Myosin heavy chain, a novel allergen for fish allergy in patients with atopic dermatitis.

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Introduction

Fish allergy (FA) is the second most frequent type of food allergy in Japan. Fish allergens listed in the database of the WHO include parvalbumin, which is known to be the major allergen, β -enolase, aldolase A, vitellogenin and tropomyosin. Patients with atopic dermatitis (AD) have an increased risk for sensitization to allergens due to their increased skin barrier permeability. Here, we enrolled six patients with FA, who had also been diagnosed with AD according to the diagnostic criteria of the Japanese guidelines.

Method

Fish protein extraction from salmon
↓
1D(A) and 2D(B) SDS-PAGE and Western blotting(WB)
↓
Silver staining and In gel digestion
↓
Mass spectrometric analysis (Nano LC-MS/MS)
↓
Data analysis → **detection of Myosin heavy chain(MYHC)**
↓
Preparation of recombinant salmon MYHC (rsMYHC)
↓
WB(C), Inhibition assay(D), Basophil activation assay(E) with rsMYHC

Clinical characteristics and serologic data.

	#	Age (y)	Sex	AD	FA symptom	Total IgE (IU/mL)	CAP for parvalbumin						WB
							CAP		rCyp c 1		rGad c 1		IgE-reactivity with rMYHC
							others*	fish**	class	(U _g /mL)	class	(U _g /mL)	
Patient	1***	21	F	Y	UR, CUR	70.5	cedar pollen	Y	1	0.759	1	0.457	Y
	2	7	M	Y	UR	2180.0	egg white, wheat	Y	4	28.2	4	26.2	Y
	3	14	M	Y	UR, EIA	4792.0	wheat, shrimp	Y	0	0.223	0	0.243	Y
	4	0	F	Y	UR, AN	45.8	N	Y	3	4.86	3	3.55	N
	5	7	M	Y	UR	267.5	Dermatophagoides	Y	3	12.1	3	7.24	Y
	6	5	F	Y	UR	538.5	egg white, milk	N	0	<0.100	0	<0.100	Y
Control §	1	61	M	N	None	91.8	N	NT	NT	NT	NT	NT	N
	2	32	M	Y		14331.0	Dermatophagoides	NT	NT	NT	NT	NT	N
	3	40	M	N		NT	NT	NT	NT	NT	NT	N	
	4	41	M	N		NT	NT	NT	NT	NT	NT	N	
	5	32	M	N		NT	NT	NT	NT	NT	NT	N	
	6	40	M	N		NT	NT	NT	NT	NT	NT	N	

AD, atopic dermatitis; UR, urticaria; CUR, contact urticaria; EIA, exercise-induced anaphylaxis; AN, anaphylaxis; FA, fish allergy

Y, yes; N, no; NT, not tested; WB, Western blot; rMYHC, recombinant myosin heavy chain

*, Representative, high class allergens are shown.

**, CAP assay for tuna, salmon, mackerel and sardine. Y indicates positive for at least one fish.

***, Patient #1 showed positive SPT results for salmon (+++), horse mackerel (+++), flounder (+++), cod (++), sardine (++), pike (++), tuna (+++) and seabass altus (+++).

§, Control individuals, including #2, have no history of allergy.

(A) Western blot using sera from FA patients and controls.

Heat-untreated protein extracts from salmon were used for immunoblotting with sera from 6 patients and 2 controls, then were developed with anti-human IgE. Arrowheads, approximately 230-kD proteins; #, non-specific bands presumably derived from the second antibody, even in the absence of serum (data not shown).

(B) Heat-untreated protein extracts separated by 2D-gel electrophoresis followed by IgE immunoblotting using sera from patient #1 and control#1.

Five fractions of approximately 230-kD proteins with different pI/s (red arrows) were isolated, then analyzed by nano LC-MS/MS and all were identified as MYHC. #, aforementioned non-specific bands.

(C) Western blot using rsMYHC.

CBB, Coomassie Brilliant Blue R-250; arrowheads, bands corresponding to rsMYHC.

(E) Basophil activation test using rsMYHC

Peripheral blood from patient #6 was used. Positive control, anti-IgE; negative control, PBS. rsMYHC, 10 μ g/mL. Numbers indicate the percentage of CRTH2+CD203c+ cells (arrows).

(D) Validation of IgE immunoreactivity to MYHC with an inhibition assay.

Sera from patients #1 and #3 were preincubated with or without the salmon protein extract, then Western blotted on rsMYHC. The specificity of MYHC was confirmed by restoration of the IgE reactivity when salmon protein extracts were used after absorption of MYHC with an anti-MYHC IgG antibody. Arrowheads, bands corresponding to rsMYHC; Asterisks, non-specific bands presumably derived from anti-MYHC IgG.

Conclusion

MYHC should be on the list of FA allergens to allow us to make a correct diagnosis of FA, particularly in cases not determined by conventional screenings with parvalbumin as the representative allergen.

Raw fish cuisine has become very popular not only in Japan but also outside of Japan. Touching raw fish might increase the risk of sensitization to MYHC in patients with AD.