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Anaphylaxis by exclusive allergy to swordfish and identification of a new fish allergen

To the Editor:

Fish allergy affects up to 7% of the worldwide population, with variations between countries mostly stemming from consumption rates and methods of allergy diagnosis. In Spain, where fish consumption is high, fish allergy affects 11% of food-allergic children under 14 years of age, who consult for the first time for food allergy.[1] Clinical presentation most frequently involves cutaneous or digestive symptoms, although anaphylaxis has been described in up to 25% of Spanish fish-allergic children.[1] The fish that most frequently produce allergic reactions are those with the highest parvalbumin content, such as megrim fish (*Lepidorhombus boscii*), hake (*Merluccius angustimanus*), and cod *(Gadus morhua*),[2] while fish-allergic children usually tolerate swordfish (SF) (*Xiphias gladius)* and tuna (*Thunnus albacares*) well.

The allergen most commonly responsible for allergic reactions to fish is parvalbumin. The concentration of parvalbumin in fish has been demonstrated to vary from one part of the fish to another and between species. Swordfish has less than 1 mg of parvalbumin per gram of fresh fillet; this concentration is the same as in tuna.[3] Other fish species, meanwhile, contain more than 2.5 mg of parvalbumin per gram, as is the case with cod (*Gadus morhua*) and carp (*Cyprinus carpio*). Swordfish is a member of the Xiphiidae fish family, a large-sized migratory fish with a characteristic sharp-edged bill. They have a lower content of white muscle and therefore contain low amounts of parvalbumin in comparison with sedentary fish.[4] This special characteristic makes swordfish one of the most well-tolerated fish among fish-allergic patients and thus have been termed "low allergenicity" fish by Kobayashi et al.[2]

Allergens other than parvalbumin have previously been identified (enolase, aldolase, and triosephosphate isomerase), though not in swordfish. Here, we describe a selective allergy to swordfish and identify its allergens, including a new fish allergen.

An 8-year-old boy developed anaphylaxis at 6 years of age shortly after playing soccer, presenting urticaria, conjunctivitis, and mild bronchospasm. He had eaten a swordfish fillet 90 minutes before the reaction. The patient required emergency management consisting of inhaled short-acting beta$_2$-agonists and oral corticosteroids. Prior to this episode, he had tolerated all fish types, including swordfish. Since then, he avoided swordfish and other fish, but tolerated panga *(Pangasius hypophthalmus*). He had been under surveillance in our outpatient clinic from age 3 years for mild intermittent asthma, though had not presented with previous food allergy.

Skin prick testing (SPT) at age 6 years using commercially available extracts of swordfish, tuna, cod, megrim, sole, anchovy, hake, and *Anisakis s*. (Leti, Madrid, Spain [1 mg/mL]) was performed on the volar surface of his arm, with negative results. Prick by prick testing with raw swordfish was 3 mm greater than SPT with 0.9% saline. Levels of specific serum IgE determinations (ImmunoCAP, Thermo Fisher, Uppsala, Sweden) were 2.3 kU/L for tuna and <0.35 kU/L for swordfish, megrim, sole, hake, salmon, sardine, trout, rGad c 1, rCyp c 1, and *Anisakis s*. With prior written informed consent signed by the parents, a controlled oral food challenge (OFC) was performed with a fillet of 250 g of cooked swordfish in increasing doses beginning with 20 g and doubling the dose every 30 minutes. Symptoms of rhinitis, oral pharyngeal pruritus, dysphonia, dry cough, and wheezing were triggered at a cumulative dose of 140 g. Intramuscular

adrenaline was administered for symptom resolution. The patient passed tuna and hake OFC. He was advised to eat all kinds of fish except swordfish.

When the patient underwent a follow-up examination at the age of 8, he reported regular intake of tuna, hake, sardine, anchovy, cod, and whiting. SPT, prick by prick, and serum specific IgE to swordfish were negative. Swordfish OFC was performed, and the patient had a similar anaphylactic reaction, with the same cumulative dose administered in the previous OFC (140 g).

Further in vitro tests were performed to investigate potential allergens responsible for the severe allergic reaction. Swordfish extract was prepared from raw swordfish. After centrifugation, the supernatant was delipidated with diethyl ether and dialyzed against 0.1 M ammonium bicarbonate, as previously described.[5,6] Protein extract was lyophilized and stored at 4°C. Proteins from swordfish extract were separated by SDS-PAGE under reducing conditions, and specific IgE-binding bands were evaluated by immunoblotting with the patient serum. Serum samples from individuals not sensitized to fish were used as negative controls and did not detect any bands. Incubation with patient serum showed 6 main IgE-binding bands with an apparent molecular weight of approximately 57, 50, 40, 38, 33, and 28 kDa (Figure 1). Only 4 IgE bands were identified by mass spectrometry, as described in the "Supporting Information" file (electronic repository). This process led to the identification of the 57-kDa band as a pyruvate kinase (SF1), the 50-kDa band as an enolase (SF2), the 40-kDa band as an aldolase (SF3), and the 28 kDa band as a triosephosphate isomerase (SF4; Figure 1).

Allergy to swordfish is very rare, especially in the absence of allergy to other fish. There is only one reported case of swordfish allergy in an adult with good tolerance to other fish.[7] Ours is the first case of isolated swordfish allergy in children presenting as anaphylaxis. It should be noted that the patient tolerated swordfish for years before the allergic reaction, which is exceptional in fish allergy.

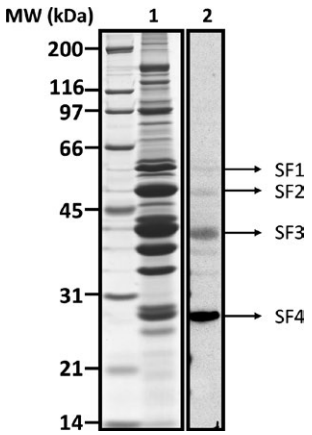

FIGURE 1 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot of swordfish extract. Coomassie blue-stained SDS-PAGE (Lane 1). Western blot (WB) assay of swordfish extract using patient's serum (Lane 2). Serum from a nonatopic patient used as negative control (not shown). Molecular weight markers are indicated as kiloDaltons (kDa). SF1, pyruvate kinase; SF2, enolase; SF3, aldolase; SF4, triosephosphate isomerase

In addition to parvalbumin, other important fish allergens have been identified and characterized in fish: fish gelatin,[8] a novel allergen identified in Japanese-fish-allergic population; enolases and aldolases in salmon, cod, and albacore tuna[9] and another enolase in long-tail tuna;[4] triosephosphate isomerase in common sole;[5,6] and creatine kinase in long-tail tuna.[4] Enolases and aldolases are native oligomers which are labile to thermal treatment. Triosephosphate isomerase plays an important role in glycolysis and is essential for efficient energy production.[5,6]

This is the first time that a pyruvate kinase has been identified as a fish allergen. Pyruvate kinase is a cytosolic protein previously identified as allergen in prawn.[10]

It is worrisome that the allergen/s responsible for the symptoms in this patient induced a severe systemic reaction, although it is also vexing that regular tests to assess sensitization (SPT, serum specific IgE, prick–prick) were unable to properly identify the swordfish allergy. Only prick by prick was slightly positive in the first—and negative in the second—followed by a positive OFC. OFC was the only reliable procedure for the diagnosis of this patient. On the other hand, the fish allergy in this child has some unusual characteristics: the selective and severe allergy to swordfish (a fish that is usually mildly allergenic), late onset after several years of good tolerance and also persisting over time with the same severity. Unlike other publications, this case shows that fish allergy is not always due to sensitization to parvalbumin. To our knowledge, this is the first description of allergy to swordfish in children. Four proteins were identified as the allergens involved: pyruvate kinase, enolase, aldolase, and triosephosphate isomerase. It is the first time that the allergens of this fish have been identified, and none is parvalbumin. Either one or all the identified allergens can trigger anaphylactic reactions but are not detected by usual allergic tests. To our knowledge, this is the first time that pyruvate kinase has been identified as a fish allergen.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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EMSY is increased and activates TSLP & CCL5 expression in eosinophilic esophagitis

To the Editor:

Eosinophilic esophagitis (EoE) is a clinicopathologic disease characterized by an increased number of eosinophils (greater than or equal to 15 eosinophils per high powered field) in the esophageal mucosa, likely due to an immune or antigenic response.[1] Specifically, there is an increased production of esophageal epithelial-derived pro-inflammatory mediators such as thymic stromal lymphopoietin (TSLP), eotaxin 3 (CCL26), RANTES (CCL5), calpain 14 (CAPN14), and interleukin (IL)-33,[2] which ultimately results in eosinophilic infiltration of the esophageal epithelium. Thymic stromal lymphopoietin is mainly secreted by epithelial cells and is a IL-7 like cytokine that is able to induce a strong T helper type 2 (Th2) inflammatory response.[3] Thymic stromal lymphopoietin has been shown to play a crucial role in EoE pathogenesis.[3] Similarly, IL-33 is an epithelial-derived Th2 cell agonist and allergenic ligand that has been associated with EoE.[4] Chemokine (C-C motif) ligand 5 (CCL5) is a cytokine that is known to contribute to several atopic and inflammatory eosinophilic diseases, including EoE.

Genomewide association studies (GWAS) data from our group show a strong association between EoE and a novel gene locus c11orf30-EMSY, whose role is unknown in esophageal cells.[5] Initially, EMSY was described as a transcriptional factor able to interact with and inhibit the function of BRCA2, a known tumor suppressor gene in mammary cancer.[6] In mammary and ovarian cancer cells, Akt1 phosphorylates EMSY at Ser209, which releases EMSY-mediated transcriptional repression of interferon stimulating genes.[7] More recently, EMSY has been shown to regulate post-translational modifications of core histones to induce gene expression[8] of its target genes and stimulate cell proliferation. Furthermore, Amaral et al proposed that the locus c11orf30 is associated with atopy.[9] In addition to mammary epithelial cells, EMSY expression has also been demonstrated in multiple types of epithelium including esophageal, ovarian, kidney, and bronchial epithelium as well as cells of hematopoietic lineages.[6,8,10] As the esophageal epithelium plays a central role in the pathogenesis of eosinophilic esophagitis, we set out to determine the tissue-specific role of EMSY in esophageal epithelium and its potential role in regulating the expression of key epithelial cytokines and chemokines.

The aim of this study was to investigate the role of EMSY in esophageal epithelial cell biology and in EoE and to determine if EMSY is involved in gene regulation in the esophageal epithelium. To investigate the function of EMSY in normal esophageal epithelium,