Vertebrate Tropomyosin as an Allergen

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Food allergy has emerged as an unexpected 'second wave' of the allergy epidemic, dramatically increasing the burden of allergic disease among children and adolescents. Tropomyosin is the major shellfish allergen and one of the allergens responsible for cross-reactivity between house dust mites and shellfish [1,2]. Although vertebrate tropomyosin has traditionally been considered a nonallergenic protein [3,4], recent studies reveal the possibility that several vertebrate tropomyosins may in fact be allergenic [3,5-7]. Parvalbumin is the main allergen in fish allergy, although new fish allergens have recently been identified, and others are waiting to be discovered and described [8].

In the present case report, we describe how clinical manifestations related to fish ingestion were due to cross-reactivity between shellfish and fish tropomyosins. Our findings reinforce those of more recent studies that conclude that vertebrate tropomyosin may also be considered an allergen.

The patient was an 11-year-old white boy with no family or personal history of atopy who began to experience respiratory difficulty with progressive cyanosis during dinner. He was assisted locally by an emergency team and transferred to the hospital. On arrival, he was confused, with cyanosis and hypoxemia, and was treated with high-concentration oxygen. No cutaneous manifestations of allergy (urticaria or angioedema) were observed. Progressive clinical worsening in the emergency room necessitated resuscitation maneuvers, intubation, and mechanical invasive ventilation. On arrival, the family reported that the child had ingested a shrimp patty for the first time. On suspicion of anaphylaxis, adrenaline was administered with progressive improvement in the patient's clinical condition.

Asthma, rhinitis and atopic eczema were subsequently excluded in the pediatric outpatient clinic. The family emphasized that it was the first time, as far as they knew, the child had eaten shrimp. They also said that he had experienced itching and mild swelling of the mouth and throat immediately after ingestion of some types of fish, mainly codfish and hake. He had eaten octopus without symptoms. The skin prick test was not performed owing to the severity of the clinical presentation and risk of a severe reaction. Serum specific IgE levels measured using ImmunoCAP (Thermo Fisher) to shrimp extract were >100 kU_A/L and positive (>0.35 kU_A/L) to other arthropods (lobster >100, crab >100), mollusk (Pacific flying squid, 55.1; squid [*Loligo* species], 3.57; octopus, 37.7; blue mussel, 23), fish (tuna, 2.77; codfish, 2.50; salmon, 2.33; sole,

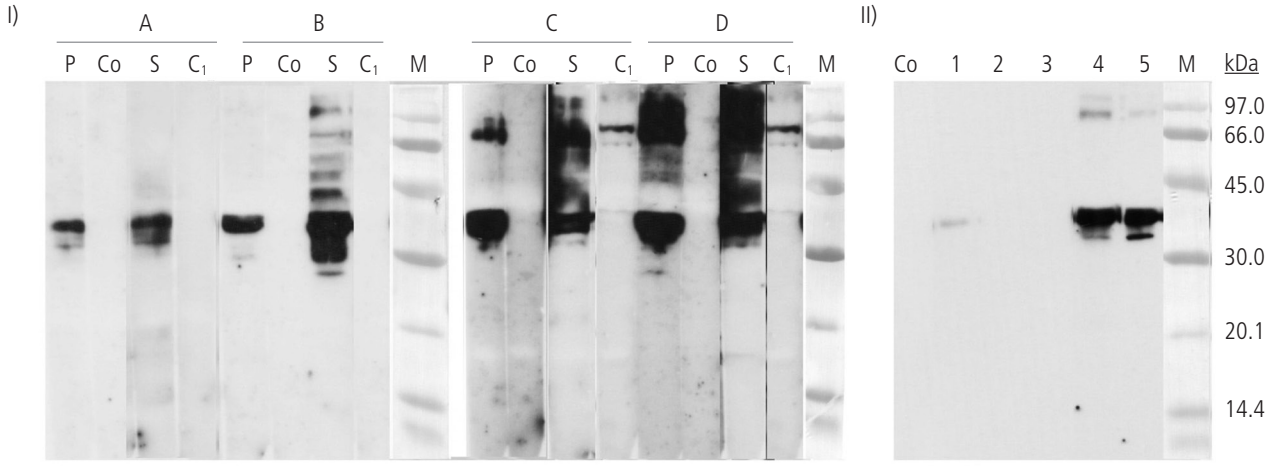

Figure. I) SDS-PAGE IgE-immunoblotting. A, Shrimp (raw) extract; B, Indian prawn (raw) extract; C, Hake (raw) extract; D, Codfish (raw) extract. Lane P, patient serum; Lane Co, control serum (pool of sera from nonatopic subjects). SDS-PAGE IgG-immunoblotting. Lane S, rabbit serum against tropomyosin from the genus *Penaeus*; Lane C_1, rabbit serum before immunization; Lane M, molecular mass standard. II) SDS-PAGE IgE immunoblotting-inhibition - Solid phase: Codfish (raw) extract. Lane Co, control serum (pool of sera from nonatopic patients); Lanes 1-5, Patient serum previously incubated with codfish (raw) extract (lane 1), shrimp (raw) extract (lane 2), shrimp (cooked) extract (lane 3), ovalbumin (lane 4), and lamb extract (lane 5); lane M, molecular mass standard.

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1.09; hake, 0.97; horse mackerel, 0.93; mackerel, 0.04; sardine, 0.02), and pollens (mixed grasses, 4.73; *Olea europaea*, 4.93). A molecular allergy study with in vitro diagnostic tests was performed for simultaneous measurement of specific IgE to 112 allergen components (ImmunoCAP ISAC, Thermo Fisher). The study revealed sensitization to tropomyosin (Pen m 1, 46 ISU-E; Der p 10, 39 ISU-E; Ani s 3, 43 ISU-E; Bla g 7, 27 ISU-E), shrimp arginine kinase (Pen m 2, 3.8 ISU-E), and polcalcins (Bet v 4, 1.6 ISU-E; Phl p 7, 6.2 ISU-E). Sensitization to parvalbumin was not detected.

To study allergy to shrimp and possible cross-reactivity with fish, protein extracts from hake (*Merluccius*), codfish (*Gadus*), shrimp (*Palaemon serratus*), and Indian prawn (*Penaeus indicus*) were prepared by homogenization in phosphate-buffered saline before dialysis and lyophilization.

SDS-PAGE immunoblotting was carried out under reducing conditions with 2-mercaptoethanol as previously described (9). The patient's serum was diluted 1:300 in crustacean extracts and 1:8 in fish extract; mouse monoclonal antibody antihuman IgE (Southern Biotech) was diluted 1:10 000, and chemiluminescent reagent was used for detection (Amersham ECL Prime Western Blotting Detection Reagent, GE Healthcare UK Limited). IgE-binding bands of 36 kDa were detected in extracts from hake, codfish, shrimp, and Indian prawn with the patient's serum, and IgG-binding bands of the same molecular mass were detected in all of these extracts with rabbit serum against tropomyosin from *Penaeus* species. The rabbit serum was obtained in our laboratory from a rabbit immunized with a sample of *Penaeus* species tropomyosin, which was obtained from an SDS-PAGE gel where a *Penaeus* extract was electrophoresed. The tropomyosin band was identified using a commercial rabbit antiserum antitropomyosin from chicken muscle (Sigma Co).

The IgE-immunoblotting-inhibition assay with codfish extract in solid phase and shrimp extract as inhibitor showed complete inhibition of IgE binding on the 36-kDa band from codfish (Figure).

These results and the serum specific IgE levels against Pen m 1 led us to believe that the 36-kDa band from codfish and hake were the tropomyosins from both types of fish.

To confirm this hypothesis, we performed liquid chromatography–mass spectrometry (MS) analysis of the 36-kDa IgE-binding band from codfish, as reported by Rosa et al [10]. MS-MS spectra were searched against the SwissProt 20160108 Database, restricting taxonomy to Actinopterygii (5210 sequences). MS revealed 36 peptides of the tropomyosin α-1 chain of *Liza aurata*, also known as golden grey mullet, and *Chelon aurata*, a fish of the Mugilidae family, with sequence coverage of 80.30% on the codfish 36-kDa IgE-binding band (sequences of the 36 peptides are presented in Supplementary Figure 1).

To date, vertebrate tropomyosins have been reported to be nonallergenic proteins [3,4], although recent studies are leading us to challenge this belief [3,5,7], because of evidence that some fish allergies could be explained by tropomyosin-specific IgE [6,7]. Tilapia tropomyosin is now included on the fish allergen list [6]. The first vertebrate tropomyosin characterized and identified as allergenic in the WHO/IUIS Allergen Nomenclature Database (www.allergen.org) was Ore m 4, from Mozambique tilapia (*Oreochromis mossambicus*). It therefore seems that cross-reactivity between fish and crustacean tropomyosins would be the cause of fish allergy in seafood-allergic patients.

Invertebrates are phylogenetically distant from vertebrates. However, homology in tropomyosin sequences from some species of fish (which are primitive vertebrates) and some crustaceans could be sufficiently high to produce cross-reactivity events that trigger allergy symptoms after ingestion of fish by shrimp-allergic patients [3].

Gonzalez-Fernandez et al [3] report that the allergenicity of fish tropomyosins could be due to the evolutionary fact that fish can live in cold water and their tropomyosins have strategic amino acid substitutions that resolve the muscle rigidity induced by cold [3]. This flexibility, as previously hypothesized in the case of tropomyosins from invertebrates, may confer epitopes that are not shown in tropomyosins from homeotherms.

In our case report, a shrimp tropomyosin–sensitized patient with seafood allergy showed positive serum levels to tropomyosin from other arthropods and nematodes. We believe that he had fish allergy caused by IgE cross-reactivity between crustacean and fish tropomyosins

Despite the clinical history (fish allergy symptoms apparently occurred before shrimp anaphylaxis), which was based on the clinical symptoms (more severe reaction with shrimp ingestion than with fish ingestion) and in vitro results (higher specific IgE levels to shrimp extract than to fish extracts), we believe that the primary sensitizing agent was shrimp tropomyosin and that the reaction with fish tropomyosin was a cross-reactivity phenomenon. An indication for avoidance of shellfish and fish was given to the family, and 2 adrenaline autoinjectors were prescribed. Community education (focusing on risks and dangers of food allergy) was discussed, and patient/family quality of life was assessed.

To our knowledge, this is a complex case of allergy involving fish and shrimp tropomyosins. Much remains to be clarified, although insightful research often leads to new discoveries.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Ayuso R, Reese G, Leong-Kee S, Plante M, Lehrer SB. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. Int Arch Allergy Immunol. 2002;129(1):38-48.
2. Iparraguirre A, Rodriguez-Perez R, Juste S, Ledesma A, Moneo I, Caballero ML. Selective allergy to lobster in a case of primary sensitization to house dust mites. J Investig Allergol Clin Immunol. 2009;19(5):409-13.

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3. Gonzalez-Fernandez J, Daschner A, Cuellar C. Allergenicity of vertebrate tropomyosins: Challenging an immunological dogma. Allergol Immunopathol (Madr). 2017;45(3):297-304.
4. Jenkins JA, Breiteneder H, Mills EN. Evolutionary distance from human homologs reflects allergenicity of animal food proteins. J Allergy Clin Immunol. 2007;120(6):1399-405.
5. Gonzalez-Fernandez J, Veleiro B, Daschner A, Cuellar C. Are fish tropomyosins allergens? Ann Allergy Asthma Immunol. 2016;116(1):74-6 e5.
6. Liu R, Holck AL, Yang E, Liu C, Xue W. Tropomyosin from tilapia (Oreochromis mossambicus) as an allergen. Clin Exp Allergy. 2013;43(3):365-77.
7. Tomm JM, van Do T, Jende C, Simon JC, Treudler R, von Bergen M, et al. Identification of new potential allergens from Nile perch (Lates niloticus) and cod (Gadus morhua). J Investig Allergol Clin Immunol. 2013;23(3):159-67.
8. Kuehn A, Swoboda I, Arumugam K, Hilger C, Hentges F. Fish allergens at a glance: variable allergenicity of parvalbumins, the major fish allergens. Front Immunol. 2014;5:179.
9. Bartolome B, Mendez JD, Armentia A, Vallverdu A, Palacios R. Allergens from Brazil nut: immunochemical characterization. Allergol Immunopathol (Madr). 1997;25(3):135-44.
10. Rosa M, Bech-Serra JJ, Canals F, Zajac JM, Talmont F, Arsequell G, et al. Optimized Proteomic Mass Spectrometry Characterization of Recombinant Human mu-Opioid Receptor Functionally Expressed in Pichia pastoris Cell Lines. J Proteome Res. 2015;14(8):3162-73.

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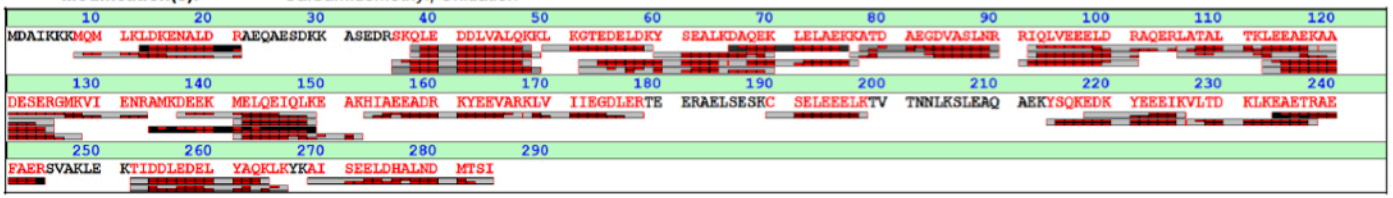
Protein 1:	Tropomyosin alpha-1 chain OS=Liza aurata PE=1 SV=1		
Accession:	TPM1_LIZAU	**Score:**	2115.12
Database:	SwissProt	**MW [kDa]:**	32.70
Seq. Coverage [%]:	80.30 %	**pI:**	4.54
		No. of Peptides:	36
Modification(s):	Carbamidomethyl, Oxidation		



Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
2160	1	579.6210	-10.60	3	14.37	42.47	2	8-21	K.MQMLKLDKENALDR.A	Oxidation: 1, 3
783	3	537.2781	-8.90	2	9.78	57.07	1	13-21	K.LDKENALDR.A	
3030	4	743.9015	-10.38	2	17.28	73.01	1	36-48	R.SKQLEDDLVALQK.K	
3559	1	807.9500	-8.35	2	19.07	70.44	2	36-49	R.SKQLEDDLVALQKK.L	
4429	9	636.3388	-10.95	2	22.17	65.67	0	38-48	K.QLEDDLVALQK.K	
3874	2	700.3870	-8.82	2	20.17	45.42	1	38-49	K.QLEDDLVALQKK.L	
828	1	574.2899	-10.05	2	9.93	35.36	1	50-59	K.LKGTEDELDK.Y	
3906	1	799.3749	-10.36	2	20.27	38.13	1	52-65	K.GTEDELDKYSEALK.D	
4269	1	723.6727	-7.33	3	21.55	32.00	2	52-70	K.GTEDELDKYSEALKDAQEK.L	
1003	1	641.3127	-10.95	2	10.52	44.57	1	60-70	K.YSEALKDAQEK.L	
2079	1	637.3279	-11.72	2	14.10	41.61	1	66-76	K.DAQEKLELAEK.K	
1630	1	701.3786	-6.03	2	12.60	45.40	2	66-77	K.DAQEKLELAEKK.A	
1846	3	723.8557	-9.87	2	13.33	40.83	1	77-90	K.KATDAEGDVASLNR.R	
2434	6	659.8095	-8.79	2	15.28	27.96	0	78-90	K.ATDAEGDVASLNR.R	
3669	1	700.3723	-11.95	2	19.43	22.56	1	91-101	R.RIQLVEEELDR.A	
4052	1	622.3240	-9.68	2	20.78	53.39	0	92-101	R.IQLVEEELDR.A	
3384	2	576.6299	-9.92	3	18.47	38.42	1	92-105	R.IQLVEEELDRAQER.L	
3723	2	708.8940	-9.70	2	19.62	97.22	1	106-118	R.LATALTKLEEAEK.A	
4442	1	725.7023	-9.96	3	22.22	37.13	2	106-125	R.LATALTKLEEAEKAADESER.G	
820	3	738.8373	-9.38	2	9.90	69.92	1	113-125	K.LEEAEKAADESER.G	
800	2	904.9130	-7.91	2	9.83	58.75	2	113-128	K.LEEAEKAADESERGMK.V	Oxidation: 15
863	1	574.2741	-12.00	3	10.05	28.69	2	119-133	K.AADESERGMKVIENR.A	Oxidation: 9
3106	3	665.6575	-9.65	3	17.53	40.84	2	134-149	R.AMKDEEKMELQEIQLK.E	Oxidation: 2, 8
3503	1	550.2684	-11.67	3	18.85	24.14	1	137-149	K.DEEKMELQEIQLK.E	Oxidation: 5
3371	2	574.2983	-11.71	2	18.42	59.34	0	141-149	K.MELQEIQLK.E	Oxidation: 1
2814	1	738.3860	-8.60	2	16.55	25.07	1	141-152	K.MELQEIQLKEAK.H	Oxidation: 1
1780	1	908.4419	-12.05	2	13.12	38.18	2	153-167	K.HIAEEADRKYEEVAR.K	
4089	1	642.8738	-9.25	2	20.92	22.64	1	168-178	R.KLVIIEGDLER.T	
2855	1	568.7540	-11.71	2	16.68	42.96	0	190-198	K.CSELEEELK.T	Carbamidomethyl: 1
1517	1	844.8973	-8.34	2	12.23	60.44	2	214-226	K.YSQKEDKYEEEIK.V	
1426	1	591.7726	-12.23	2	11.92	23.84	1	218-226	K.EDKYEEEIK.V	
1987	1	701.8931	-7.86	2	13.80	55.41	2	227-238	K.VLTDKLKEAETR.A	
1260	1	654.8065	-9.24	2	11.37	37.85	1	234-244	K.EAETRAEFAER.S	
3664	9	776.8660	-8.40	2	19.43	89.27	0	252-264	K.TIDDLEDELYAQK.L	
4800	1	897.4554	-7.32	2	23.90	33.42	1	252-266	K.TIDDLEDELYAQKLK.Y	
4874	4	887.9039	-8.89	2	24.28	53.61	0	269-284	K.AISEELDHALNDMTSI.-	Oxidation: 13