Identification of allergens in Azuki (Adzuki) bean allergy

Kitamura K[1]*, Sato N[2,3]*, Nakamura M[2,3], Iwawaki Y[1], Matsui T[1], Takasato Y[1], Sugiura S[1], Matsunaga K[2], Ito K[1,2,4]

[1]Department of Allergy, Allergy and Immunology Center, Aichi Children's Health and Medical Center, Obu, Japan.
[2]Department of Integrative Medical Science for Allergic Disease, Fujita Health University School of Medicine, Nagoya, Japan.
[3]General Research and Development Institute, Hoyu Co., Ltd., Nagakute, Japan.
[4]Comprehensive Pediatric Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan.
*Equal contributions.

Corresponding Author:

Katsumasa Kitamura
Department of Allergy, Aichi Children's Health and Medical Center
Address: 7-426 Morioka-cho, Obu, Aichi, 474-8710, Japan
Email address**:** katsumasa_kitamura@sk00106.achmc.pref.aichi.jp

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Vigna angularis, or azuki or adzuki bean, is widely consumed in East Asia and traditionally used in various sweets in Japan, Korea, and China [1]. Although azuki beans belong to the same *Fabaceae* family as soybeans, they are usually consumed by those allergic to soybeans. There have only been a few case reports of azuki bean allergy, and no recorded allergens for azuki beans in the WHO/IUIS nomenclature database [2-4]. Here, we present the antigen analysis of azuki beans in a patient with azuki bean and soybean allergy, who was considered sensitized to each allergen independently, without cross-reactivity, based on antigen analysis.

A 2-year-old girl with mild atopic dermatitis and food allergies to shrimp and macadamia nuts visited our hospital with oral itchiness and urticaria after consuming azuki beans and soybeans at different times, both of which she had previously consumed without any symptoms. Serological testing revealed a total nonspecific immunoglobulin E (IgE) level of 368 IU/mL and a soybean-specific IgE level of 18.1 kU_A/L (ImmunoCAP®, Thermo Fisher Scientific, Sweden). The skin-prick test (SPT) showed positive results for azuki bean paste (azuki beans and sugar; wheal diameter, 14 × 5 mm) and soybean extract (Torii Pharmaceutical, Japan; 11 × 7 mm). The results for the positive (10 mg/mL histamine dihydrochloride) and negative control (glycerin 50% [w/w] and sodium chloride 5% [w/w]) were 9 × 6 mm and 0 × 0 mm, respectively. A positive SPT

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reaction was defined as a wheal of ≥3 mm or more than half the diameter of the positive control. Informed consent was obtained from the guardians of the patient. This study was approved by the ethics committees of Aichi Children's Health and Medical Center (2022009) and Fujita Health University (HM22-269).

Oral food challenges (OFC) were performed for diagnosis [5], and wheezing and perioral urticaria were observed with 33 g of azuki bean paste (protein dose, 3.2 g); transient cough, local urticaria, swollen lips, and irritability were observed with 60 g of tofu (soybean protein dose, 4.0 g). Based on these results, the patient was diagnosed with a food allergy to azuki beans and soybeans. Azuki beans were restricted in her diet, whereas she continued to consume soybeans in small amounts.

To investigate the antigens and cross-antigenicity of each legume, we performed SDS-PAGE followed by western blotting (1D-WB) and inhibition tests using azuki beans and soybeans as previously described (Figure) [6]. WB confirmed specific reactions to azuki beans and soybeans. In the inhibition test, azuki beans inhibited reactions to azuki beans, soybeans inhibited reactions to soybeans, and there was no inhibition between azuki beans and soybeans.

To identify the antigens of azuki beans and confirm the cross-antigenicity with soybeans, we performed a two-dimensional gel electrophoresis of azuki beans followed by WB (2D-WB), an inhibition test using soybean, and mass spectrometric analysis using the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/) (*Vigna angularis*) as described previously [6]. 2D-WB showed significant IgE antibody responses against azuki beans (Supplementary Figure S1). Among these responses, soybeans did not inhibit proteins whose IgE antibody response was inhibited

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by azuki beans. These proteins were identified by mass spectrometry as beta-conglycinin beta subunit 1 (7S globulin) and glycinin G4-like (11S globulin), both previously reported as azuki bean allergens, and three unreported proteins, including protein ADP-ribosyltransferase PARP3, alpha-1,4 glucan phosphorylase L isozyme, and sucrose binding protein, were newly identified (Supplementary Table S1).

To our knowledge, this is the first report of a patient with combined azuki and soybean allergies. Inhibition analysis revealed no cross-antigenicity between azuki and soybeans, suggesting that the patient developed allergic symptoms due to independent sensitization to azuki and soybeans. We also report new candidates for azuki bean allergens.

If a patient has allergies to multiple legumes, an inhibition test can contribute to clinical patient instructions by confirming whether they are independently sensitized or if there is a risk of symptom induction due to cross-reactivity. The inhibition test showed no cross-antigenicity in the present case, and independent instructions were provided for azuki and soybean intake.

Antigen analysis of azuki beans identified several proteins, including 7S and 11S globulins. Some of these azuki bean allergen candidates were consistent with those identified in a previous report by our group in a patient with azuki bean allergy who cross-reacted with runners and white pea beans [3]. Since these proteins, including previously unreported antigen candidates, were not inhibited by soybeans in the present inhibition 2D-WB, and runners and white peas did not inhibit some proteins in the previous report, they are likely to be independent allergens of the azuki bean allergy. The three newly identified candidates have not been reported as food allergens to date, and further investigation is needed to determine whether they are the major antigens of azuki beans.

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WHO/IUIS database describes six allergens (Vig r 1 to 6) from *Vigna radiata* (Mung bean), with the same genus as the azuki bean; however, none were identical to the proteins identified in this study.

Four cases of azuki bean allergy have been reported [2-4]. One was a 9-year-old boy who had allergic reactions to runners and white peas, likely due to cross-antigenicity from azuki bean 11S globulin. The other three patients were considered allergic to azuki beans only. Although our patient had independent sensitization to azuki beans and soybeans, antigen analysis revealed sensitization to 11S globulin in azuki beans. 11S globulin has been previously reported to be responsible for cross-reactivity in azuki beans, runners, and white peas [3]. The amino acid sequence homology of 11S globulin was 72.7% for azuki beans and runner/white pea beans and 50.6% for azuki beans and soybeans [3]. Additional studies, such as epitope analysis, are needed to clarify why some patients show cross-reactivity whereas others do not.

Here, we describe a case of azuki bean and soybean allergy and the azuki bean antigen analysis results. Because cross-reactivity between legumes varies among patients, a differential diagnosis of cross-antigenicity is helpful. Additional cases and analyses are required to identify the causative antigens of azuki bean allergy.

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Conflicts of Interest

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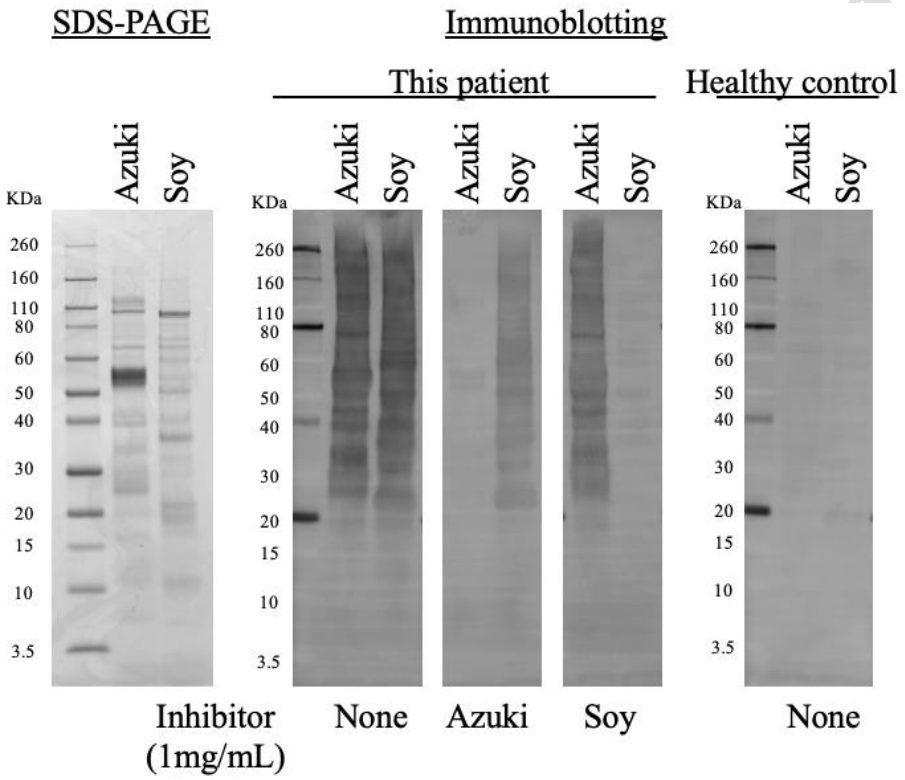
Figure. Antigen analysis of azuki beans and analysis of cross-antigenicity with soybeans Azuki and soybean proteins were isolated using SDS-PAGE and immunoblotted with serum from the patient and healthy control. Cross-antigenicity between legumes was evaluated based on the inhibition of each legume. Healthy control was a volunteer without legume allergy.



Soy, soybean; azuki, azuki bean.

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