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Letter to the Editor

Identification of a novel food allergen in lotus root

Dear Editor,

Nelumbo nucifera, known by various common names, including Indian lotus, sacred lotus, bean of India or simply lotus, is one of two species of aquatic plant in the family Nelumbonaceae belonging to the order Proteales. The flowers, seeds, young leaves and roots are all edible. Lotus root ("renkon" in Japanese, "yeon geun" in Korean and "ŏu" in Chinese) is a popular vegetable in East Asia, however, hypersensitivity reactions to it have not been reported. We report, for the first time, a case of hypersensitivity reactions after consumption of lotus root.

A 6-year-old girl had outgrown food allergies to egg, milk and wheat, mild atopic dermatitis and allergic rhinitis. She had experienced mild perioral erythema after eating a hamburger containing lotus root, ground meat and potato when she was 1 year old and had avoided lotus root during the following 5 years. She suffered urticaria on her face and body immediately after she ate fried lotus root at 6 years old. On this occasion, her mother thought lotus root presented no current risk of allergy because the girl had outgrown her earlier major food allergies. She later developed erythema on her neck where her mother touched with her hands after having handled raw lotus root for cooking.

We suspected lotus root as the source of the causative allergen but the family refused to undergo oral food challenge. We then, instead performed the skin prick test (SPT) with raw and boiled (100 °C, 20 min) lotus root extracts, and considered positive to it, with wheal diameter being 8.5 mm with histamine positive control, 0 mm with saline negative control, 17 mm with raw lotus root and 4.5 mm with boiled lotus root. Four control subjects showed negative reactions to the extracts.

We further performed a basophil activation test using a commercial kit (Allergenicity Kit, Beckman Coulter, Tokyo, Japan), as described elsewhere.[1] Extracts of boiled (100 °C, 5 min) and raw lotus root were prepared as described elsewhere.[2,3] The extracts enhanced CD203c expression on the patient's basophils, but no significant enhancement of CD203c was observed for two control subjects (Fig. 1A).

To identify allergenic proteins in lotus root, immunoblot analysis was perfomed. The lotus root extract at 0.1 mg/ml was electrophoresed on 4–20% SDS-PAGE mini gels (TFCO, Tokyo, Japan) under reducing conditions. As shown in Figure 1B, three prominent bands between 15 and 30 kDa and several minor bands were detected in the raw lotus root extract. The fractionated proteins were then transferred to Immobilon-P Transfer membranes (Millipore, Bedford, MA, USA) and blocked with SuperBlock blocking buffer (Thermo Fisher Scientific, Yokohama, Japan). Serum samples from the patient and two healthy control subjects at 15-fold dilution were applied and incubated overnight. After washing, phosphatase-labeled antihuman IgE antibody (KPL Europe, Guildford, UK), then BCIP/NBT phosphatase substrate (1-component) were applied. Serum from the patient reacted with one of the main electrophoretic bands between 20 and 30 kDa from the raw lotus root extract (Fig. 1B, lane 1). Serum from each of two healthy control individuals did not react to the protein band (Fig. 1B, lanes 2–3).

Lastly, we determined the N-terminal amino acid sequences of the protein from the abovementioned band by Edman degradation. The 10-base N-terminal amino-acid sequence of this band (Thr-Ser-Val-His-Phe-Phe-Leu-His-Ala-Gln) was a perfect match to extracellular ribonuclease LE-like in *Nelumbo nucifera*. Also, this sequence was highly homologous to the storage protein in *Nelumbo nucifera* (Fig. 2). According to a full homology search using NCBI BLAST, the amino acid sequence of extracellular ribonuclease LE-like had 96% homology to the storage protein (Fig. 2).

Lotus root is not commonly consumed as an edible vegetable in Western countries, but it is in East Asian countries, including Japan. Hypersensitivity reactions to the vegetable, however, may have been either overlooked or simply under-diagnosed because of unavailability of specific IgE tests and standardized allergen extracts. In addition, since some vegetables, including the lotus root, are known to contain bioactive substances such as acetylcholine and serotonin, any allergy-like episodes after ingestion of lotus root may have been diagnosed as "pseudoallergy" or non-immunologic reactions. With this first report of IgE-mediated lotus root allergy, we hope that patients with a similar history will be investigated more closely at allergy clinics in countries where lotus root is consumed as a food.

In this study, we identified an IgE-binding 15- to 30-kD protein in crude extracts of lotus root. This protein was considered to be extracellular ribonuclease LE-like in *Nelumbo nucifera*. Extracellular ribonuclease LE-like is a member of the ribonuclease T2 (RNase T2) family. RNase T2 is a widespread family of secreted RNases found in every organism examined to date. T2 family RNases have been implicated in nutrition, phosphate remobilization, self-incompatibility, senescence, and defense against pathogens.[4] Other ribonuclease like pathogenesis-related proteins of the PR10 class have been identified as allergens.[5–7] The extracellular ribonuclease LE-like protein we identified may also be a novel allergen. Further characterization of it is warranted.

The identified sequence was highly homologous to the storage protein in *Nelumbo nucifera*. Moreover, the amino acid sequence of the extracellular ribonuclease LE-like had 96% homology to the storage protein. Various storage proteins in plants have been identified as major allergens in vegetable allergies. For example, 11s globulin (Gly m 5) and 7s globulin (Gly m 6) in soybeans[8] and 2S

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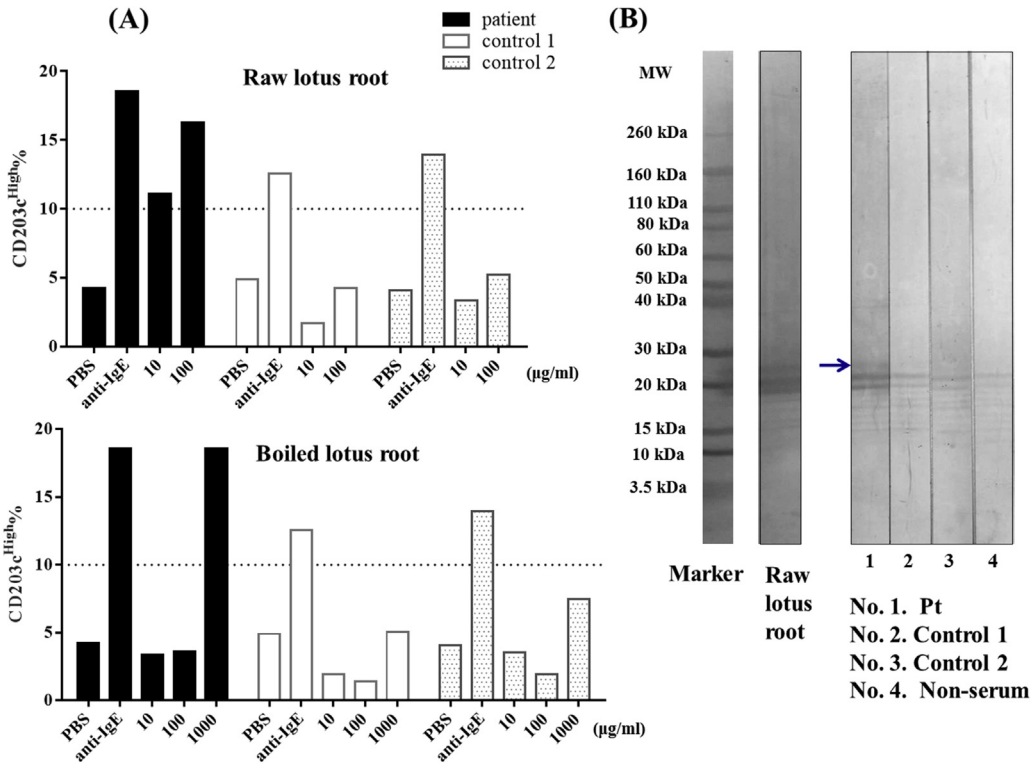


Fig. 1. **(A)** Expression of CD203c on basophils incubated in the presence of raw and boiled lotus root extracts. **(B)** SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis of raw lotus root. MW, molecular weight.

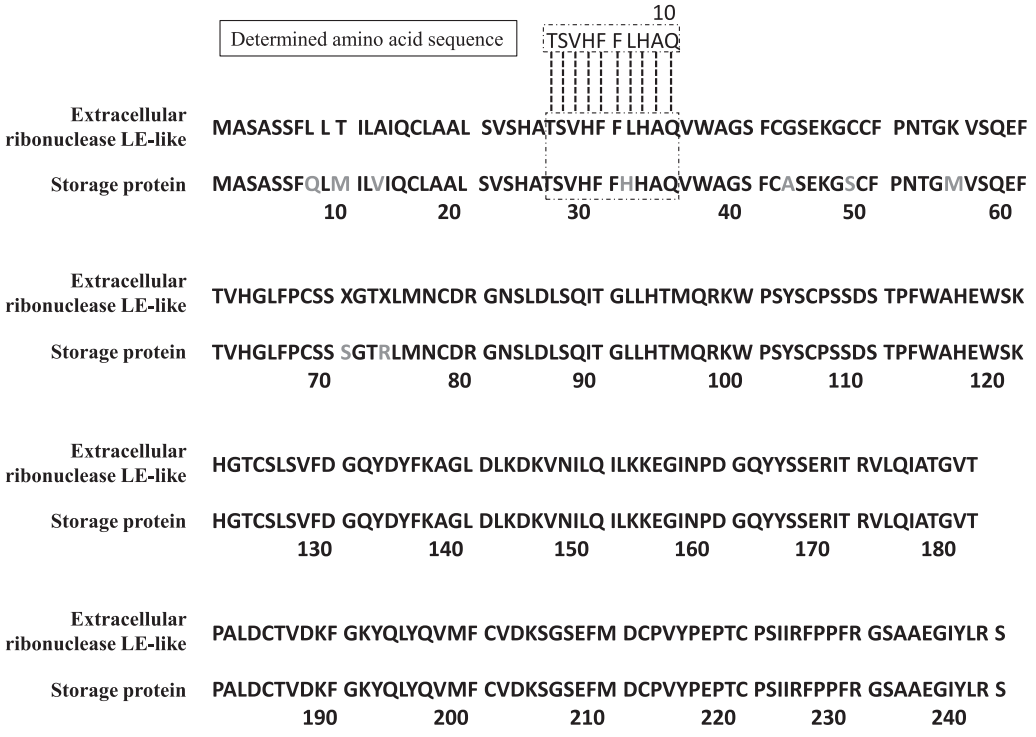


Fig. 2. Sequence similarity between the determined N-terminal of this allergen, extracellular ribonuclease LE-like and the storage protein of *Nelumbo nucifera*.

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albumin (Ara h 2) in peanuts[9] are storage proteins reported to be major allergens in the corresponding foods.

Lotus root is a popular, healthy food consumed in East Asia, and recently in Western countries. For this reason, this first reported case of hypersensitivity reactions and identification of the offending allergen should be of particular interest to allergists, who need to be aware of this “new” food allergen.

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Conflict of interest

The authors have no conflict of interest to declare.

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