Identification of Allergens in Azuki (Adzuki) Bean Allergy

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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 Fabaceae family, as do soybeans, they are usually consumed by persons who are allergic to soybean. There have only been a few case reports of azuki bean allergy, and no allergens for azuki beans have been recorded in the WHO/IUIS nomenclature database [2-4]. Here, we present the results of antigen testing in a patient with azuki bean and soybean allergy. The patient was considered sensitized to each allergen independently, without cross-reactivity, based on the results of the test.

A 2-year-old girl with mild atopic dermatitis and food allergy to shrimp and macadamia nuts visited our hospital with oral itchiness and urticaria after consuming azuki bean and soybean at different times. She had previously consumed both types of bean without 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). 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 control (10 mg/mL histamine dihydrochloride) and negative control (glycerin 50% [wt/wt] and sodium chloride 5% [wt/wt]) were 9 × 6 mm and 0 × 0 mm, respectively. A positive SPT 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 patient's guardians. 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 challenge (OFC) for diagnosis [5] with 33 g of azuki bean paste (protein dose, 3.2 g) revealed wheezing and perioral urticaria; OFC with 60 g of tofu (soybean protein dose, 4.0 g) revealed transient cough, local urticaria, swollen lips, and irritability. Based on these results, the patient was diagnosed with allergy to azuki beans and soybeans. Azuki beans were restricted in her diet, although 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 bean and soybean, as previously described (Figure) [6]. WB confirmed specific reactions to azuki bean and soybean. In the inhibition test, azuki bean inhibited reactions to azuki bean, soybean inhibited reactions to soybean, and there was no inhibition between azuki bean and soybean.

To identify the antigens of azuki bean and confirm cross-antigenicity with soybeans, we performed 2-dimensional gel electrophoresis of azuki bean 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*),

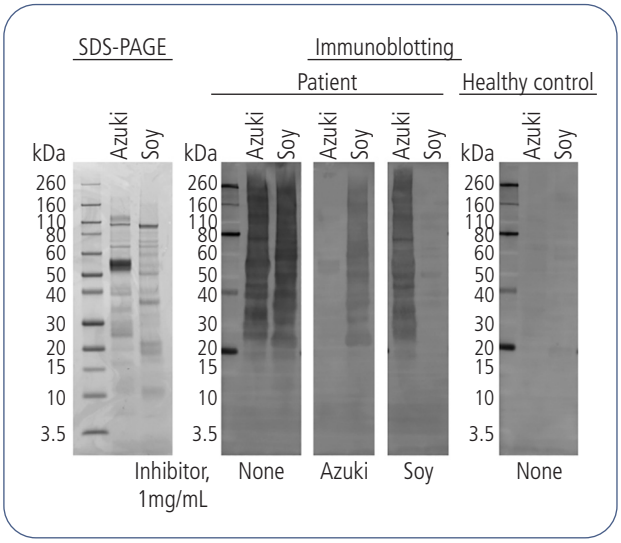

Figure. Antigen testing of azuki bean and analysis of cross-antigenicity with soybean.
Azuki and soybean proteins were isolated using SDS-PAGE and immunoblotted with serum from the patient and a healthy control. Cross-antigenicity between legumes was evaluated based on the inhibition of each legume.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

as described previously [6]. 2D-WB showed significant IgE antibody responses against azuki bean (Supplementary Figure S1). Among these responses, soybean did not inhibit proteins whose IgE antibody response was inhibited by azuki bean. These proteins were identified by mass spectrometry as ß-conglycinin ß subunit 1 (7S globulin) and glycinin G4–like (11S globulin), both previously reported as azuki bean allergens. Three unreported proteins, namely, protein ADP-ribosyltransferase PARP3, α-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 bean and soybean allergy. Inhibition analysis revealed no cross-antigenicity between azuki bean and soybean, suggesting that the patient developed allergic symptoms owing to independent sensitization to azuki bean and soybean. We also report new candidates for azuki bean allergens.

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

Antigen analysis of azuki bean 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 runner bean and white pea bean [3]. Since these proteins, including previously unreported antigen candidates, were not inhibited by soybean in the present 2D-WB inhibition, and runner bean and white pea bean did not inhibit some proteins in the previous report, they are likely to be independent allergens in azuki bean allergy. The 3 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 bean. The WHO/IUIS database describes 6 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 involved a 9-year-old boy who had allergic reactions to runner and white pea, likely due to cross-antigenicity from azuki bean 11S globulin. The other 3 patients were considered allergic to azuki bean only. Although the patient we report was independently sensitized to azuki bean and soybean, antigen analysis revealed sensitization to 11S globulin in azuki bean. 11S globulin has previously been reported to be responsible for cross-reactivity in azuki bean, runner bean, and white pea bean [3]. The amino acid sequence homology of 11S globulin was 72.7% for azuki, runner, and white pea bean and 50.6% for azuki bean and soybean [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 report the results of azuki bean antigen testing. Because cross-reactivity between legumes varies from patient to patient, 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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