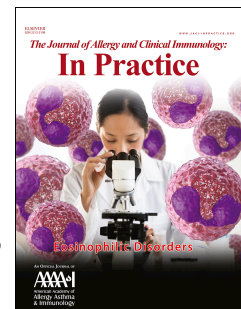


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Natural history of IgE-mediated fish allergy in children

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1 **TITLE**

2 **Natural history of IgE-mediated fish allergy in children**

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Contribution to the article

Paraskevi Xepapadaki: Writing and critical revision of the manuscript

Georgia Christopoulou: Contribution to drafting the paper and interpretation of data

George Stavroulakis: Contribution to design and acquisition of data

Raphaela Freidl / Birgit Linhart / Laurian Zuidmeer: Contribution to the molecular (laboratory) data

John Lakoumentas: Statistical analysis and table/figure formation

Ronald van Ree and Rudolf Valenta: Critical review for important intellectual content

Nikolaos G. Papadopoulos: Conception, design and critical review of the study and final approval of the manuscript.

ABSTRACT

Background: Fish allergy is not uncommon -especially in countries with high fish consumption-, can frequently be severe and may affect dietetic and lifestyle choices. Nevertheless, data on its clinical course and natural history are scarce.

Objective: To describe the natural history of IgE-mediated fish allergy and the potential differential reactivity to various fish species and identify prognostic markers in children with confirmed disease.

Methods: Clinical history, specific-IgE and skin prick tests (SPTs) to various fish were recorded in 126 children with confirmed IgE-mediated fish allergy. IgE reactivity was also evaluated by immunoblotting. Eligible participants proceeded to a series of food challenges to tuna, swordfish and codfish. In total, 234 challenges were performed.

Results: Fifty-eight children (9.7 ± 3.9 years) were included in the analysis. Age at first reaction was 0.5-5 years (median: 1.3). Thirteen children (22%) tolerated all fish tested - including cod- 1-14 years (mean 8.2 ± 4.2 years) following their first reported reaction. Complete fish tolerance increased with age, ranging from 3.4% in preschool children to over 45% in adolescents (95% CI: 26.3%-79.7%). Most children were able to tolerate swordfish (94%) and tuna (95%). Pre-challenge sIgE to cod >4.87 kUA/L was the best positive predictive marker for fish allergy persistence (94%), followed by SPT to sardine >6.5 mm (92%).

Conclusion: A considerable proportion of fish-allergic children develop tolerance around adolescence. Most fish-allergic children can consume tuna and swordfish, which, thus, provide safe alternatives for a balanced diet.

Highlight box

- What is already known about this topic?

Fish allergy is usually persistent and involves multiple fish species.

- What does this article add to our knowledge?

Fish allergy usually appears at the age of 1-2 years. Fish allergy resolves by adolescence in a substantial proportion of children. Some fish species may be tolerated by most fish-allergic children.

- How does this study impact current management guidelines?

Children with fish allergy should be periodically evaluated for resolution of the allergy and for the ability to include specific fish species in their diet.

Keywords: Fish Allergy, IgE, Natural history, Fish species, Food challenge

Abbreviations:

IgE, Immunoglobulin E;

DBPCFC, Double-Blind Placebo-Controlled Food Challenge;

FC, Food Challenge;

OFC, Open Food Challenge;

SPT, Skin Prick Test.

INTRODUCTION

Fish allergy is one of the most common causes of food allergy, especially in children and young adults, with prevalence rates ranging from 0.1% to 0.5%, as assessed by oral challenges^{1,2}, more so in countries where fish consumption or the number of people working in the fish industry is high³. The implicated major fish allergen is beta (β)-parvalbumin, a well-studied protein characterized as Ca^{2+} -binding of molecular weight 12kDa, resistant to heat and digestion⁴. Structural homology among β -parvalbumin isoforms of different fish species has been reported in significant rates (62-74%), thus, a high degree of cross-reactivity has been reported between different bony fish in the Osteichthyes class⁵. Indeed, most patients with an allergic reaction to one fish will also react upon ingestion of other fish⁶. However, a lower parvalbumin content and respective IgE reactivity is potentially associated with partial or complete tolerance to tuna and salmon in a considerable proportion of cod-allergic patients^{6,7}. Chondrichthyes include cartilaginous fishes, i.e., sharks and rays, and they mainly contain α -parvalbumin, which has been shown to be less allergenic in *in vitro* studies⁸⁻¹⁰.

Data on the natural history of fish allergy are scarce, with the majority of reports suggesting long-term clinical reactivity^{11,12}. Previous studies showed that only 15% of children outgrow their fish allergy within a period of 2-5 years¹³, while lower rates (~3.5%) have been reported in a wide telephone survey in the US¹⁴. It is also well accepted that some patients allergic to certain fish may tolerate other species⁶.

We hypothesized that a considerable proportion of children may overcome their fish allergy and that biological and clinical characteristics, such as fish-specific immunoglobulin E (sIgE) and parvalbumin-specific IgE levels, wheal responses to fish as assessed by skin prick tests or clinical characteristics of IgE-mediated reactions to fish, may help predict the medium- or long-term outcome. The aim of this study was to describe the natural history of IgE-mediated

- 125 fish allergy, as assessed by objective measurements of clinical reactivity to fish in children,
126 deriving from a national referral center for pediatric allergy and asthma in Greece.

MATERIALS AND METHODS

Patients

The study included children with reported IgE-mediated fish allergy, aged 4-18 years, derived from the database of the Allergy Department, 2nd Pediatric Clinic, University of Athens, from April 2009 to December 2012. Participants were either new patients referred to our department because of symptoms strongly suggestive of IgE-mediated fish allergy or patients previously diagnosed with fish allergy by a specialist. Inclusion criteria were a definite history of IgE-mediated symptoms, i.e., immediate-type symptoms within two hours following fish ingestion and either a positive open food challenge (OFC) to the implicated fish or a positive SPT to cod fish extract and Cod specific (s)IgE antibodies >0.35 kU/L in the preceding 12 months. Children with chronic diseases (except atopic dermatitis, allergic rhinitis or asthma) or receiving allergen immunotherapy and/or anti-IgE were excluded.

The study was approved by the P. A. Kyriakou Children's Hospital ethics committee. Written consent was obtained from parents.

Study design

The primary aim of the study was to describe the natural course of fish allergy. Secondary outcomes included a. identification of prognostic predictors of fish allergy resolution and b. differential reactivity patterns to various fish species.

At baseline, a detailed history including demographic data, detailed history of the type and symptoms during reactions to fish and personal and family history of allergy-associated diseases was obtained. SPTs to commercial fish extracts and fresh fish were performed, if such tests had not been recorded in the preceding 6 months. A blood sample was obtained for determination of total/sIgE and parvalbumin-specific IgE by Western blot assays.

After inclusion, patients were monitored prospectively according to a predefined care pathway, depending on sensitization and reactivity responses. We hypothesized that children

would most probably tolerate fish/preparations of lower allergenicity; in case of a reaction to such challenges, the higher step or steps were not undertaken, assuming reactivity. A series of food challenges to evaluate differential clinical reactivity to different fish species was planned, based on previous published allergenicity data ^{6,7}, as follows: (1) open food challenge (OFC) to canned tuna, (2) OFC to fresh swordfish or fresh tuna fish, depending on parent's preferences, and (3) DBPCFC to codfish. In case of a negative DBPCFC to cod or refusal, an OFC to codfish was offered to the child on a separate day. OFCs to other fish (salmon, mackerel or chub mackerel) were performed on parental request.

Despite DBPCFC being the gold standard for food allergy diagnosis¹⁵, we opted to use it only at the final step, in order to increase adherence to the suggested multi-challenge follow-up schedule.

In case of a positive OFC (with objective symptoms) to any fish, the child was characterized as having an "active fish allergy" and was not challenged further. Children with a negative OFC to canned tuna and/or swordfish but sIgE to cod >20 kU/L, which has previously been reported as having >95% positive predictive value for a fish challenge¹⁶, were not challenged. Tolerance was defined by a negative food challenge to cod, whereas a positive outcome to any fish challenge or sIgE value to cod (f3) >20 kU/L defined an "active fish allergy".

Assessment of sensitization

Skin Prick Tests (SPTs)

SPTs were performed at baseline and at 6 month-intervals using commercial extracts for cod, tuna, salmon, sardine, white-fish mix and shrimp extracts (ALK-Abelló, Madrid, Spain) and prick-to-prick to implicated raw fish, according to history. SPTs were considered positive in case of a wheal size [calculated as the mean value of the longest (*D*) and the midpoint orthogonal diameter (*d*) of the wheal] being ≥ 3 mm compared to negative control ^{17,18}.

Blood samples

Total and sIgE antibodies to cod (f3), tuna (f40), salmon (f41), sardine (f61), and rGad c 1 (f426) were analyzed with the Thermo Fisher ImmunoCAP system (Phadia AB, Uppsala, Sweden) and were considered positive if values were >0.35 kUA/L. Fish-specific IgE levels were recorded as exact values if <100 or as 101 kU/L in case of >100 kU/L¹⁹. Initial and peak fish-IgE levels correspond to the values recorded at initial presentation and the highest values among all available for the participant respectively.

Immunoblotting using fish extracts

Cod, mackerel, tuna and swordfish were purchased at a local fish market. Fish muscle tissue was frozen in liquid nitrogen, homogenized and extracted in phosphate-buffered saline (pH 7.4)²⁰. Fish extracts (90 μ g total protein/cm gel) were separated on a 14 % SDS-PAGE gel. Strips (4 mm) of nitrocellulose-blotted extracts (Whatman Protran nitrocellulose membrane, Sigma-Aldrich, St Louis, Mo) were incubated with serum from fish-allergic children (volume 900 μ L, 1: 10). Bound IgE was detected by ¹²⁵I-iodine-labeled anti-human IgE antibodies (1: 20, Demeditec Diagnostics, Kiel, Germany) and visualized by autoradiography at varying exposition times (1 day, 3 days, 1 week). The signals of bound IgE on images of the X-ray film were analyzed using the ImageJ software and are expressed as relative values²¹. Parvalbumin levels were quantified using the ImageJ software.

Sequence analysis

Multiple sequence alignment was performed using the GeneDoc software. The parvalbumin protein sequences were derived from the UniProt database and sequence identities were determined using the Protein BLAST multiple-sequence-alignment tool.

Oral Food Challenges

All fish challenges were performed according to standardized procedures, established in the EuroPrevall study²²⁻²⁴. The challenge was considered positive in case of objective symptoms from the skin or respiratory, cardiovascular or systemic manifestations within 2h following

the final dose. Reactions were graded from 1 to 5 according to severity²⁵. In case of an inconclusive outcome, children were re-challenged 1-4 weeks later. In case of tolerance, parents were instructed to incorporate the food into the child's diet. For fish potentially containing significant amounts of mercury, parents were advised against consuming them more than once a week²⁶.

DBPCFCs were performed only to codfish. The matrix used for the challenges was chicken burger²⁷. All challenges (active and placebo) consisted of 5 escalating doses with 20-minute intervals. Unblinding was done after the completion of the last challenge day. Blinding and unblinding were performed by a trained dietitian.

The cumulative dose of fish offered during a DBPCFC was 90-95 gr (equal to ~20gr fish protein), while for an OFC a full serving according to child's age and preference was offered.

Statistical Analysis

Statistical analysis was performed with R (version 3.5.0), with the assistance of the RStudio interface (version 1.1.383). Our quantitative variables (specific and total IgE, as well as SPT diameters) did not follow the normal distribution, as obtained by the Shapiro-Wilk test for composite normality, and thus, non-parametric tests were used. Statistical significance was set at <0.05 .

In order to assess correlations between the subjects' status (tolerant vs. allergic) and quantitative variables, we grouped each variable per status level and performed Wilcoxon's rank-sum test for median comparisons of the two distributions. Moreover, we computed ROC curves and then estimated their area under the curve (AUC). By discovering the optimal thresholds of these quantitative variables, we finally estimated their positive and negative predictive values (PPV and NPV respectively). Optimal thresholds were discovered with the Youden criterion. Dependencies between qualitative variables (the subjects' status and atopic dermatitis, asthma, allergic rhinitis and other food allergies) were estimated using Pearson's

chi-squared test of independence. For post-hoc comparisons, we conducted pairwise chi-squared tests with Bonferroni correction. Subjects' age and specific IgE were correlated using Spearman's correlation. Both linear and polynomial fitting was applied, to estimate the dependency between the two variables. A Kaplan-Meier curve was used to depict the development of tolerance to fish over time.

RESULTS

Study population

Files from 126 children with fish allergy were reviewed; from those, 96 were eligible for the study and 75 (78%) accepted our invitation and were enrolled. Seventeen participants dropped out during the follow-up period, mainly due to the long and demanding nature of the protocol including several FCs. Therefore, 58 children (mean aged 9.7 ± 3.9 years, 35 males) were included in the final analysis; 47 completed all challenges per protocol, while 11 performed part of the challenges (Figure 1).

No differences were found in respect to all baseline characteristics between participants and drop-outs, except for allergy-associated reported diseases, i.e., atopic dermatitis, asthma and other food allergies, which were significantly more prevalent in participants, as shown in Table 1.

The age of first reported reaction ranged between 0.5-5 years (median: 1.25, interquartile range (IQR)=1.5) (Figure 2). Reaction on first fish consumption was reported in 36 children (62%). The number of reactions to fish before evaluation by a specialist ranged from 1 to 11, (median, 3, IQR=2). Less than three reactions were observed in 37 children (64%), whereas 20 children had 4-6 (34%) and only one (2%) reported ≥ 7 reactions. Almost all children (56, 97%) had a history of atopic dermatitis (80% moderate-severe), 39 (67%) had asthma -49% early wheezing- and 22 children (38%) reported allergic rhinitis. At least one other food

allergy was present in 40 children (69%). Detailed data on food allergy frequencies, symptoms and elicitors are shown in Table 1. Twenty-nine children reported moderate or severe reactions following fish consumption (Grade 3-5). Almost half of the participants - 28/58 (48%)- reacted only to one fish species (usually cod), while respective numbers/ratios to two, three and four fish species were 19/58 (33%), 7/58 (12%) and 4/58 (7%).

Fish allergy resolution

Enrolled participants were subjected to a total of 188 food challenges: 67 tuna OFC, 46 swordfish OFC, 14 cod DBPCFC and 38 cod OFC. In addition, 9 challenges were performed to different fish: 2 OFCs to mackerel, 3 to chub mackerel and 4 to salmon. Overall, 13/58 (22%) children developed tolerance to cod throughout the follow-up period.

The time interval between first and last reported reaction was 6.5 years (median, min=0, max 17 years). The duration of follow-up (time in years from first reported reaction to final diagnosis) did not differ significantly between the two groups (tolerant 8.5 vs allergic 7.5, $p=0.63$). The median time interval between challenges was 3 months (range: 0.55 - 30 months) between canned tuna and fresh tuna or swordfish and 6 months (range 0.75-24 months) for cod.

Baseline clinical characteristics, including pre-cod challenge cod SPT and sIgE between adolescent and younger patients did not differ, except for adolescents presenting increased SPT max diameter to cod compared to younger children (median: 10.5 (7.75-13.25) mm vs 8 (6-9.5) mm, $p=0.04$), while children younger than 12 years old had increased cod-IgE values: 7.75 (3.885-23.25) KU/lt compared to adolescents [3.76 (2.46-6.4), $p=0.03$]. No differences were observed in respect to baseline characteristics between included and drop-out adolescents. The mean duration of fish allergy in those who acquired tolerance was 8.2 ± 4.2 years (range: 1-14 years). Tolerance increased with increasing age, starting from 3.4% in 4- to 5-year-old children to 45.6% in adolescents (Figure 3). Survival analysis predicted

resolution of 45.8% (95%, CI: 26.3%-79.7%) 18 years after the initial reaction (Figure 4). The model did not change even after excluding children with cod IgE $\geq$ 20 KU/LT, who were not subjected to the cod challenge.

The number of reported reactions prior to initial presentation did not differ significantly between tolerant and persistent allergic children either within the whole group (tolerant: 3, IQR: 2 vs allergic: 3, IQR: 2, p=0.96) or in the adolescents (tolerant: 3, IQR: 1, allergic: 3 IQR: 2, p=0.26). Of the 45 fish-allergic children, 39/41 (95%) tolerated tuna and 33/35 (94%) swordfish. No placebo reactors were recorded in the blinded challenges.

Predictors of fish allergy resolution

Detailed data on initial, peak, pre-challenge cod-sIgE and rGad c 1 levels for all challenged patients are provided in Table E1 in the online repository. Peak cod-IgE levels were significantly lower in tolerant children compared to actively allergic ones (median: 4.49, IQR: 7.85 vs 18.3, IQR: 42.36 kUA/L respectively, p < 0.01) (see Figure E1A), while similar trends were observed for pre-challenge cod-IgE and rGad c 1 levels (median: 3.76, IQR: 3.02 vs 8.23, IQR: 19.78 kUA/L, p< 0.01 and 2.48, IQR: 6.25 vs 7.74, IQR: 22.07 kUA/L, p <0.05) (Figure E1B, E1C). Cod-sIgE levels tended to decrease over time in tolerant children, while no change was noted in the allergy-persistent group (Figure E2). No differences were noted in the initial cod-IgE levels between groups (median, IQR=4.07, 4.82 vs 7.32, 16 kUA/L respectively, p=0.218).

Cumulative SPT values were significantly associated with a positive cod challenge (median: 5, IQR: 3.8 vs 8, IQR: 3 mm, p=0.02 and 24.5, IQR: 31.8 vs 34, IQR: 10 mm, p=0.04 respectively) (see Table E2 in the online repository).

In respect to clinical characteristics, cod-tolerant children reported less severe reactions compared to reactive children (Grade 4 reactions; 8% vs 49%, p<0.001) and increased prevalence of allergy-associated diseases (asthma, rhinitis, atopic dermatitis and concurrent food allergy to one or multiple foods), as depicted in Table 1.

Age at first reaction did not differ between fish-tolerant and allergic patients (median: 1.5, IQR: 7.5 vs. 1.25, IQR: 1.5 years, $p=0.53$).

When several predictors were assessed in respect to cod-challenge outcome, pre-challenge cod-sIgE values of 4.87 kUA/L had the best positive predictive value (PPV) (94%), followed by SPT to sardine: 6.5 mm, PPV=92%, f_{426} ; 2.66 kUA/L, PPV=89%; SPT to fish mix: 5 mm, PPV=90%; and cumulative fish SPT: 24 mm, PPV=88%. Respective PPV, NPV and AUC are depicted in Table 2. A cut-off pre-challenge cod-sIgE value of 4.87 KU/lt was the best predictor for fish-allergy persistence (AUC=0.8). Respective AUCs for all predictors assessed are provided in online repository Figure E5.

Sensitization patterns

Immunoblotting to natural extracts from cod, mackerel, tuna and swordfish was performed in 47/58 patients for whom sera were available. The protein content of the various fish extracts was analyzed by SDS-PAGE and Western blot. Fish extracts contained different amounts of parvalbumin (MW 12 kD), as shown by Coomassie staining (Fig. E4A), which reacted with Cyp c 1-specific antibodies to a variable extent (Fig. E4B). Parvalbumin from swordfish and tuna was detected only as a faint band. When patient sera were exposed to cod extract, all tested patients reacted to Gad c 1, confirming parvalbumin as the major fish allergen (Figure 5A). In the case of patients 8 and 27, the band became visible after 1-week exposure time (data not shown), as both patients had very low levels of Gad c 1-specific IgE in ImmunoCAP measurements (0.25 and 0.53 kUA/L respectively). Few patients reacted to additional bands. IgE from 5/47 patients recognized 2-3 bands (patients 15, 17, 34, 46, 54) and 4/47 had IgE specific to more than 3 bands (patients 3, 6, 12, 33). All these patients belonged to the allergic group; none of the tolerant children recognized other allergens apart from parvalbumin. IgE antibodies from all tested patients also recognized parvalbumin from mackerel (Fig. 5B), while recognition of swordfish and tuna parvalbumins was low, which could be explained by the low concentration of parvalbumin in these extracts. Fish-tolerant

patients had significantly lower cod- and mackerel-parvalbumin relative values compared with those with persistent allergy (0.15 vs 0.42, $p < 0.05$ and 0.17 vs 0.36, $p < 0.05$ respectively), while marginal differences were observed for tuna parvalbumin (0.08 vs 0.1, $p = 0.09$).

DISCUSSION

To the best of our knowledge, this is the largest study on the natural history of fish allergy using food challenges and objective markers. It shows that a considerable proportion of children with fish allergy acquire tolerance, frequently during adolescence, despite the commonly accepted perception that fish allergy persists for life^{28,29}. Our data deriving from a large population subjected to multiple challenges following a predefined pathway with a wide range of observation time demonstrate considerably higher resolution rates compared with previous studies with a smaller number of participants, which presented fish-allergy resolution in only 15-17% of children^{13,30}. Nevertheless, recent data from a retrospective study are in line with our findings, showing that up to 63% of patients may overcome fish allergy³¹. In our cohort, fish allergy most often appeared in the 1-2 first years of life, while none of the children included presented fish allergy after the age of 5 years. The 95% confidence intervals of the estimated resolution rate ranged from a minimum of 26% to a maximum of 79%, depending on age. To a considerable extent, this is influenced by the high-resolution rate in the relatively small number of adolescents ($n = 11$) in the study. Similar time trends were previously shown in children with shrimp allergy, regarding which, although it is typically considered a persistent long-lived allergy, sensitization rates to respective proteins decrease with age³². Clearly, adolescents are a challenging group in regard to study retention and compliance. Nonetheless, the risk of bias in recruitment or retention is towards more severe forms of disease that have a lower chance of tolerance development, as also supported by our data. Therefore, our findings represent a conservative estimate of the true resolution rate.

Moreover, this study demonstrates that, despite cross-reactivity between fish species³³, there are at least some fish that can be tolerated by the majority of the children (tolerance rates to swordfish: 94% and tuna: 95%), probably in reflection of their parvalbumin content and/or composition. These findings are clinically relevant, taking into account that in other studies the tolerance rates to other fish are lower^{6,31}. Tolerance to at least one fish species can be very important for allergic children, as they can in fact consume fish, which has beneficial effects on health due to the high omega-3 content of fish³⁴. The European Food and Safety Authority (EFSA) recommends the consumption of 1-2 servings of fish per week, which is associated with a lower risk of coronary heart disease (CHD)²⁶.

We identified several laboratory features predicting fish allergy resolution. Pre-challenge cod-specific IgE (f3), cod rGad c 1 (f426), as well as SPT to sardine and cumulative SPT were the most predictive ones in this population. Tolerance development is associated with generally lower (or dropping) IgE, while more severe/polyallergic cases tend to persist. Moreover, a value of cod IgE ≥ 4.87 kU/L was associated with a high probability of a positive food challenge to cod in the children being challenged (positive predictive value: 94%).

Participants and drop-outs did not differ in respect to most baseline characteristics, except for allergy-associated conditions that were more prevalent in participants, suggesting that patients with a less complex/milder disease profile may have had less motivation to follow such a demanding food-challenge schedule. A high percentage of children reported a reaction during their first fish consumption, which might be due to recall bias or sensitization through skin, since 97% of the cases had a positive history of atopic dermatitis.

Selective tolerance to some fish species -associated with parvalbumin content- may be linked to their phylogenetic distance. The phylogenetic tree of ray-finned fish (Figure E3) reveals a long distance between cod and tuna/swordfish (different order), as tuna and swordfish belong to different families of the Perciformes order.

There are some limitations to our study that may underrepresent the true rate of fish-allergy resolution. We classified children with sIgE value to cod (f3) >20 kU/L (n=10) as fish-

allergic without a challenge, based on previously published >95% positive predictive value of reaction to fish¹⁶; however, these data derive from a different population. Moreover, the possibility of a negative challenge to cod and, thus, tolerance to fish cannot be completely excluded. We used mainly commercial extracts for SPTs for availability reasons, although these might have a lower sensitivity. Also, children who had dropped out of the study had a milder disease profile and this may have resulted in an underestimation of tolerance rate. Furthermore, it is possible that some patients with a positive reaction to cod might have overcome their allergy, but no such information was recorded, since they were not followed-up. Although the initial diagnosis was not always challenge-confirmed, age at first reaction, number of reported episodes prior to inclusion and reaction following skin contact with fish were not different between eventually tolerant and persistently allergic patients, and so, they did not support a true diagnosis.

In conclusion, we show that fish allergy in children starts early, mostly during the first two years of life; a considerable proportion of children will outgrow it, particularly those with less severe reactions and a lower level of sensitization. Those who continue being allergic may still tolerate several fish species such as tuna and swordfish.

Studies are underway to evaluate the relative allergenicity of other fish species, in order to provide more options for our patients, as well as to observe prospectively if the consumption of fish with low allergenicity could interfere with the natural history of the disease.

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Table 1. Patient characteristics including demographic and clinical data.

Patient Characteristics	Fish-allergic (n=45)	Fish-tolerant (n=13)	Total (n=58)	Declined/ Dropped-out (n=38)	P values
Sex, n (%)					
Male	27 (60)	8 (62)	35 (60)	23 (61)	ns
Age in years, mean (SD)	9.5 (3.9)	10.2 (4.2)	9.7 (3.9)	10.1 (4.2)	ns
Age at first reaction, median IQR)	1.25 (1.5)	1.5 (1.75)	1.25 (1.5)	1.3 (1)	ns
Number of children who consumed fish before their first reaction, n (%)	18 (40)	4 (31)	22 (38)	20 (52)	ns
Number of reactions, median (range)	3 (1-11)	3 (1-6)	3 (1-11)	3 (1-9)	ns
Number of reactions, n (%)					
1-3	29 (65)	8 (62)	37 (64)	28 (74)	ns
4-6	15 (33)	5 (38)	20 (34)	7 (18)	ns
>7	1 (2)	0 (0)	1 (2)	3 (8)	ns
Other atopic conditions, n (%)					
Atopic dermatitis	43 (96)	13 (100)	56 (97)	15 (40)**	<0.001
Mild	8 (19)	3 (23)	11 (20)	4 (27)	ns
Moderate/severe	35 (81)	10 (77)	45 (80)	11 (73)	ns
Asthma	30 (67)	9 (69)	39 (67)	10 (26)**	<0.001
Early wheezing	15 (50)	4 (44)	19 (49)	6 (60)	ns
Allergic rhinitis	17 (38)	5 (38)	22 (38)	8 (21)	ns
Any food allergy besides fish, n (%)	31 (69)	9 (69)	40 (69)	9 (24)**	<0.001
Egg	19 (42)	4 (44)	23 (58)	4 (44)	ns
Nuts	16 (36)	4 (44)	20 (50)	5 (56)	ns
Cow's milk	14 (31)	3 (33)	17 (35)	3 (33)	ns
Legumes (lentil)	13 (29)	0 (0)	13 (33)	1 (11)	ns
Wheat	5 (11)	0 (0)	5 (13)	0 (0)	ns
Fruits	4 (9)	1 (11)	5 (13)	1 (11)	ns
Allergy to (only) 1 food besides fish, n (%)	6 (13)*	5 (56)	11 (27)	3 (33)	0.04
Allergy to (only) 2 foods besides fish, n (%)	10 (22)	4 (44)	14 (35)	3 (33)	ns
Allergy to >2 foods besides fish, n (%)	15 (33)*	0 (0)	15 (38)	3 (33)	<0.001
Food allergy to mollusks/crustaceans, n (%)	4 (9)	0 (0)	4 (7)	2 (5)	ns
Type of symptoms of reaction, n (%)					
Grade 1	7 (16)*	9 (69)	16 (27)	8 (21)	<0.001
Grade 2	11 (24)	2 (15)	13 (22)	10 (26)	ns
Grade 3	4 (9)	1 (8)	5 (9)	2 (5)	ns
Grade 4	22 (49)*	1 (8)	23 (40)	18 (48)	0.008
Grade 5	1 (2)	0 (0)	1 (2)	0 (0)	ns
Reaction after skin contact with fish, n (%)	26 (58)	5 (38)	31 (53)	19 (32)	ns
Reaction after inhalation of fish, n (%)	18 (40)	3 (23)	21 (36)	8 (21)	ns

* Significantly different from fish-tolerant group (p<.05)

** Significantly different from total group (p<.05)

Abbreviations: SD, Standard Deviation; IQR, Interquartile Range

Table 2. Measures of allergen sensitization as predictors of persistent fish allergy.

	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC
f3 (cod) (kU/L)	4.87	0.73	0.85	0.94	0.48	0.8
f426* (kU/L)	2.66	0.85	0.64	0.89	0.54	0.74
SPT cod (mm)	6.5	0.82	0.46	0.84	0.43	0.62
SPT tuna (mm)	2.5	0.93	0.31	0.82	0.57	0.61
SPT salmon (mm)	2.5	0.97	0.4	0.86	0.8	0.66
SPT fish mix (mm)	5	0.88	0.6	0.9	0.55	0.66
SPT cumulative (mm)	24	0.92	0.5	0.88	0.33	0.72

Abbreviations: SPT, Skin Prick Test; PPV, Positive Predictive Value; NPV, Negative Predictive Value; AUC, Area Under the Curve

*f426 rGad c1 from cod fish

Figure 1. Flowchart of participants included in the study.

Challenges performed and drop-outs are presented in detail. Number of participants eligible for evaluation of the natural history of fish allergy are depicted at each step. OFC: open food challenge, DBPCFC: double-blind, placebo-controlled food challenge.

Figure 2. Age of first reaction to fish.

The age of first reaction ranges between 0.5-5 years (median age: 1.25 years).

Figure 3. Tolerance acquisition in children with fish allergy per age group.

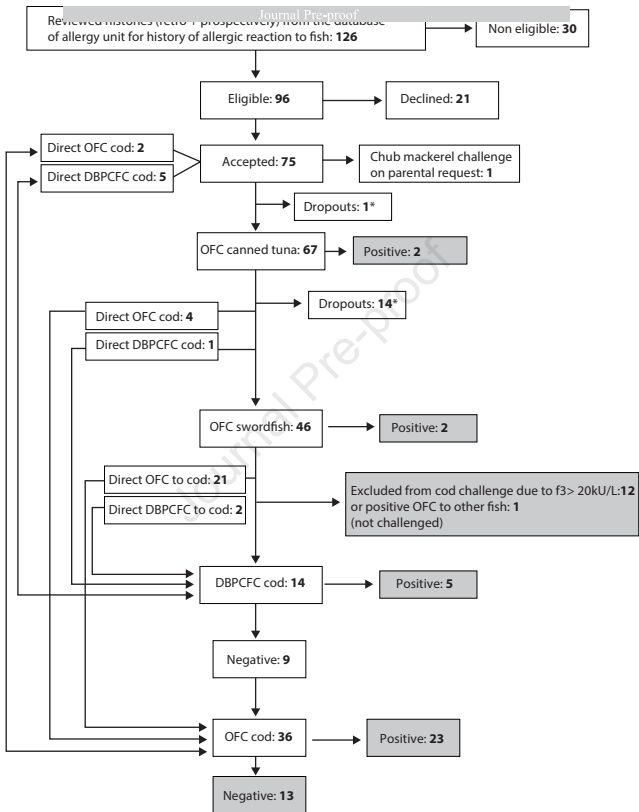
In each age group, the number of participants included, challenged (according to the study protocol) and tolerated (negative) or reacted (positive) as well as the resolution rate (%) are shown.

Figure 4. Fish-allergy resolution over time.

A Kaplan-Meier survival curve depicting the time from first reaction (in years) to tolerance induction in children with fish allergy.

Figure 5. Serum-IgE reactivity to proteins in different fish extracts

IgE reactivity to proteins in fish extracts from a) cod, b) swordfish, c) mackerel and d) tuna. Nitrocellulose-blotted extracts were incubated with sera from fish-allergic patients (lanes 1-50), serum from a fish-allergic patient (P) and non-allergic (N) control and buffer control (B). Bound IgE was detected with ¹²⁵I-iodine-labeled anti-IgE antibodies and visualized by autoradiography. The exposition time of immunoblots was 1 day.



* Dropped out due to demanding protocol

Figure 2. Age of first reaction to fish.



Figure 3: Tolerance acquisition in children with of fish allergy per age group.

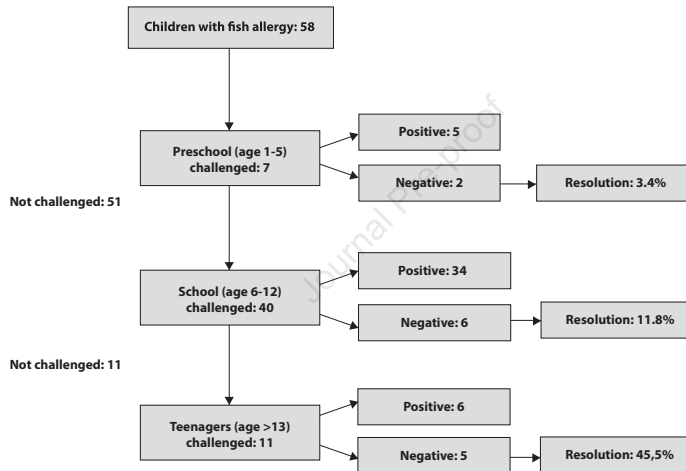


Figure 1. Fish allergy resolution over time.

