IgE Antibodies to Galactose-α-1,3-galactose, an Epitope of Red Meat Allergen, Cross-react With a Novel Flounder Roe Allergen

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To the Editor:

Sensitization to galactose-α-1,3-galactose (α-Gal) through tick bites causes allergies to various red meats and cetuximab [1]. Cases of α-Gal syndrome have been reported in several countries, and the identity of implicated tick species varies geographically [1-4]. We previously described 30 individuals with red meat allergy, 24 of whom were found to have IgE against α-Gal-containing water-soluble salivary gland proteins of *Haemaphysalis longicornis* [4]. Interestingly, most of the patients also experienced allergic reactions after ingesting flounder in winter [5]. In this study, we aimed to clarify the mechanism of flounder allergy found in patients allergic to red meat.

Thirty patients with red meat allergy (19 men and 11 women; age range, 37-88 years) were enrolled in the study (Supplementary Table 1). Twenty of the 30 patients had experienced allergic reactions after ingesting flounder with roe. The episodes of flounder roe allergy followed the episodes with red meat allergy in most cases, and patients experienced no allergic symptoms after ingesting flounder without roe. Five healthy individuals without food allergy were enrolled as negative controls. The methods used in this study are described in Supplementary Methods.

All the participants had specific IgE to beef (f27) and α-Gal (o215) (Supplementary Table 1). None of the patients examined had specific IgE to flounder meat (f254). Skin prick testing showed that all 5 patients (patients 3, 13, 14, 15, 16) had positive reactions to flounder roe but negative reactions to flounder meat. Five healthy controls reacted neither to heated nor to unheated flounder roe.

IgE immunoblotting of the sera of 5 patients with red meat allergy (patients 1, 3, 4, 5, 16) showed a similar reaction pattern: IgE reacted with the water-soluble beef fraction, water-insoluble beef fraction, and water-insoluble flounder roe fraction, but not with the flounder meat fraction or water-soluble flounder roe fraction (Supplementary Figure 1). Two bands (240 kDa and 140 kDa) were common for the water-

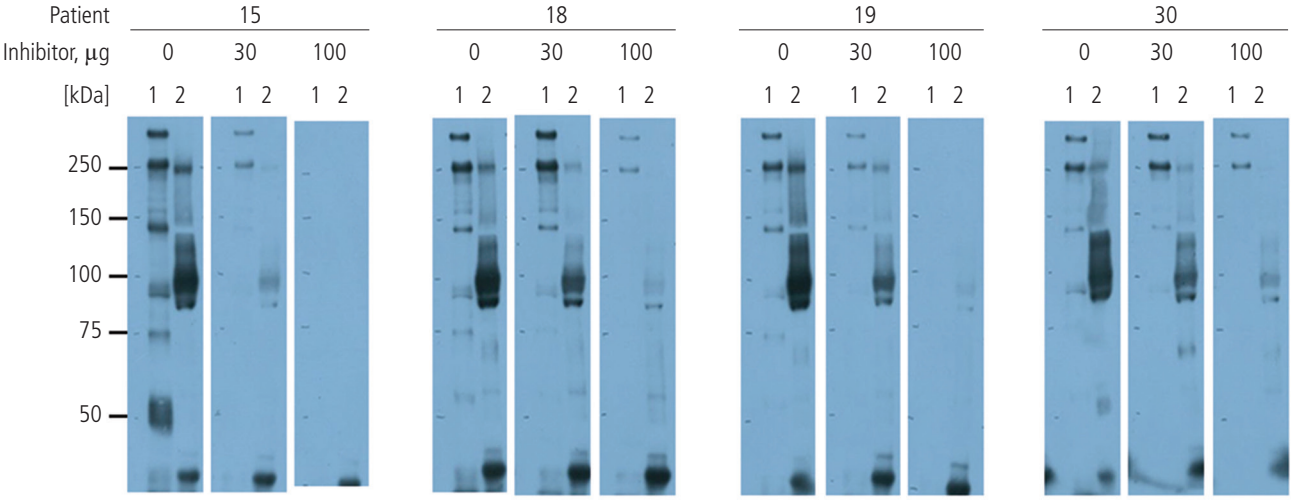

Figure. Cross-reactivity of water-soluble beef proteins and water-insoluble flounder roe proteins in the immunoblot inhibition experiment. Electrophoresed membranes were blotted using patients' sera preincubated with the water-soluble beef fraction as an inhibitor (final concentrations, 0 µg, 30 µg, and 100 µg). Lane 1, water-soluble beef fraction (30 µg/lane); lane 2, water-insoluble flounder roe fraction (30 µg/lane).

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soluble beef fraction, as previously reported [6], and 3 bands (100, 84, and 75 kDa) were commonly observed for the water-insoluble flounder roe fraction. IgE from 23 out of 30 patients (patients 1, 3, 5, 7–9, 10, 12, 13, 15–25, 27, 29, 30) reacted to the water-soluble beef fraction (Supplementary Figure 2). Furthermore, IgE from 27 out of 30 patients (patients 1, 3–5, 7–27, 29, 30) reacted to the water-insoluble flounder roe fraction. IgE-binding to water-insoluble flounder roe proteins was dose-dependently inhibited by the preincubation of sera with water-soluble beef proteins (Figure), indicating a cross-reaction between the two.

To investigate whether IgE bound to carbohydrate moieties of flounder roe proteins, the latter were removed by periodate treatment, as previously described [6]. This approach markedly decreased the density of IgE-binding bands of water-insoluble flounder roe proteins in the samples of all patients tested (patients 15, 18, 19, 23, 30) compared with when periodate treatment was not applied (Supplementary Figure 3). To check for the presence of α-Gal, water-insoluble flounder roe proteins were separated using SDS-PAGE, and glycoproteins were visualized using glycoprotein staining. Several proteins of various sizes were stained as shown. However, no remarkable staining was observed by immunoblotting with an anti–α-Gal monoclonal antibody, indicating that water-insoluble flounder roe proteins are not associated with modification of α-Gal.

IgE immunoblotting of the patients' sera (patients 1 and 5) with water-insoluble flounder roe proteins showed 3 dominant spots (Supplementary Figure 4) corresponding to relative molecular mass values of 100, 84, and 75 kDa. Because the 84 and 75 kDa spots were also detected using the serum of healthy control 2, the 100-kDa protein was further analyzed as the possible dominant flounder roe allergen. The N-terminal amino acid sequence of the 100-kDa protein was NSQSGSNLXADXAGNLM, which was highly matched (identities, 13/17 [76%]; positive, 14/17 [82%]) with the sequence of a protein of the flounder *Platichthys flesus* (accession no. DV56602) based on the DNA Data Bank of Japan (DDBJ). Furthermore, DV56602 has 56% homology with the zona pellucida protein ZPAX in the Japanese rice fish *Oryzias latipes* (accession no. AF331670) in the DDBJ.

In order to determine the entire amino acid sequence of the protein identified at the N-terminus, the full-length cDNA sequence was obtained using the 3′-RACE and 5′-RACE methods. cDNA cloning yielded a clone of 2938 DNA base pairs (bp) (Supplementary Figure 5). The coding region comprised 2739 bp, and the estimated amino acid sequence length was 913 amino acids. Its amino acid sequence was homologous to that of *Oryzias latipes* ZPAX (identities, 568/913 [62%]; positive, 725/913 [79%]), suggesting that the protein identified by full-length cDNA sequencing and N-terminal analysis was ZPAX.

In this study, we report evidence of a novel aspect of α-Gal syndrome, in which patients who already allergic to red meat develop flounder roe allergy owing to a cross-reaction. Interestingly, episodes of flounder roe allergy appeared only during winter and early spring. Since flounder lay roe in winter, we hypothesized that the culprit allergen was flounder roe protein.

Fish roe, including salmon roe and cod roe, is a common food in Japan. The major allergen of salmon roe is the β′-component of vitellogenin, and its homologous β'-components have been identified in rainbow trout roe, flounder roe, and cod roe [7]. Of the 30 patients examined in this study, 20 experienced allergic symptoms to flounder roe, although none had an episode of allergy to other fish roe. In addition, allergen-specific IgE to salmon roe and cod roe was not detected in all the patients we examined. These findings suggest that the IgE against flounder roe allergen identified in this study does not cross-react with other fish roe allergens. A homology search identified *Hippoglossoides dubius* roe allergen in the DDBJ, suggesting that the protein involved could be ZPAX, a member of the zona pellucida protein family. ZPAX has also been identified as a zona pellucida glycoprotein in *Gallus* and *Xenopus* species [8].

In conclusion, we report on patients with red meat allergy who developed flounder roe allergy because of the cross-reaction of their anti–α-Gal IgE to flounder roe allergens, possibly an immunoreactive carbohydrate group(s) structurally mimicking α-Gal in ZPAX.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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