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Allergy International

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Letter to the Editor

Specific immunoglobulin E to Ani s 1 is rare in patients with Anisakis allergy living in Hokkaido, northern Japan

Dear Editor,

Consumption of raw fish contaminated with live larvae of the Anisakidae family leads to Anisakidosis. The Larvae attach to the mucosa of the gastrointestinal tract, resulting in abdominal pain, vomiting, and nausea, although some patients remain asymptomatic. In addition, some sensitized individuals develop hypersensitivity reactions, which is known as Anisakis allergy. *Anisakis simplex* is considered the only Anisakis parasite associated with allergic reactions in humans. To date, 14 proteins (Ani s 1–14) have been identified as *A. simplex* allergens (Table 1). Ani s 1 is particularly important because it could be a possible biomarker to identify patients at risk of severe allergic reaction.¹

The prevalence of antigen recognition by patients with Anisakis allergy was estimated at the time of antigen identification, with reports originating primarily from Spain and Japan (Table 1). Regional differences in the prevalence of antigen recognition remain unclear. This study aimed to estimate the prevalence of antigen recognition among patients with Anisakis allergies living in Hokkaido.

Eight patients who visited the Sapporo Medical University Hospital were included in this study (4 women; age range, 31–87 years). All patients resided in Hokkaido and experienced acute allergic reactions after fish consumption. Seven of the 8 patients had anaphylaxis. Written consent to participate was obtained from all patients. The study was approved by the Ethics Committee of the Sapporo Medical University Hospital (registration number: 292–3443).

IgE specific to *A. simplex*, assessed using the ImmunoCAP system (Thermo Fisher Scientific, Waltham, MA, USA), was detected in all patients (level range, 1.89 to >100.00 UA/mL, median level 22.4 UA/mL). The median interval between the allergic episode and the specific IgE determination was 3.5 weeks (range, 2–84 weeks). Fish allergy was excluded because of the negative result of the fish-specific IgE tests.

Six of the 14 allergens from *A. simplex* were prepared as recombinant allergens (rAni s 1, 4, 5, 6, 9 and 12), and one was a purified natural allergen obtained from 3rd stage larvae (nAni s 3) (Supplementary Methods and Supplementary Reference). IgE specific to each *A. simplex* allergen was detected by ELISA (Supplementary Fig. 1). The concentrations of all the recombinant and natural *A. simplex* allergens were adjusted to 1 µg/ml for ELISA (Supplementary Methods and Supplementary Reference).

The rate at which each allergen was detected is presented in Table 1. The prevalence of IgE specific to rAni s 1 in patients living

in Hokkaido was notably lower than that reported elsewhere. IgE specific to rAni s 1 was detected in one (13%) patient in this study. For comparison, the prevalence of IgE specific to natural Ani s 1 (nAni s 1) has been estimated at 85% in Spain.² In addition, Shimakura reported that 88% of patients from the central part of Japan had IgE specific to nAni s 1.³ Although rAni s 1 has somewhat less IgE reactivity than nAni s 1 at low coating concentrations (1–10 nM), our previous data show that coating concentrations of 30–300 nM provided sufficient IgE reactivity against rAni s 1.⁴ In this study, rAni s 1 was coated at 1 µg/ml (equivalent to 50 nM), no or extremely low reactivity to rAni s 1 was observed, which led us to conclude that the lower prevalence for Ani s 1 is not attributable to differences in protein type (natural or recombinant).

Another notable result is that the prevalence of IgE specific to rAni s 9 (63%) in this study was higher than that reported in Spain (14%).⁵

The prevalence of IgE specific to each allergen was compared between patients with and without anaphylaxis, using Fisher's exact test. There was no inter-group difference in the prevalence of IgE reactivity to any of the studied allergens.

This study reports a lower prevalence of IgE specific to rAni s 1 in patients with Anisakis allergy living in Hokkaido than that reported for patients living in Spain and other parts of Japan. This difference may come from the geographic distribution of sensitizing agents of Anisakis allergy. Most patients with Anisakis allergy are thought to be sensitized by ingestion of Anisakis larvae. Therefore, sensitizing agents of Anisakis allergy can be strongly associated with the causative agents of Anisakidosis. In Japan, there is a geographic variation in the specific pathogens causing Anisakidosis. In Tokyo, most cases of Anisakidosis are associated with *A. simplex*; in contrast, in Hokkaido, 40% cases are associated with *Pseudoterranova decipiens* (sensu. lato.) (Fig. 1, Supplementary Reference). The main fish species associated with Anisakidosis in Tokyo is the Pacific chub mackerel (*Scomber japonicus*), whereas, in Hokkaido, the Pacific cod (*Gadus macrocephalus*) is considered a major carrier of pathogens. Fish species that live in cold currents, such as cod, are intermediate hosts of *P. decipiens*, commonly referred to as cod worms. As aforementioned, a regional difference in fish species may be related to different etiological agents of Anisakidosis and Anisakis allergy.

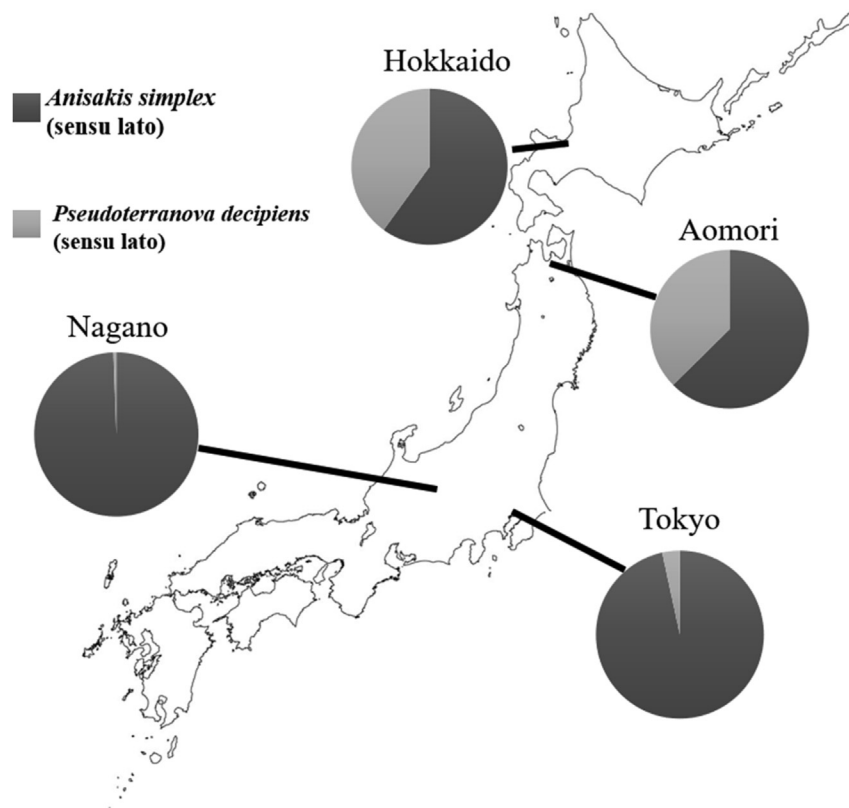
The allergenic potential of *P. decipiens* is being investigated. In mice, *P. decipiens* (sensu. stricto) infection can cause sensitivities and trigger allergic reactions during an oral challenge.⁶ Another mouse study has shown that the antibody responses induced by the crude extract of *A. simplex* and *P. decipiens* are cross-reactive.⁷ Recently, proteomic profiling detected the homologous proteins

Peer review under responsibility of Japanese Society of Allergy.

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Table 1
Comparisons of the prevalence of IgE specific to each *Anisakis simplex* allergen between previous and present studies.

Allergen	Biochemical function	MW (kDa)	Type	IgE prevalence (country, reference)	IgE prevalence (the present study, N = 8)
Ani s 1	Similar to the Kunitz serine protease inhibitor	24	E/S [†]	85% (Spain, Moneo <i>et al.</i> ²) 88% (Japan, Shimakura <i>et al.</i> ³)	13%
Ani s 2	Paramyosin	97	Somatic	88% (Spain, Pérez-Pérez <i>et al.</i> Int Arch Allergy Immunol 2000; 123: 120–9.)	—
Ani s 3	Tropomyosin	41	Somatic	13% (Spain, Asturias <i>et al.</i> Allergy 2000; 55: 898–9.)	50%
Ani s 4	Cysteine protease inhibitor	9	E/S	27% (Spain, Moneo <i>et al.</i> Parasitol Res 2005; 96: 285–9.)	0%
Ani s 5	SXP/RAL-2 family protein	15	E/S	25% (Japan, Kobayashi <i>et al.</i> [Supplementary Reference 3])	25%
Ani s 6	Serine protease inhibitor	15	E/S	18% (Japan, Kobayashi <i>et al.</i> [Supplementary Reference 3])	38%
Ani s 7	Unknown function	139	E/S	94% (Spain, Rodríguez <i>et al.</i> Allergy 2008; 63: 219–25.)	—
Ani s 8	SXP/RAL-2 family protein	15	E/S	25% (Japan, Kobayashi <i>et al.</i> Mol Biochem Parasitol 2007; 155: 138–45.)	—
Ani s 9	SXP/RAL-2 family protein	14	E/S	14% (Spain, Rodríguez-Pérez <i>et al.</i> ⁵)	63%
Ani s 10	Unknown function	21	Somatic	39% (Spain, Caballero <i>et al.</i> Parasitol Int 2011; 60: 209–12.)	—
Ani s 11	Unknown function	27	Unknown	50% (Japan, Kobayashi <i>et al.</i> [Supplementary Reference 5])	—
Ani s 12	Unknown function	31	Unknown	57% (Japan, Kobayashi <i>et al.</i> [Supplementary Reference 5])	50%
Ani s 13	Hemoglobin	37	E/S	64% (Spain, González-Fernández <i>et al.</i> Int J Parasitol 2015; 45: 399–407.)	—
Ani s 14	Unknown function	24	Unknown	54% (Japan, Kobayashi <i>et al.</i> Shokuhin Eiseigaku Zasshi 2015; 56: 194–199.)	—

[†] E/S, Excretory/secretory allergen.**Fig. 1.** Etiological agents of Anisakidosis in Japan. The data included in the Supplementary Reference 7–10 are summarized here.

of Ani s 2, Ani s 3, Ani s 5, Ani s 8, and Ani s 9 in *P. decipiens*.⁸ The missing detection of the Ani s 1 homologue in *P. decipiens* may be associated with the fact that rAni s 1 is rarely detected in patients with Anisakis allergy living in Hokkaido. The prevalence of rAni s 9 in Hokkaido was higher than that reported in Spain where *Pseudoterranova* is rarely located.⁹ This trend might also support our hypothesis in Anisakis allergy.

In summary, the major allergen, Ani s 1, was detected in only 13% of patients with Anisakis allergy living in Hokkaido. Geographical differences in the etiological agents causing human Anisakidosis

may account for the differences in sensitizing allergens in patients with Anisakis allergy.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2021.11.007>.

Conflict of interest

The authors have no conflict of interest to declare.

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Received 14 May 2021

Received in revised form 10 November 2021

Accepted 14 November 2021

Available online xxx

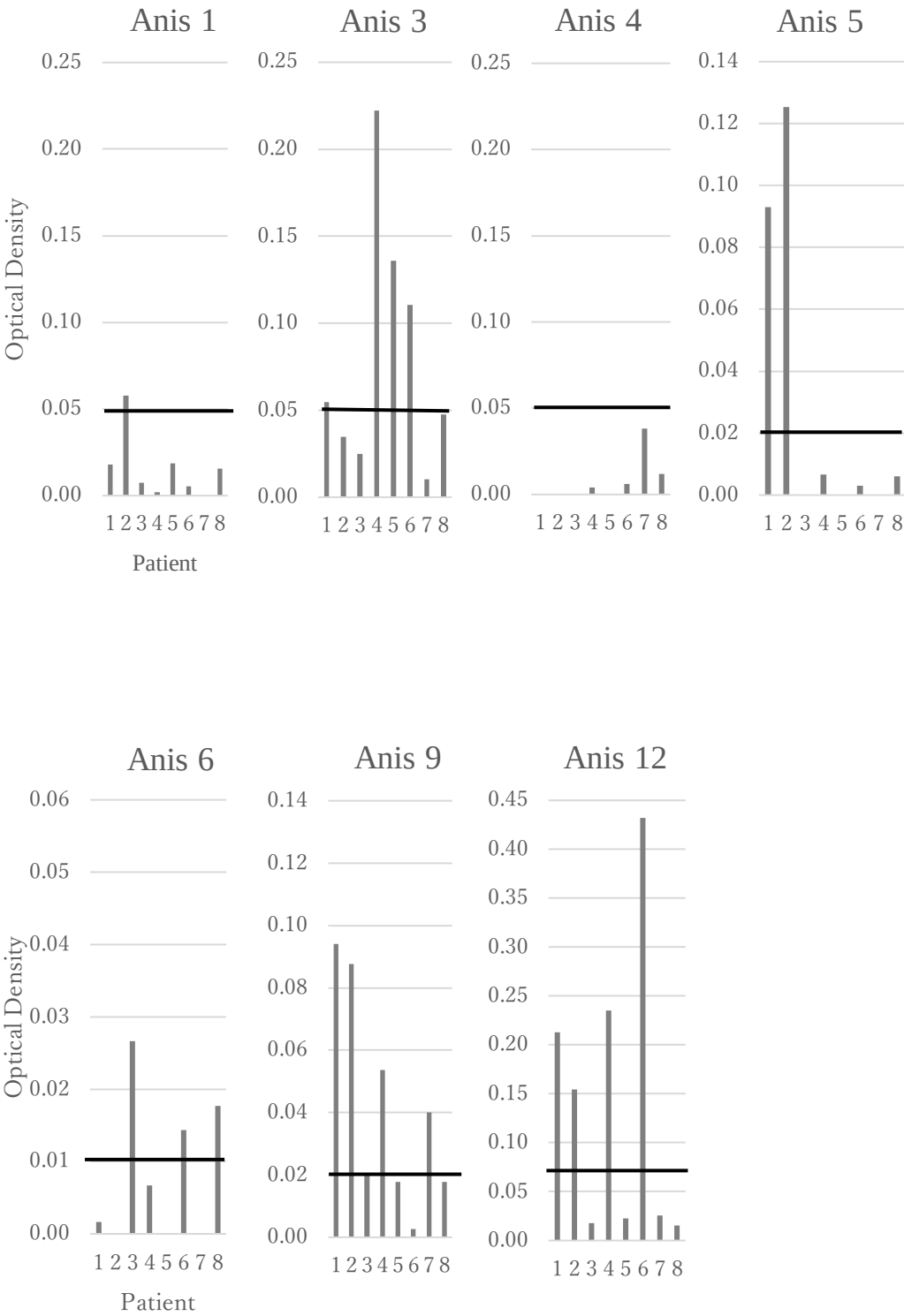
Supplementary Methods

Six of the 14 allergens from *A. simplex* were prepared as recombinant allergens (rAni s 1, 4, 5, 6, 9 and 12), as reported previously [Supplementary References 1,3,4,5], one was purified from 3rd stage larvae (nAni s 3) [Supplementary References 2], according to the modified methods for obtaining disc abalone paramyosin. IgE specific to each *A. simplex* allergen was detected by the ELISA [Supplementary References 6].

The concentrations of all the recombinant or natural *A. simplex* allergens were adjusted to 1 µg/ml for ELISA. Optical density (OD) was calculated at 450 nm. The cut-off values for IgE determination were 0.05 for rAni s 1, nAni s 3, and rAni s 4; 0.02 for rAni s 5 and rAni s 9; 0.01 for rAni s 6; and 0.07 for rAni s 12.

Supplementary Figure 1

The IgE reactivities of eight patients to each allergen. The bars indicate the cut off value for each allergen.



Supplementary Reference

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prepared according above (not reported)
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