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Cross-reactivity to fish and chicken meat – a new clinical syndrome

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Abstract

Background: Fish is one of the most allergenic foods. While clinical cross-reactivity among different fishes is a widely accepted feature of fish allergy, associations with other food allergies are not well understood. This study aims at analyzing the relevance of clinical cross-reactivity between fish and chicken meat in patients with allergy to chicken meat without sensitization to hen's eggs.

Methods: Patients with food allergy to fish and chicken meat ($n = 29$) or chicken meat only ($n = 7$) were recruited. IgE-reactive chicken proteins were identified (Edman, MS analysis) and quantified (ELISA). Allergens were used in IgE ELISA and skin testing.

Results: Chicken parvalbumin and two new allergens, aldolase and enolase, were identified at 12, 40, and 50 kDa, respectively. They were recognized by sIgE of 61%, 75%, and 83% of all patient sera which were in the majority of the cases positive for the fish homologues as well. Fish and chicken meat allergens were highly cross-reactive while high inhibition rates with fish or chicken allergens correlated with the patients' primary sensitization to fish or chicken. In cooked or roasted foods, enolase and aldolase were detectable in chicken breast while parvalbumin was detectable in chicken legs and wings.

Conclusions: Fish and chicken meat are cross-reactive foods; both fish-allergic and chicken meat-allergic patients might be at risk of developing a food allergy to chicken meat or to fish, respectively. This clinical phenomenon is proposed to be termed 'fish–chicken syndrome' with cross-reactive allergens involved being parvalbumins, enolases, and aldolases.

Both fish and chicken meat are common components of human nutrition, and as such, they are widely consumed. However, food from fish and poultry origin can also provoke allergic reactions in sensitized patients (1–4).

Fish is considered as one of the eight most important elicitors of food-allergic reactions (1, 2). The prevalence is estimated to range below 1% of the general population (5, 6). Generally, patients present with allergic symptoms upon

ingestion of fish manifesting with symptoms such as urticaria, angioedema, rhinitis, asthma, and digestive and cardiovascular symptoms (7). Clinical cross-reactivity among various fish species has been reported as a mainstay of this food allergy (8, 9). While most patients react to a wide variety of fish species, further clinical phenotypes with a more restricted reactivity profile have been identified. These individuals react to single or specific fish species only (10, 11). Parvalbumins, the major fish allergens, are important triggers of fish-adverse reactions (2, 12). The high IgE cross-reactivity among parvalbumins has been used to explain the phenomenon of the high clinical cross-reactivity. However, these allergens are also implicated in isolated reactions to single or specific fish species. Patients with monosensitization to salmonid fishes have specific IgE antibodies recognizing the salmonid parvalbumins only (10). A similar case has been reported for food allergy to pangasius (13). Clinical reactivity to fish is not necessarily linked to the presence of specific IgE to parvalbumins. Other allergenic molecules such as enolases, aldolases, and fish gelatin seemed to be involved in food-allergic reactions of patients with poly-, oligo- or monosensitization to fish (11, 14, 15). Besides cross-reactivities among fishes, other food allergies have been observed in the context with this hypersensitivity. The clinical cross-reaction between fish and frog meat has been explained by specific IgE recognition of both fish and frog parvalbumins (16). A case study on a fish-allergic patient with concomitant food allergy to chicken meat seemed to further suggest cross-reactivity (17). In this case, parvalbumins from fish and chicken have been suggested as the causal allergens.

Food allergy to chicken meat is less common than fish allergy. The most frequent clinical picture is the so-called bird–egg syndrome (4). These subjects experience symptoms after repeated exposure to birds (respiratory sensitization) and subsequently develop symptoms of food allergy upon ingestion of chicken eggs and chicken meat. The major allergen is Gal d 5, chicken serum albumin, which is highly present in egg yolk and avian meat and serum (3). Clinical cross-reactions have been reported for several avian meats such as chicken and turkey (18). Hypersensitivity to chicken meat without sensitization to eggs has been only rarely addressed (19–22). Among these studies, some cases reported identified chicken meat allergens such as hemoglobin or myosin light chain while others described allergens in a molecular weight range of 20–70 kDa without further insights about their molecular identities and properties. One case study reported on the allergen identification of parvalbumin in a patient with chicken meat allergy (20). A single case of clinical cross-reactivity between fish and chicken has been reported more recently (17). So far, the identification of a new clinical phenotype of fish-allergic patients with cross-reactivity to chicken meat has not yet been addressed before.

The objective of this study was to analyze the relevance of the clinical association between fish allergy and chicken meat allergy in patients without sensitization to chicken eggs and furthermore to analyze the molecular basis for this clinical cross-reactivity. Our study focuses on the identification and characterization of allergens causing clinical cross-reactivity between fish and chicken.

Methods

Patients and patient sera

Twenty-nine patients with food allergy to both fish and chicken meat were recruited at clinical centers in Luxembourg (n = 18), France (n = 4), Belgium (n = 4), Germany (n = 2), and Sweden (n = 1). Twenty-three patients were males and six were females (age 4–54 years; mean age 19.3 years). Seven patients with chicken meat allergy only were recruited in Luxembourg (n = 3), Germany (n = 2), France (n = 1), and Belgium (n = 1). The age of these five male and two female individuals ranged from 7 to 30 years (mean age 16.1 years). Criteria for study participation were a documented clinical history of codfish allergy and/or chicken meat allergy without symptoms to bird feathers and chicken eggs. Further criteria were positive skin prick tests with cod and/or chicken meat as well as specific IgE to cod and chicken extract. Skin prick tests were performed using commercial cod/chicken extracts (Allergopharma, Reinbek, Germany) or fresh cod/chicken meat samples. They were rated positive if the wheal diameter was 3 mm larger than the negative control. Single patients were tested for skin reactivity with native, purified allergens (0.1, 1, 10, und 50 μg allergen/ml sterile saline containing 0.03% human serum albumin; ALK, Hørsholm, Denmark). Specific IgE was detected by ImmunoCAP (>0.35 kU_A/l positive; Phadia-ThermoFisher, Uppsala, Sweden). All patients experienced several allergic episodes upon ingestion, inhalation, and/or contact with fish and/or chicken meat; clinical symptoms were recorded for each food. Most patients refused to undergo oral provocation tests with the argument that allergic symptoms to the culprit food had been elicited at several occasions. Nevertheless, oral challenges were performed in five patients with chicken meat according to published procedures (23). The study was approved by the National Committee for Medical Research Ethics in Luxembourg (Ref. 201307/04), and all patients gave informed consent.

IgE reactivity to cod and chicken extracts by immunoblot

Proteins were extracted from cod (*Gadus morhua*) and chicken (*Gallus domesticus*) muscle, followed by immunoblot analysis as described before (14, 20). Briefly, patient sera were 3×, 5×, and 10× diluted in blocking buffer containing 3% bovine serum albumin (BSA; Sigma-Aldrich, Bornem, Belgium). Sera from five nonatopic subjects were applied as negative controls.

Biomolecular characterization of cod and chicken meat allergens

Edman N-terminal protein sequencing and mass spectrometric analysis of IgE-reactive proteins were performed as previously established (14). Then, the identified allergens – parvalbumins, enolases, and aldolases from cod and chicken – were purified by column chromatography using a combination of ion exchange and gel filtration matrices already used in previous studies to isolate cod allergens and chicken

parvalbumin (14, 20). Protein purity was analyzed by silver-stained sodium dodecyl–polyacrylamide gel electrophoresis (SDS-PAGE) and 2D-gel analysis (24). Protein identity was confirmed by allergen-specific antibody detection in immunoblot using two antiparvalbumin antibodies (cod: murine anti-cod parvalbumin polyclonal antibody (24); chicken: rabbit anti-rat parvalbumin polyclonal antibody; Abcam, Cambridge, MA, USA), an antienolase (rabbit anti-human enolase polyclonal antibody; Lifespan Biosciences, TE Huissen, the Netherlands) and an antialdolase antibody (goat anti-rabbit aldolase polyclonal antibody; Rockland, Limerick, PA, USA) shown to cross-react with homologues from fish and chicken. The presence of native structures of purified parvalbumins was established by circular dichroism spectroscopy or enzymatic activity assays for enolases and aldolases (14). Enolase enzyme activity was tested in a coupled enzymatic assay (2-phosphoglycerate to phosphoenolpyruvate by enolase, followed by phosphoenolpyruvate conversion to pyruvate by pyruvate kinase, followed by reduction in pyruvate to lactate by lactic dehydrogenase with concomitant conversion of NADH to NAD) while the last enzymatic step was followed by absorbance decrease at 340 nm. Aldolase enzyme activity was measured by a coupled colorimetric assay (fructose-1,6-diphosphate to dihydroxyacetone phosphate/glyceraldehyde 3-phosphate by aldolase, followed by reaction with 2,4-dinitrophenylhydrazine) while the hydrazone formation was recorded at 540 nm.

IgE reactivity to cod and chicken meat allergens by ELISA

Determination of specific IgE was performed using enzyme-linked immunosorbent assay (ELISA) including an IgE standard curve as detailed in a previous study (14). In short, allergens were coated at a concentration of 5 μg/ml to ELISA plates. Patient sera were 3×, 5×, and 10× diluted in blocking buffer containing 3% BSA. Fifteen sera of nonatopic individuals were used as negative controls. IgE titers below 0.35 kU_A/l were considered negative. The negative control sera gave a mean background that was several fold lower than the cutoff value.

For IgE cross-inhibition, a competitive ELISA method was used and sera (where >2 ml was available: no. 16, 19, 27) were pre-incubated at final concentrations of 50–500 μg inhibitor/ml patient serum. Following, IgE reactivity was tested with a homologue allergen coated onto the ELISA plate. As an inhibitor concentration of 200 μg/ml turned out to be in the saturation phase of the inhibition curve, all other sera were inhibited at this inhibitor concentration as follows: Sera were pre-incubated with purified parvalbumin, enolase, and aldolase from cod and chicken, and then mixes containing patient sera and inhibitor proteins were added to an ELISA plate coated with the homologue. To exemplify, if cod parvalbumin was used as inhibitor, chicken parvalbumin was coated to the microtiter plate. In addition, selected sera inhibitions (patient sera nos 1, 2, 8, 11, and 28) were titrated at final concentrations of 0.01–100 μg inhibitor/ml patient serum. Cod or chicken enolase and cod or chicken aldolase were used as inhibitors in this setting.

Detection of chicken meat allergens in authentic food samples

Quantitative detection of poultry allergens was modified from a method previously reported (24, 25). Briefly, authentic food samples (three individual samples each) of raw but also cooked and roasted chicken (breast, leg, and wing) were obtained, followed by protein extraction in triplicate and buffer exchange in phosphate-buffered saline (pH 7.2). For comparison, three cod samples were extracted by the same method. A calibration curve was established by ELISA using purified cod and chicken allergens coated in duplicate at 2–200 ng protein/ml. Cod and chicken extracts were coated in a dilution series at 0.5–5 μg/ml (triplicate). Allergens were detected using an anti-rat parvalbumin polyclonal antibody (Abcam), anti-human enolase polyclonal antibody (Lifespan Biosciences), and anti-rabbit aldolase polyclonal antibody (Rockland) (1 : 10 000 dilution). Allergen contents were estimated as 'milligram per gram muscle tissue'. Quantification limits were calculated for each antibody as the mean of the lowest allergen concentration detected in 10 experiments. Detection limits for cod allergens were 8 ng enolase/ml and 12 ng aldolase/ml. Chicken allergens were detected down to 2 ng parvalbumin/ml, 9 ng enolase/ml, and 15 ng aldolase/ml.

Results

Clinical characteristics of patients with fish and chicken meat allergy

Twenty-nine patients with documented clinical history of food allergy to fish and chicken meat were recruited (Table 1). Clinical symptoms varied from mild over moderate to severe. Clinical reactivity to chicken meat was confirmed by oral provocation in three representative patients with fish/chicken meat allergy and in two representative patients with chicken meat allergy only (Table 2). Skin prick tests to cod and chicken meat were positive (except for one patient, no. 21, where the sensitization was confirmed by specific oral food challenges). Specific IgE titers for cod and for chicken meat extract (ImmunoCAP) were positive in most patients (90% and 97%, respectively).

Identification of new chicken meat allergens

In IgE immunoblotting experiments using total extracts, most patient sera detected IgE-reactive protein bands at 12, 40, and 50 kDa in cod and chicken meat. IgE reactivity to chicken proteins is shown in Fig. 1A. For a minority (about 15% of the patients), additional IgE reactivity to other chicken proteins (20–35 kDa) was found. Immunodetection by specific antibodies against parvalbumin, enolase, and aldolase as well as N-terminal sequencing identified main IgE-reactive bands as parvalbumin (12 kDa), aldolase (40 kDa), and enolase (50 kDa) (Table S1). Minor chicken meat allergens were found to be myoglobin (20 kDa) and triosephosphate isomerase (28 kDa). Further analysis was focused on allergens from fish and chicken presumably

Table 1 Twenty-nine cases of fish and chicken meat allergy and seven cases of chicken meat allergy: patients' clinical characteristics and relevant IgE profiles

				sIgE by CAP (kU_A/l)‡		sIgE by ELISA (kU_A/l)‡					
						Parvalbumin		Enolase		Aldolase	
No.	Sex/Age	Clinical symptoms*	SPT to cod/chicken†	Cod	Chicken	Cod	Chicken	Cod	Chicken	Cod	Chicken
Patients with allergy to fish and chicken meat											
1	M/4	Fish: U Chicken: AE	Pos/Pos	12	11	0.8	Neg	2.1	3.0	1.5	Neg
2	M/5	Fish: OAS Chicken: U	Pos/Pos	0.4	2.0	0.4	Neg	0.5	0.4	0.4	Neg
3	M/7	Fish: A, AE, V Chicken: AE	Pos/Pos	188	8.0	140	22	70	Neg	13	0.6
4	M/7	Fish: AP, N, OAS Chicken: AP, N, U, V	Pos/Pos	0.9	3.4	0.4	0.4	0.9	1.1	0.9	1.0
5	M/7	Fish: U, V Chicken: V	Pos/Pos	5.4	8.9	Neg	Neg	4.3	4.6	Neg	Neg
6	F/7	Fish: N, U Chicken: AE, U	Pos/Pos	3.5	4.0	Neg	Neg	2.1	1.1	Neg	1.0
7	F/8	Fish: OAS, Co Chicken: AE, U, V	Pos/Pos	1.0	2.8	0.5	0.4	1.3	0.5	0.8	0.4
8	M/9	Fish: AP, U, V Chicken: AP, D	Pos/Pos	1.2	0.9	2.1	1.9	2.3	0.5	2.0	0.5
9	M/10	Fish: AP, OAS Chicken: AP, OAS	Pos/Pos	0.9	1.1	Neg	Neg	0.6	0.4	0.5	0.4
10	M/12	Fish: A, AE, U Chicken: A, U	Pos/Pos	16	3.2	18	1.0	10	2.1	7	2.7
11	M/12	Fish: AE Chicken: Pr	Pos/Pos	17	2.8	14	0.4	18	Neg	15	0.4
12	M/13	Fish: AE, U Chicken: AE	Pos/Pos	3.2	2.4	2.7	2.3	3.5	Neg	3.7	0.9
13	M/14	Fish: OAS Chicken: AE, AP, D, V	Pos/Pos	1.1	1.6	Neg	1.0	Neg	Neg	4.1	1.0
14	M/14	Fish: OAS, U Chicken: A, AE, U	Nd/Nd	1.5	40	Neg	108	1.8	0.5	Neg	Neg
15	M/19	Fish: A, AE, OAS Chicken: OAS, Pr	Pos/Pos	1.3	0.9	Neg	0.4	1.9	0.6	2.1	0.8
16	M/20	Fish: A, AE, U Chicken: nd	Pos/Pos	19.8	1.5	21	7.2	5.7	Neg	9.6	0.9
17	M/21	Fish: AE Chicken: U, V	Pos/Pos	9.8	10	Neg	1.1	3.9	2.3	2.1	0.5
18	M/21	Fish: A, AE, U Chicken: AE, U	Pos/Pos	0.5	2.2	Neg	Neg	Neg	0.5	Neg	0.4
19	F/22	Fish: AE, U Chicken: OAS	Pos/Pos	>100	1.6	25	18	5.0	1.2	Neg	1.4
20	M/23	Fish: AP, OAS, V Chicken: AP, V	Pos/Pos	Neg	0.5	0.4	1.4	0.7	0.4	0.6	0.5
21	F/25	Fish: AE, OAS, U Chicken: A, AE, U	Neg/Neg	Neg	Neg	Neg	Neg	1.9	0.6	2.1	0.8
22	F/26	Fish: AE, U Chicken: AE, U	Pos/Pos	0.4	0.8	Neg	0.4	Neg	Neg	Neg	Neg
23	M/27	Fish: AP Chicken: AP, D, Pr	Neg/Pos	0.5	4.6	0.5	Neg	0.5	Neg	0.5	0.4
24	M/30	Fish: A, OAS Chicken: A, Pr	Pos/Pos	0.5	0.6	0.4	0.4	1.3	0.4	0.4	0.4

Table 1 (continued)

No.	Sex/Age	Clinical symptoms*	SPT to cod/chicken†	sIgE by CAP (kU_A/l)‡		sIgE by ELISA (kU_A/l)‡					
						Parvalbumin		Enolase		Aldolase	
				Cod	Chicken	Cod	Chicken	Cod	Chicken	Cod	Chicken
25	M/33	Fish: A, AE Chicken: AE, D, U	Pos/Pos	1.1	10	Neg	1.0	1.3	Neg	Neg	1.4
26	M/35	Fish: AE Chicken: AE	Neg/Pos	0.4	0.4	Neg	Neg	0.4	0.4	Neg	0.4
27	M/36	Fish: AE Chicken: AE, OAS	Pos/Pos	33	0.8	40	1.6	3.1	0.4	Neg	0.5
28	M/38	Fish: AE, U Chicken: A, AE, U	Pos/Pos	Neg	0.3	Neg	0.8	0.6	2.1	Neg	0.8
29	M/54	Fish: OAS Chicken: OAS, V, H	Pos/Pos	3.0	12	Neg	1.7	5.0	1.3	3.0	11.3
Mean	19.3	–	–	38.0	13.0	10.0	2.6	5.3	0.8	2.7	1.1
Median	19.3	–	–	1.2	2.3	0.4	0.4	1.9	0.5	0.6	0.5
Patients with allergy to chicken meat only											
1	M/7	Chicken: AE	Neg/Pos	Neg	1.7	Neg	0.5	Neg	Neg	Neg	0.9
2	M/8	Chicken: A, AE, U, V	Nd/Pos	Neg	6.8	Neg	Neg	Neg	6.1	Neg	0.4
3	M/10	Chicken: D, OAS, U	Neg/Pos	Neg	4.9	Neg	Neg	Neg	0.5	Neg	Neg
4	M/15	Chicken: D, OAS, U	Neg/Pos	Neg	3.7	Neg	2.8	3.2	1.4	Neg	0.5
5	F/19	Chicken: A, AE, U, V	Neg/Pos	Neg	3.7	Neg	Neg	Neg	0.4	Neg	0.4
6	M/24	Chicken: A, AE, U	Nd/Pos	Neg	Neg	Neg	Neg	0.7	0.4	Neg	0.4
7	F/30	Chicken: U	Nd/Neg	Neg	Neg	Neg	Neg	Neg	0.5	Neg	1.1
Mean	16.1	–	–	0.35	3.1	0.35	0.7	0.8	1.4	0.35	0.6
Median	15.0	–	–	0.35	3.7	0.35	0.35	0.35	0.5	0.35	0.4

*A, asthma; AE, angioedema; AP, abdominal pain; Co, conjunctivitis; D, diarrhea; H, hypotension; N, nausea; Pr, pruritus; U, urticaria, V, vomiting; nd, no data.

†SPT: Nd, not determined; Neg, negative; Pos, positive; SPT, skin prick test.

‡IgE determination by ImmunoCAP system and ELISA: Neg, negative.

Table 2 Five patients with diagnostic food challenges to chicken meat: eliciting doses and clinical symptoms

No.	Chicken breast (g)*	Clinical symptoms
1	100	AE, AP, OAS
21	30	OAS, U
24	100	OAS
5	0.1	AE, V
7	120	U, OAS

AE, angioedema; AP, abdominal pain; OAS, oral allergy syndrome; U, urticaria, V, vomiting.

*Lowest dose giving a first objective symptom.

involved in clinical cross-reactivity – parvalbumins, aldolases, and enolases. Indeed, cod parvalbumin, enolase, and aldolase as well as chicken parvalbumin had been isolated and described as allergens before (14, 20). For the present study on fish–chicken cross-reactivity, these allergens in addition to chicken enolase and aldolase were extracted from cod and chicken and purified to homogeneity by column chromatography (Fig. 1B). Protein quality was substantiated for parvalbumins by evidence of the alpha-helical protein structure and for enolases/aldolases by determination of enzymatic activities (Fig. S1). By 2D-gel analysis, the isoelectric points of purified cod and chicken meat allergens were found to be similar, both for parvalbumin at about pH 5.0, enolase at about pH 7.0, and aldolase at about pH 9.0 (Fig. S2).

Pure chicken muscle enolase and aldolase were further characterized as to their protein sequence by MS analysis with sequence coverages of 74% and 63%, respectively (Tables S2 and S3).

Patients with fish and chicken meat allergy: IgE reactivity, skin reactivity to purified native allergens, and cross-reactivity among homologues

Cod and chicken meat allergens were tested in ELISA for IgE quantification in sera from patients with both food allergies. Concerning parvalbumins, 15 of 29 patients (52%) had specific IgE to cod parvalbumin (0.4–140.0 kU_A/l) and 20 of 29 (69%) to chicken parvalbumin (0.4–108.0 kU_A/l) (Table 1). Focusing on specific IgE to enolases, 26 of 29 patients (90%) had IgE to cod (0.4–70.0 kU_A/l) and 21 of 29 (72%) to chicken enolase (0.4–4.6 kU_A/l). For aldolases, 19 of 29 patients (66%) were positive in IgE ELISA with cod aldolase (0.4–15.0 kU_A/l) and 24 of 29 (83%) with the chicken homologue (0.4–11.3 kU_A/l).

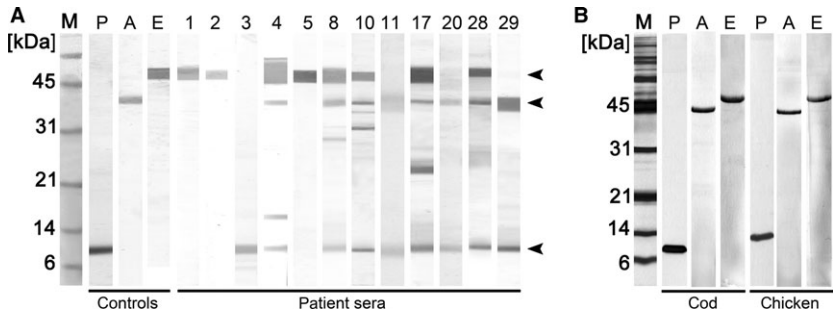


Figure 1 (A) Chicken meat allergens were detected by patient sera-specific IgE (for instance, patient nos 1–5, 8, 10, 11, 17, 20, 28, and 29). As controls, allergen-specific antibodies detected parvalbumin (P), aldolase (A), and enolase (E) in chicken leg extract at 10, 40, and 50 kDa. M, molecular weight marker; arrows, major allergens. (B) Cod and chicken allergen purity was shown by SDS-PAGE followed by silver staining. P, parvalbumin; A, aldolase; E, enolase.

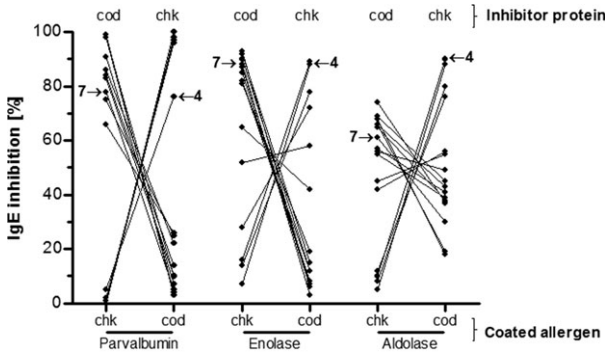


Figure 2 Serum IgE antibodies of fish and chicken meat-allergic patients are cross-reactive to parvalbumins, enolases, and aldolases from cod and chicken (chk). High inhibition rates correlate with patient's primary sensitization (for instance, patient no. 7 to fish and patient no. 4 to chicken).

Table 3 Five patients with fish and chicken meat allergy: primary sensitization and results of skin testing using purified enolases and aldolases

		Prick test (μg/ml)†			
		Enolase		Aldolase	
No.	Primary sensitization*	Cod	Chicken	Cod	Chicken
1	Fish	10	10	10	Neg
2	Fish	10	10	Neg	Neg
8	Fish	10	50	1	50
11	Fish	10	Neg	10	50
28	Chicken	50	10	Neg	Neg

*Self-report based.

†Lowest allergen concentration giving a first positive skin reaction.

Cross-reactivity among fish and chicken meat allergens was confirmed by a competitive IgE inhibition ELISA method (Fig. 2). IgE cross-reaction was found for parvalbumins (15 patients), enolases (16 patients), and aldolases (16 patients) in individuals with IgE to both homologues. In patients with cross-reactive IgE to parvalbumins and enolases, either fish or chicken allergens were potent inhibitors in all patients (except for two patients with moderate inhibition rates of about 50% for enolases). In patients with specific IgE to aldolase homologues, chicken aldolase was a strong inhibitor in four patients while other inhibition rates were in the medium range (about 30–50%). The inhibition rates correlated with the self-report of primary sensitization in most patients. In patients with primary sensitization to fish, fish allergens inhibited effectively the IgE binding to chicken homologues (Fig. 2, for instance, patient no. 7). In patients with primary sensitization to chicken meat, chicken allergens were strong inhibitors of IgE reactivity to fish homologues (Fig. 2, for instance, patient no. 4).

In vivo reactivity to fish and chicken enolases and aldolases was verified in five patients by skin testing using the purified allergens (Table 3). In case of positive skin testing with homologous enolases and aldolases, IgE cross-reactivity was further analyzed by establishing titration curves using various inhibitor concentrations (Fig. 3). Skin test results and IgE cross-reaction rates correlated to the self-reported initial source for food allergy of these selected patients as follows. In patients primarily sensitized to fish (nos 1, 2, 8, and 11), positive skin reactivity was found either equally strong or stronger for fish allergens than for chicken meat homologues while all IgE inhibition rates were clearly stronger with the fish allergens (enolase and aldolase). In patient no. 28 with reported primary sensitization to chicken meat, the skin reactivity to chicken enolase was stronger with the chicken meat allergen than with the fish allergen correlating with the results of the corresponding high IgE inhibition rates using the chicken allergen as the inhibitory protein.

New chicken meat allergens were named Gal d 8 for parvalbumin, Gal d 9 for enolase, and Gal d 10 for aldolase (Allergen Nomenclature Sub-committee of the World Health Organization and International Union of Immunological Societies, WHO/IUIS; www.allergen.org).

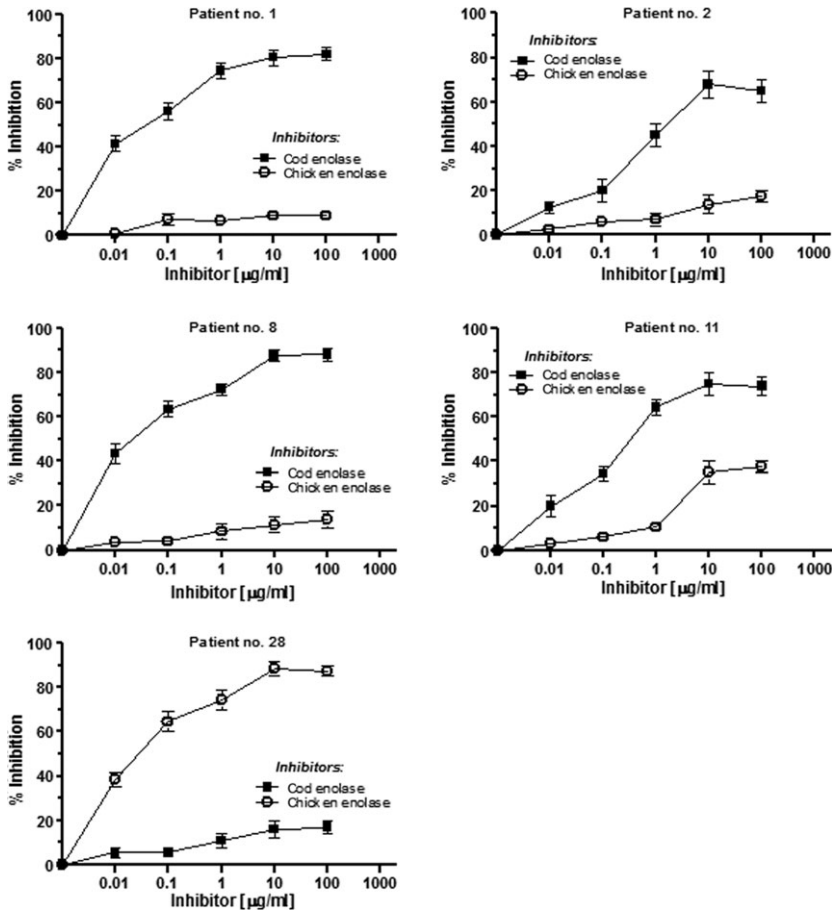


Figure 3 Serum IgE antibodies of fish and chicken meat-allergic patients are cross-reactive to varying degrees to allergens from cod and chicken positively tested for skin reaction. High inhibition rates correlate with patient's primary sensitization (patient nos 1, 2, 8, 11 fish and patient no. 28 chicken meat).

Patients with chicken meat allergy: clinical characteristics and IgE reactivity

Seven chicken meat-allergic patients were included in the study (Table 1). These patients tolerated hen's eggs and fish. IgE reactivity to chicken meat was confirmed by positive skin tests and/or positive IgE titers with chicken (except for patient no. 7 with clinical reactivity confirmed by a positive oral challenge to chicken). Two of seven patients had specific IgE to chicken parvalbumin Gal d 8 and six of seven to each enolase Gal d 9 and aldolase Gal d 10. In patient nos 4 and 6, who had IgE against cod enolase, IgE binding to the cod protein could be eliminated completely by pre-incubation of the serum with the chicken homologue (data not shown).

Allergen levels in chicken samples

As allergen levels and stability of allergens toward food processing are important factors contributing to the allergenicity of food proteins, chicken allergen detection was performed in authentic food samples (Table S4). Chicken allergen levels varied in different pieces (breast, legs, and wings) as well as in differently processed samples (raw, cooked/boiled, and roasted). Parvalbumin was detected in all leg and wing samples irrespective of the food processing method (0.05–0.2 mg parvalbumin/g muscle tissue), whereas this allergen was not detectable in any of the breast samples. Enolase and aldolase were both found in all native samples from chicken breast, leg, and wing (0.6–1.5 mg enolase or aldolase/g muscle tissue). In processed samples, only the breast tissue samples were positive with detectable enolase or aldolase contents, which were reduced up to 15 times compared to the raw muscle samples. For comparison, cod enolase and aldolase levels were determined at 1.5–2.0 mg/g raw muscle tissue each and were up to seven times lower in processed fish muscle tissue.

Discussion

In the present study, we identified three new chicken allergens, parvalbumin (Gal d 8), enolase (Gal d 9), and aldolase (Gal d 10) and could show that these allergens, which have

homologues in fish, are responsible for the clinical cross-reactivity between fish and chicken meat. We demonstrated different allergen contents in chicken breast, leg, and wing samples leading to the conclusion that these tissues have a variable allergenicity, not only for patients with food allergy to fish and chicken meat but also for chicken meat-allergic patients without concomitant fish allergy. While clinical cross-reactions among different fish species are very common, the knowledge about cross-reactivity between fish and other foods is still limited (26–29). Only a single case of clinical cross-reactivity between fish and chicken meat has been described in the literature (17).

The present study used Atlantic cod (*G. morhua*) as a representative fish as all study participants had a history of allergic reactions to this fish species. Cod is also one of the most commonly consumed fishes in Europe. Parvalbumin, Gad m 1, is the well-established main cod allergen which is highly expressed in fish muscle (24, 30). Cod enolase, Gad m 2, and aldolase, Gad m 3, have been identified as relevant allergens more recently (14). Our identified homologous chicken parvalbumin (Gal d 8), enolase (Gal d 9), and aldolase (Gal d 10) are important food allergens being involved in the clinical cross-reaction between fish and chicken meat. The majority of the study participants ($n = 36$) had specific IgE binding to these allergens.

Among study participants with both fish and chicken meat allergy, we found IgE reactivity to chicken parvalbumin in 69%, to chicken enolase in 72%, and to chicken aldolase in 83% of the cases. Remarkably, nine patients (31%) had no specific IgE to chicken parvalbumin but to chicken enolase and/or aldolase only. The clinical reactivity of these parvalbumin-negative patients was confirmed in two representative individuals by food challenges (patient no. 1 and no. 21). IgE cross-reactivity to cod parvalbumin, enolase, and aldolase was found in 43%, 66%, and 59%, respectively, of these patients. IgE cross-inhibition assays revealed that *in vitro* cross-reactions were variable: in some cases the fish allergen and in other cases the chicken meat allergen being the strongest inhibitor. We explained this finding by the primary sensitization of the individual patients either to fish or to chicken. Despite the fact that the chronological onset of different food allergies is mostly difficult to define, it was possible to estimate their first food allergy based on which food the patients experienced the first allergic reactions to (fish 62%, chicken meat 31%, and fish/chicken meat 7%), and indeed, these reports matched with the results from IgE cross-inhibition assays. A further confirmation was obtained from skin test results correlating with IgE cross-reaction rates in comparison with the primary sensitization in a subgroup of selected patients. In summary, the present study suggests that both fish-allergic and chicken meat-allergic patients might be at risk to develop a food allergy to chicken meat or fish, respectively. In addition, chicken parvalbumin, enolase, and aldolase are relevant food allergens in patients with chicken meat allergy only, as shown in our study.

In general, high content and high stability of a food protein to processing are crucial features of potent food allergens. While fish parvalbumins have been described as very stable proteins, fish enolases and aldolases seem to have a lower resistance to food preparation methods (14, 31). In the present study, we found that cod enolase and aldolase levels in native fish muscle were in the range of previously reported parvalbumin levels (24). Enolase and aldolase were still traceable by immunodetection in cooked cod, but levels of intact allergens were several fold lower than in raw cod. A similar result was obtained for enolase and aldolase levels in chicken meat samples. All raw chicken meat samples were positive at similar levels as the fish samples, but in processed samples, enolases and aldolases were only detectable in breast but not in leg or wing samples. Interestingly, we could detect chicken parvalbumin only in leg and wing samples (raw, processed) but not in chicken breast, suggesting very low allergen amounts in the latter muscle tissue being below the detection level of parvalbumin-specific ELISA. As a translational aspect to clinical practice, we hypothesize that chicken meat-allergic patients might react differently to breast and leg/wing samples depending on their individual allergen-specific IgE-binding profiles. Patients with specific IgE to chicken parvalbumin might experience more severe allergic reactions upon consumption of chicken leg/wing than breast, irrespective of the processing method. Individuals with sensitization to chicken enolases or aldolases might react stronger to chicken breast than leg or wing samples. In the present study, the clinical work-up did not include the systematic analysis of clinical reactivity to different parts of chickens. However, one patient (no. 19) reported to react to chicken leg which correlated to high specific IgE to chicken parvalbumin. It might be important to raise the awareness of clinicians about varying *in vitro* and *in vivo* reactivity profiles in chicken meat allergy, especially for the design of diagnostic prick-to-prick testing or the interpretation of extract-specific IgE titer.

In summary, we could for the first time substantiate the clinical profile of patients with cross-reactivity between fish and chicken meat which will be of importance in food allergy. Analogous to the 'bird–egg syndrome', we propose to define this clinical entity as 'fish–chicken syndrome' with cross-reactive allergens involved being parvalbumins, enolases, and aldolases.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

AK led the study and wrote the manuscript. AK, FH, MM, and CH designed the study. All co-authors critically were involved in the critical review of the manuscript. FC, CL, VD, SG, FB, JF, NB, MH, JP, SS, HC, FH, and MM recruited study participants, provided clinical data/patient material, and, together with MO, were substantially involved in the analysis and interpretation of all data.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (A) Alpha-helical structures of purified cod and chicken parvalbumin were visualized by far UV CD spectra as two characteristic absorbance minima at 208 and 222 nm. MRME, mean residue molar ellipticity. (B) Catalytic activity of purified enolases was confirmed in a coupled enzyme assay by absorbance decreases at 340 nm. (C) Enzyme activity of purified aldolases was verified by colorimetric analysis using absorption measurement at 540 nm.

Figure S2. Isoelectric points of purified cod and chicken parvalbumin (P), aldolase (A) and enolase (E) were determined by 2D-gel followed by silver staining.

Table S1. N-terminal sequences of putative chicken meat allergens by microsequencing from IgE-reactive bands in total extracts.

Table S2. Chicken beta enolase peptides matching with enolase of chicken, *Gallus gallus* (gi/46048765) sequence after request in the NCBInr database (74.4% sequence coverage).

Table S3. Chicken aldolase peptides matching with fructose-bisphosphate aldolase A of falcon, *Falco cherrug* (gi/541954690) sequence after request in the NCBInr database (63.3% sequence coverage).

Table S4. Allergen contents in raw and authentic chicken samples by quantitative ELISA (24, 25).

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