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) due to tick bites causes allergies to various red meats and cetuximab [1]. The α-Gal syndrome cases have been reported in different 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 the 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 showed allergic reactions after ingesting flounder with roe. The episodes of flounder roe allergy followed the episodes with red meat allergy in most of the patients, and they had no allergic symptoms after ingesting flounder without roe. Five healthy subjects without food allergy were enrolled as negative controls. Methods used in this study was described in Supplementary Methods.

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All the subjects had specific IgE to beef (f27) and α-Gal (o215) (Supplementary Table 1). No patient we could examine had specific IgE to flounder meat (f254). Skin prick test showed that all five patients (patients 3, 13, 14, 15, 16) exhibited positive reactions to flounder roe but negative reactions to flounder meat. Five healthy subjects reacted to neither heated nor unheated flounder roe.

IgE immunoblotting of the sera of five patients with red meat allergy (patients 1, 3, 4, 5, 16) showed similar reacting pattern: IgE reacted with water-soluble beef fraction, water-insoluble beef fraction, and water-insoluble flounder roe fraction, but not with flounder meat fraction or water-soluble flounder roe fraction (Supplementary Figure 1). Two bands, 240 kDa and 140 kDa, were commonly seen for the water-soluble beef fraction as previously reported [6], and three bands, 100 kDa, 84 kDa, and 75 kDa, were commonly seen for the water-insoluble flounder roe fraction. IgE of 23 out of 30 patients (patients 1, 3, 5, 7–9, 10, 12, 13, 15–25, 27, 29, 30) reacted to water-soluble beef fraction (Supplementary Figure 2). Furthermore, IgE of 27 out of 30 patients (patients 1, 3–5, 7–27, 29, 30) reacted to water-insoluble flounder roe fraction. IgE-binding to the water-insoluble flounder roe proteins was dose-dependently inhibited by the preincubation of sera with water-soluble beef proteins (Figure 1), 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 manipulation 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 to the density in the condition without periodate treatment (Supplementary Figure 3). To check for α-Gal presence, water-insoluble flounder roe proteins were separated by SDS-PAGE, and glycoproteins were visualized by glycoprotein staining. Several proteins of various sizes were stained as shown. However, no

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remarkable staining was observed by immunoblotting with an anti-α-Gal monoclonal antibody, indicating that water-insoluble flounder roe proteins do not feature α-Gal modification.

IgE immunoblotting of the patients' sera (patients 1, 5) with water-insoluble flounder roe proteins showed three 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 subject 2, the 100 kDa protein was analyzed further 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%) and positives: 14/17 (82%)) to the sequence of a protein of the flounder *Platichthys flesus* (accession No. DV56602) using DNA Data Bank of Japan (DDBJ). Furthermore, DV56602 has 56% homology to zona pellucida protein ZPAX of Japanese rice fish *Oryzias latipes* (accession No. AF331670) on 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 by the 3′-RACE and 5′-RACE methods. cDNA cloning yielded a clone of 2,938 DNA base pairs (bp) (Supplementary Figure 5). The coding region comprised 2,739 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%); positives: 725/913 (79%)), suggesting that the protein identified by the full-length cDNA sequencing and N-terminal analysis was ZPAX.

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

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Fish roe, commonly ingested in Japan, includes salmon roe and cod roe. The major allergen of salmon roe is 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 patients showed allergic symptoms to flounder roe, but neither of them had any episode of allergy to other fish roe. In addition, allergen-specific IgEs to salmon roe and cod roe were not detected in all patients whom we could examine. These findings suggest that the IgE against flounder roe allergen identified in this study does not cross-react to other fish roe allergens. Homology search for our identified *Hippoglossoides dubius* roe allergen in DDBJ suggested that it could be ZPAX, a member of the zona pellucida protein family. ZPAX has also been identified in *Gallus* and *Xenopus* as a zona pellucida glycoprotein [8].

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

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Conflict of interest

The authors have no conflicts of interest to declare.

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References

1. Platts-Mills TAE, Li RC, Keshavarz B, Smith AR, Wilson JM. Diagnosis and Management of Patients with the α-Gal Syndrome. J Allergy Clin Immunol Pract 2020; 8: 15-23. e1.
2. Hamsten C, Starkhammar M, Tran TA, Johansson M, Bengtsson U, Ahlen G et al. Identification of galactose-a-1,3-galactose in the gastrointestinal tract of the tick *Ixodes ricinus*; possible relationship with red meat allergy. Allergy 2013; 68: 549–552.
3. Chinuki Y, Morita E. Alpha-Gal-containing biologics and anaphylaxis. Allergol Int 2019; 68: 296-300.
4. Chinuki Y, Ishiwata K, Yamaji K, Takahashi H, Morita E. *Haemaphysalis longicornis* tick bites are a possible cause of red meat allergy in Japan. Allergy 2016; 71: 421-425.
5. Chinuki Y, Takahashi H, Morita E. Clinical and biological evaluation of twenty patients with red meat allergies. Jpn J Dermatol 2013; 123: 1807-1814 (Japanese).
6. Takahashi H, Chinuki Y, Tanaka A, Morita E. Laminin γ-1 and collagen α-1 (VI) chain are galactose-α-1,3-galactose-bound allergens in beef. Allergy 2014; 69: 199-207.
7. Shimizu Y, Nakamura A, Kishimura H, Hara A, Watanabe K, Saeki H. Major allergen and its IgE cross-reactivity among salmonid fish roe allergy. J Agric Food Chem 2008; 57: 2314-2319.
8. Wong JL, Wessel GM. Defending the zygote: search for the ancestral animal block to polyspermy. Curr Top Dev Biol 2006; 72: 1-151.

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Figure Legends

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