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LETTER TO THE EDITOR

CROSS REACTIVITY BETWEEN RECOMBINANT PARVALBUMIN OF CARP AND COD AND RECOMBINANT GRASS MOLECULES

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To the Editor,

Pollen allergy is very common in clinical practice. Some allergenic proteins are highly conserved and are present in homologous forms that may be unrelated between them. These molecules are defined panallergens: they are responsible for IgE cross-reactivity between unrelated pollen and plant food allergen. Sensitization to panallergens might be problematic as it implicates the risk of developing multiple sensitizations. However, other molecules have an allergenic specificity and are defined as primary or "genuine" molecules (1, 2). Panallergen structures may be conserved among proteins with similar function (3). Recently, the European Academy of Allergy and clinical Immunology (EAACI) published evidence-based guidelines for Food Allergy and Anaphylaxis (4). The allergens were classified in different groups. Grass pollens, belonging to *Poaceae* family, are classified in many genera (5). There are several molecules belonging to *Phleum pratense* (Phl p) species. The different Phl p sensitization patterns depend on the geographic area. Patients sensitized to the epitopes Phl p1, Phl p 2, Phl p5, and Phl p 6 are considered primarily sensitized to *Gramineae*; whereas patients sensitized only to nCyn d 1 are sensitized to *Cynodon dactylon* or *Zea mays*; patients sensitized to Phl p 6 are sensitized to *Lolium perenne*. In clinical practice, sensitization to Phl p 5 is considered a marker for genuine grass sensitization. Phl p 7 and Phl p 12 are the main cross-reactive components: Phl p 7 is a calcium-binding protein and Phl p 12 is a profilin (6). Sensitization to Phl p 1, 2, and 5 predicts successful response to allergen immunotherapy (7).

Gad c 1 is the main allergen of cod (*Gadus callaria*) and is a heat-resistant and gastro-resistant parvalbumin. Gad c 1 is a sarcoplasmic protein belonging to the family of muscular calcium-binding proteins.

Cyp c 1 is the main allergen of carp (*Cyprinus carpio*) and is a parvalbumin. Cyp c 1 share 70% epitopic homology with cod (Gad c 1), tuna (Thu a 1), and salmon (Sal s 1) parvalbumins (8). In clinical practice, sensitization to the carp parvalbumin is detectable in over 95% of people with allergy to seafood. Therefore, Cyp c 1 sensitization is

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an excellent marker of seafood allergy. There is evidence that various species of fish significantly cross-react with grass pollen, especially in patients sensitized to cod parvalbumin (9). In this regard, molecular diagnostics is fundamental in poly-sensitized patients (10).

The current study investigated the possible relationship between cod/carp and grass sensitizations and the pattern of grass sensitization based on parvalbumin sensitization.

MATERIALS AND METHODS

In this study, 387 pediatric patients (228 males and 159 females, median age 11.9 years) were enrolled. The parents (or the guardians) signed an informed consent and the procedure was approved by the Ethics Committee of the Policlinico San Matteo. Thorough medical history was performed. Moreover, *in vivo* tests (skin prick test) and *in vitro* molecular analysis were performed. The subjects were included in the study if they had at least one sensitization to recombinant grass (Cyn d1, Phl 1, Phl 2, Phl 4, Phl 5, Phl 6, Phl 7, Phl 11, Phl 12) and/or cod and carp parvalbumin (Cyp c 1 and Gad c 1).

Serum IgE were measured by ImmunoCAP solid-phase allergen chip (ISAC) test according to the manufacturer's recommendations (Thermo-Fisher, Milan, Italy). The test comes in a four reaction sites glass holder, each of them of 7 x 7 mm. Each site contains 103 individual molecules immobilized on the surface of the slide in triplicate. Analysis of the results was automatically evaluated using a micro-array Image Analyser, using only 20 μL of the patient's serum. The ISAC score was reported as ISAC Standardized Units (ISU-E). Levels < 0.3 were defined as negative test results.

Statistical analysis was made using the statistical toolbox package from Matlab®. Data were described as mean, median, and 25th-75th percentile. The association of categorical variables was assessed by the Wilcoxon test. Categorical variables were compared using *Chi*-squared test. A value of $p < 0.05$ was considered statistically significant.

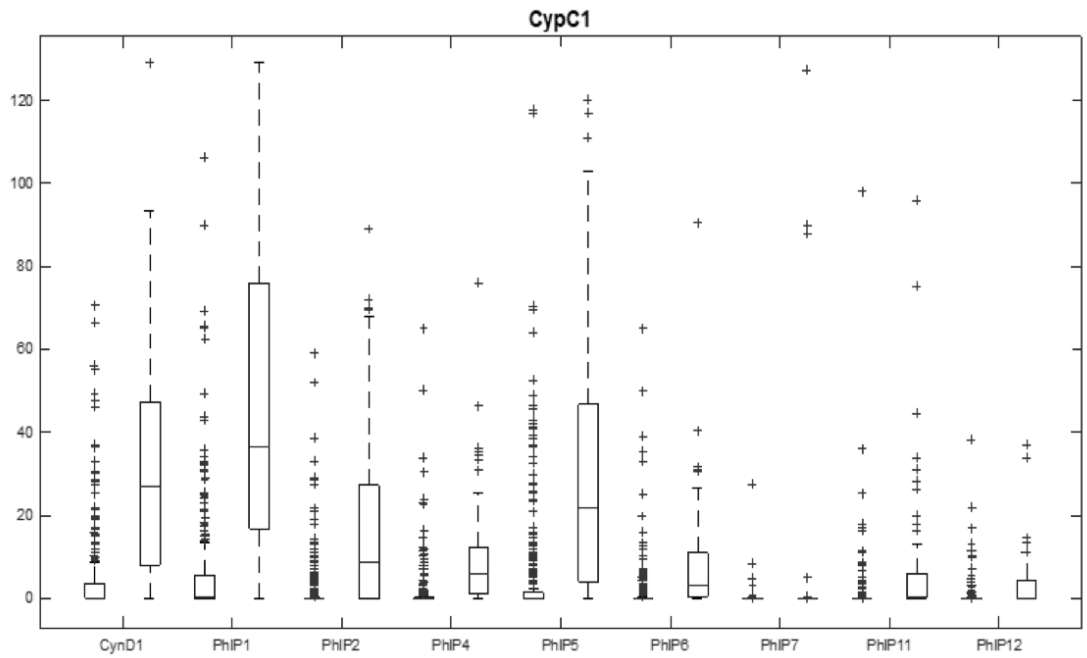

Fig. 1. *Difference in the recombinant grass molecules between the two groups of patients, with and without positivity to Cyp c 1.*

RESULTS

The patients were divided into 4 groups: 321 patients without Cyp c1 sensitization (Group 1), 66 patients sensitized to Cyp c1 (Group 2), 373 patients without Gad c 1 sensitization (Group 3), 14 patients sensitized to Gad c1 (Group 4). Groups 1 and 2 showed a significantly different profile of sensitization to the recombinant grass molecules (Fig. 1).

Groups 3 and 4 did not show any statistically significant difference in the sensibilization profile for any of the recombinant grass molecules. In detail, Wilcoxon test, performed between Group 1 and Group 2, showed significant results for all the molecules, with p-values <0.001 in all cases, except for Phl p 7 (p-value = 0.04). Considering Gad c 1 (Groups 3 and 4), we found no significant association with grass molecules. Notably, all the subjects tolerated fish. In other words, they were only sensitized and not allergic to seafood molecules.

DISCUSSION

Carp is rarely present in the Mediterranean diet, but the current globalization involves considerable variability in the diet of the population. Cross-reactivity between carp and grass allergens was found for Cyp c 1 molecule, although this phenomenon was exclusively immunological as all the subjects tolerated the seafoods to which were sensitized, i.e. they were not allergic. It would be helpful to investigate the immunological mechanisms involved in the cross-reactivity cod/carp-grass, considering the structural analogy between the grass family carp/cod parvalbumin.

In conclusion, a careful interpretation of clinical history and *in vitro* molecular analysis provides an accurate diagnosis of the causative allergen, so the patient can be correctly informed about his/her condition and advised appropriately in relation to the risk of potential cross-reactions to ensure a normal quality of life without dietary restrictions, if not justified.

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REFERENCES

1. Asero R. Looking for sensitization profiles in different populations by recombinant allergens. Int Arch Allergy Immunol. 2014; 164:106-8.
2. Muraro A, Roberts G, Worm M, et al. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. Allergy 2014; 69:1026-45.
3. Andersson K, Lidholm J. Characteristics and immunobiology of grass pollen allergens. Int Arch Allergy Immunol 2003; 130:87-107.
4. Ciprandi G, Scala E, Ariano R, et al. Phelumpratense molecular pattern across Italy. Eur Ann Allergy Clin Immunol 2017: 49:176-80.
5. Das Dores S, Chopin C, Villaume C, Fleurence J, Guéant JL. A new oligomericparvalbumin allergen of Atlantic cod (Gad m I) encoded by a gene distinct from that of Gad c I. Allergy 2002; 57(S. 72):79-83.
6. De Martino M, Novembre E, Galli L, de Marco A, Botarelli P, Marano E, Vierucci A. Allergy to different fish species in cod-allergic children: in vivo and in vitro studies. J Allergy Clin Immunol 1990; 86:909-14.
7. Werfel T, Asero R, Ballmer-Weber BK, et al. Position paper of the EAACI: food allergy due to immunological cross-reactions with common inhalant allergens. Allergy 2015; 70:1079-90.
8. Griesmeier U, Vazquez-Cortés S, Bublin M, et al. Expression levels of parvalbumins determine allergenicity of fish species. Allergy 2010; 65:191-98.
9. Mari A, Alessandri C, Bernardi ML, Ferrara R, Scala E, Zennaro D. Microarrayed allergen molecules for the diagnosis of allergic diseases. Curr Allergy Asthma Rep 2010; 10:357-64
10. Barocci F, De Amici M, Marseglia GL. Molecular evolution in food allergy diagnosis. Minerva Ped 2016; 68:374-81.