Aasmoe, L., Bang, B. E., & Lopata, A. L. (2014). Molecular and immunological approaches in quantifying the air-borne food allergen tropomyosin in crab processing facilities. *International Journal of Hygiene and Environmental Health*, 217(7), 740–750.
- Khanaruksombat, S., Srisomsap, C., Chokchaichamnankit, D., Punyarnit, P., & Phiriyangkul, P. (2014). Identification of a novel allergen from muscle and various organs in banana shrimp (*Fenneropenaeus merguensis*). *Annals of Allergy Asthma & Immunology*, 113(3), 301–306.
- Lee, C. H., Wu, C. C., Tyan, Y. C., Yu, W. T., Huang, E. S., & Yu, H. S. (2018). Identification of pyruvate kinase as a novel allergen in whiteleg shrimp (*Litopenaeus vannamei*) by specific-IgE present in patients with shrimp allergy. *Food Chemistry*, 258, 359–365.
- Lemos, D., Salomon, M., Gomes, V., Phan, V., & Buchholz, E. (2003). Citrate synthase and pyruvate kinase activities during early life stages of the shrimp *Farfantepenaeus paulensis* (Crustacea, Decapoda, Penaeidae): Effects of development and temperature. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*, 135(4), 707–719.
- Leung, P. S. C., Chen, Y. C., Gershwin, M. E., Wong, H., Kwan, H. S., & Chu, K. H. (1998). Identification and molecular characterization of *Charybdis feriatus* tropomyosin, the major crab allergen. *Journal of Allergy and Clinical Immunology*, 102(5), 847–852.
- Leung, N. Y. H., Wai, C. Y. Y., Shu, S. A., Wang, J. J., Kenny, T. P., Chu, K. H., & Leung, P. S. C. (2014). Current immunological and molecular biological perspectives on sea-food allergy: A comprehensive review. *Clinical Reviews in Allergy & Immunology*, 46(3), 180–197.
- Li, Z. X., Lin, H., Cao, L. M., & Jamil, K. (2007). The influence of gamma irradiation on the allergenicity of shrimp (*Penaeus vannamei*). *Journal of Food Engineering*, 79(3), 945–949.
- Li, Z. X., Lin, H., Cao, L. M., & Jamil, K. (2006). Reduction of allergenic properties of shrimp

- (*Penaeus Vannamei*) allergens by high intensity ultrasound. *European Food Research and Technology*, 223(5), 639–644.
- Liu, G. M., Cheng, H., Nesbit, J. B., Su, W. J., Cao, M. J., & Maleki, S. J. (2010). Effects of boiling on the IgE-binding properties of tropomyosin of shrimp (*Litopenaeus vannamei*). *Journal of Food Science*, 75(1), T1–5.
- Long, F. Y., Yang, X., Wang, R. R., Hu, X. S., & Chen, F. (2015). Effects of combined high pressure and thermal treatments on the allergenic potential of shrimp (*Litopenaeus vannamei*) tropomyosin in a mouse model of allergy. *Innovative Food Science & Emerging Technologies*, 29, 119–124.
- Ma, T., Patel, H., Babapoor-Farrokhran, S., Franklin, R., Semenza, G. L., Sodhi, A., & Montaner, S. (2015). KSHV induces aerobic glycolysis and angiogenesis through HIF-1-dependent upregulation of pyruvate kinase 2 in Kaposi's sarcoma. *Angiogenesis*, 18(4), 477–488.
- Rahman, A. M. A., Kamath, S. D., Gagne, S., Lopata, A. L., & Helleur, R. (2013). Comprehensive proteomics approach in characterizing and quantifying allergenic proteins from northern shrimp: Toward better occupational asthma prevention. *Journal of Proteome Research*, 12(2), 647–656.
- Rahman, A. M. A., Kamath, S. D., Lopata, A. L., Robinson, J. J., & Helleur, R. J. (2011). Biomolecular characterization of allergenic proteins in snow crab (*Chionoecetes opilio*) and de novo sequencing of the second allergen arginine kinase using tandem mass spectrometry. *Journal of Proteomics*, 74(2), 231–241.
- Ramos-Martinez, J. I. (2017). The regulation of the pentose phosphate pathway: Remember Krebs. *Archives of Biochemistry and Biophysics*, 614, 50–52.
- Sahabudin, S., Misnan, R., Yadzir, Z. H., Mohamad, J., Abdullah, N., Bakhtiar, F., & Murad, S. (2011). Identification of major and minor allergens of black tiger prawn (*Penaeus monodon*) and king prawn (*Penaeus latisulcatus*). *The Malaysian Journal of Medical Sciences*, 18(3), 27–32.
- Salomon, M., Mayzaud, P., & Buchholz, F. (2000). Studies on metabolic properties in the Northern Krill, *Meganyctiphanes norvegica* (Crustacea, Euphausiacea): Influence of nutrition and season on pyruvate kinase. *Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology*, 127(4), 505–514.
- Schubert-Ullrich, P., Rudolf, J., Ansari, P., Galler, B., Fuhrer, M., Molinelli, A., & Baumgartner, S. (2009). Commercialized rapid immunoanalytical tests for determination of allergenic food proteins: An overview. *Analytical and Bioanalytical Chemistry*, 395(1), 69–81.
- Seiki, K., Oda, H., Yoshioka, H., Sakai, S., Urisu, A., Akiyama, H., & Ohno, Y. (2007). A reliable and sensitive immunoassay for the determination of crustacean protein in processed foods. *Journal of Agricultural and Food Chemistry*, 55(23), 9345–9350.
- Shanti, K. N., Martin, B. M., Nagpal, S., Metcalfe, D. D., & Rao, P. V. S. (1993). Identification of tropomyosin as the major shrimp allergen and characterization of its ige-binding epitopes. *Journal of Immunology*, 151(10), 5354–5363.
- 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*. *International Archives of Allergy and Immunology*, 146(2), 91–98.
- Usui, M., Harada, A., Ishimaru, T., Sakumichi, E., Saratani, F., Sato-Minami, C., ... Hanaoka, K. (2013). Contribution of structural reversibility to the heat stability of the tropomyosin shrimp allergen. *Bioscience Biotechnology and Biochemistry*, 77(5), 948–953.
- Vang, B., Maehre, H. K., Jensen, I. J., & Olsen, R. L. (2013). Detection of tropomyosin and determination of proteins in crustacean oils. *Food Chemistry*, 141(1), 72–76.
- Werner, M. T., Faeste, C. K., & Egaas, E. (2007). Quantitative sandwich ELISA for the determination of tropomyosin from crustaceans in foods. *Journal of Agricultural and Food Chemistry*, 55(20), 8025–8032.
- Wu, T. C., Tsai, T. C., Huang, C. F., Chang, F. Y., Lin, C. C., Huang, I. F., ... Tang, R. B. (2012). Prevalence of food allergy in Taiwan: A questionnaire-based survey. *Internal Medicine Journal*, 42(12), 1310–1315.
- Yu, H. L., Cao, M. J., Cai, Q. F., Weng, W. Y., Su, W. J., & Liu, G. M. (2011). Effects of different processing methods on digestibility of *Scylla paramamosain* allergen (tropomyosin). *Food and Chemical Toxicology*, 49(4), 791–798.
- Yu, C. J., Lin, Y. F., Chiang, B. L., & Chow, L. P. (2003). Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. *Journal of Immunology*, 170(1), 445–453.
- Yu, H. S., Tu, H. P., Hong, C. H., & Lee, C. H. (2017). Lifetime increased risk of adult onset atopic dermatitis in adolescent and adult patients with food allergy. *International Journal of Molecular Sciences*, 18(1), 42.
- Zhang, H., Lu, Y., Ushio, H., & Shiomi, K. (2014). Development of sandwich ELISA for detection and quantification of invertebrate major allergen tropomyosin by a monoclonal antibody. *Food Chemistry*, 150, 151–157.

Supplementary materials

Table S1 Levels of tropomyosin in different parts of three-spot swimming crab

Name	Part	Tropomyosin content (mg/g) ^a
Three-spot swimming crab	Claw meat	33.11±1.09
	Abdomen meat	18.17±1.46
	Gills	N.D
	Internal organs	2.40±0.22

^a: Results are the mean ± SD of two independent experiments.

N.D: Not detected.