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Kidney-bean (*Phaseolus Vulgaris*) Dependent, Exercise-induced Anaphylaxis in Patients Comorbid with Mugwort (*Artemisia Vulgaris*) Pollinosis

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ABSTRACT

Background: The cross-reactive allergen between mugwort (*Artemisia vulgaris*) and kidney bean (*Phaseolus vulgaris*) has not yet been identified.

Methods: A total of 24 patients were included in this study. The sera of patients were analyzed for the concentrations of specific IgE antibodies. The allergenicity and cross-reactivity were investigated by Western blotting and immunoblot inhibitory experiments.

Results: The immunoblotting indicated the binding of patients' IgE to crude mugwort extract at ~26 kDa protein (15 cases), ~60 kDa (15 cases), and 10–15 kDa proteins (12 cases). The results of the immunoblot-inhibition assay showed that kidney bean seed extract inhibited specific IgE binding to mugwort at 10–15 kDa, ~26 kDa, and ~60 kDa in 4 (16.7%), 1 (4.2%) and 2 (8.3%) cases, respectively. On the other hand, mugwort extract was demonstrated to inhibit specific IgE binding to kidney bean seed at 10–15 kDa, 15–20 kDa, ~30 kDa, and 60 kDa in 1 (4.2%), 3 (12.5%), 4 (16.7%), and 3 (12.5%) cases, respectively.

Conclusion: The 26–30 kDa, 10–15 kDa, and 60 kDa proteins are potential causative agents of the cross-reactivity between mugwort and kidney beans. The findings of this study improved the current understanding on the allergenicity of kidney beans and would provide insights into the refinement of treatment strategy for anaphylaxis.

KEYWORDS

Exercise-induced anaphylaxis; mugwort pollinosis; cross-reactivity; serum IgE

Introduction

Anaphylaxis is a systemic hypersensitivity reaction involving several organs and systems that could be life-threatening. Some of the anaphylaxis cases are food-dependent and exercise-induced, meaning the hypersensitivity reaction occurs only with the combination of a specific food and exercise (Barg et al. 2011). Many types of foods have been shown to cause food-dependent and exercise-induced anaphylaxis, such as nuts, eggs, fish, peanuts, pork, shellfish (Castells et al. 2003; Povesi Dascola and Caffarelli 2012).

Pollen-food allergy syndrome, or oral allergy syndrome, is a food allergy caused by cross-reacting allergens found in both pollen and food (Price et al. 2015). The most well-known symptoms of pollen-food allergy syndrome include irritation or swelling of the mouth and throat (Muluk and Cingi 2018). Mugwort pollen is a common cause of allergic rhinoconjunctivitis and asthma in China, and it is known to cross-react with some food (Popescu 2015). Mugwort

pollen-food allergy is often observed in clinical practice. Mugwort-fennel, celery-mugwort-spice, mugwort-peach, mugwort-chamomile, and mugwort-mustard associations have been reported (Egger et al. 2006; Figueroa et al. 2005). Another association is mugwort-kidney bean syndrome, in which patients sensitized to mugwort may develop an allergy to kidney beans and experience anaphylaxis. The reaction is considered as a consequence of immunologic cross-reactivity between mugwort pollen allergen and structurally related protein from kidney bean. To date, cross-reactive allergen has not yet been identified.

In the present study, we investigated the potential causative agents of the cross-reactivity between mugwort and kidney beans, using clinical samples from 24 patients with kidney-bean-dependent, exercise-induced anaphylaxis, and these patients were also allergic to mugwort pollen.

Patients and methods

Study cohort and sample collection

Twenty-four patients with both kidney-bean-induced anaphylaxis and mugwort pollinosis were enrolled in this study. All these patients admitted to the outpatient allergy clinic of Peking Union Medical Hospital (Beijing, China) from 2011 to 2014. They experienced anaphylaxis for at least one time, of which the onsets were developed after they had ingested kidney beans and exercised for 10 to 30 minutes. In the emergency room patients presented blurred vision, amaurosis, nasal obstruction, wheezing, dyspnea, and urticaria over the whole body and were diagnosed with severe anaphylaxis. Mugwort-pollen-induced allergic asthma and rhinitis were concurrent with anaphylaxis. Blood samples were drawn from the enrolled patients and were immediately centrifuged. The resulting sera were aliquoted into new tubes for storage at -80°C until later analysis. All procedures were approved by the ethics committee of Peking Union Medical College (no. 2015-4-21).

Mugwort and kidney bean extract

Mugwort pollen samples, collected from agricultural areas around Beijing, were dried, crushed, and defatted in acetone (w/v: 1:100) at 4°C . To extract the antigens, the defatted samples were continuously stirred for 8 hours at 4°C in phosphate-buffered saline (PBS, pH 7.4) (w/v 1:20).

The suspension was then centrifuged at 12,000 rpm for 15 minutes at 4°C . The resulting supernatant was filtered through a nitrocellulose membrane with a pore size of 0.2 μm , dialyzed against distilled water for 24 hours, aliquoted in small vials (2.15 ml) and lyophilized. The extract was then resuspended with 100 μl of water at a concentration of 2.75 $\mu\text{g}/\mu\text{l}$.

Fresh kidney beans (seeds and pods) purchased from a local supermarket in Beijing were homogenized in PBS (pH 7.4, w/v: 1:100). Extraction, centrifugation, filtration, dialysis, and lyophilization processes were the same as above.

Determination of specific IgE in sera

The concentrations of specific IgE antibodies in sera were determined using a solid phase immunoassay ImmunoCAP 250™ (Thermo Fisher Scientific, Uppsala, Sweden). The tests

were performed per the manufacturer's instructions. The levels of serum IgE were expressed as Level 0 to 6: Level 0, 0-0.35 KUA/L; Level 1, 0.35-0.70 KUA/L; Level 2, 0.70-3.5 KUA/L; Level 3, 3.5-17.5 KUA/L; Level 4, 17.5-50 KUA/L; Level 5, 50-100 KUA/L; Level 6, ≥ 100 KUA/L.

Immunoblotting

The cross-reactivity of patients' sera toward mugwort pollen and kidney beans was confirmed using immunoblotting. In brief, crude extracts of mugwort pollen and kidney beans were resolved (20 μ l per well, 1.5 μ g/ μ l), and were transferred to polyvinylidene fluoride (PVDF) membrane. After blocking with 3% BSA, the PVDF strips were probed with patient's serum that was diluted with BSA by four folds for 2 hours at room temperature. A normal human serum pool served as the control. The strips were washed with PBS-Tween 20 and incubated with anti-human IgE-peroxidase (1:1000 v/v) (Abcam) for 2 hours at room temperature. The IgE binding was then detected using tetramethylbenzidine.

Immunoblot-inhibition assay

To examine the inhibitory effect of kidney bean seed extract on the binding between mugwort and specific IgE antibodies in patients' sera, the kidney bean extract was preincubated with patient's serum for 3 hours at room temperature before incubation with membrane onto which the mugwort extract proteins had been transferred. On the other hand, to investigate the inhibitory effect of mugwort on the binding between kidney bean seed extract and specific IgE antibodies, the mugwort extract was preincubated with patient's serum for 3 hours at room temperature before incubation with membrane onto which the kidney bean seed extract had been transferred. The bound IgE antibodies were detected as the abovementioned.

Results

Serum levels of IgE specific to mugwort and kidney beans

The serum levels of IgE specific to mugwort and kidney beans were quantitatively determined using ImmunoCAP 250™ and were expressed as KUA/L (Table 1). All patients were tested positive with differential levels of IgE antibodies. For mugwort, 5 of 24 patients showed Level 6 (≥ 100 KUA/L); while for kidney beans, the maximum one was Level 4 (≥ 17.5 KUA/L), and there were 5 patients showing this maximal level.

Allergenicity assessment by immunoblotting

The specific binding of patients' IgE to crude mugwort extract was further confirmed using immunoblotting (Figure 1 and Table 2). All patients showed specific IgE binding of crude mugwort extract. Among the cases, there were 18 cases (75%) showing binding to ~26 kDa protein. The number of cases binding to ~60 kDa and 10–15 kDa protein was 15 (62.5%) and 12 (50%), respectively.

Table 1. Serum IgE (sIgE) against mugwort (w6) and kidney beans (f315) as determined using ImmunoCAP250™.

Patient Identification number	Mugwort (w6) KUA/L	Kidney beans (f315) KUA/L
1	100	25
2	58	3.25
3	29.5	7.25
4	0.94	20
5	0.96	22.1
6	35.8	5.5
7	30.2	7.48
8	29	15.4
9	39.2	0.54
10	1.01	2.17
11	74.1	1.42
12	42.8	1.21
13	10.8	6.37
14	32.4	2.54
15	100	7.98
16	21.1	28.3
17	100	22.3
18	4.05	0.69
19	4.33	0.51
20	19.6	3.52
21	100	4.79
22	100	5.22
23	61.9	17
24	5.98	5.4

Remark: sIgE levels of patients were classified as Level 0, 0-0.35 KUA/L; Level 1, 0.35-0.70 KUA/L; Level 2, 0.70-3.5 KUA/L; Level 3, 3.5-17.5 KUA/L; Level 4, 17.5-50 KUA/L; Level 5, 50-100 KUA/L; Level 6, ≥ 100 KUA/L

For the IgE binding to crude kidney bean seed extract, there were 13 positive cases (Figure 2 and Table 3). Ten cases (41.7%) displayed binding to ~30 kDa protein, and 5 cases (20.8%) exhibited binding to ~60 kDa protein. Binding to 10–15 kDa and 15–20 kDa proteins was found in 1 (4.2%) and 5 (20.8%) cases, respectively.

The specific IgE binding of crude kidney bean pod extract was also examined (Figure 3 and Table 4). Out of the 24, 16 patients (66.7%) showed specific IgE binding of crude kidney bean pod extract. There were 5 (20.8%) cases showing scattered binding to 30–80 kDa protein, 11 (45.8%) cases exhibiting binding to 8–15 kDa protein, and a single case (4.2%) displaying a binding to ~20 kDa protein.

Cross-reactivity between mugwort and kidney bean seed

The cross-reactivity between mugwort and kidney bean seed was examined using immunoblot-inhibition assay. Kidney bean seed extract was found to inhibit specific IgE binding to mugwort at 10–15 kDa, ~26 kDa, and ~60 kDa in 4 (16.7%), 1 (4.2%) and 2 (8.3%) cases, respectively (Figure 4 and Table 5). On the other hand, mugwort extract was demonstrated to inhibit specific IgE binding to kidney bean seed at 10–15 kDa, 15–20 kDa, ~30 kDa, and 60 kDa in 1 (4.2%), 3 (12.5%), 4 (16.7%), and 3 (12.5%) cases, respectively (Figure 5 and Table 6).

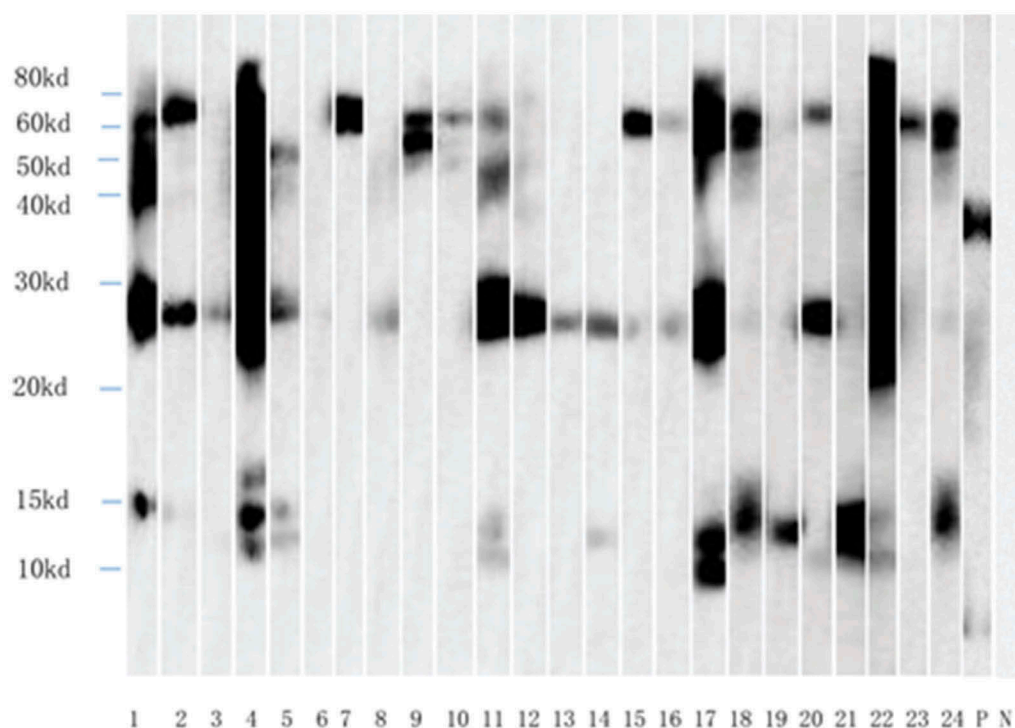


Figure 1. Binding of patients' IgE antibodies to mugwort extract was examined using immunoblotting. Each numbered lane represents a single patient. Lane P and N are positive and negative control, respectively.

Table 2. Molecular weight (MW) and percentage of positive staining of mugwort proteins in immunoblotting.

MW (mugwort)	Number	Percentage
≈26 kDa	18	75%
≈60 kDa	15	62.5%
10–15 kDa	11	45.8%

Total percentage of positivity to mugwort extract is 100%.

Discussion

The present study evaluated the allergenicity of patients with kidney-bean-induced anaphylaxis that was comorbid with mugwort pollinosis. Patients' sera were sensitive to both mugwort pollen and kidney bean extracts. Immunoblotting analysis revealed a 100% positivity of crude mugwort pollen extract, and a 83.3% positivity of crude kidney bean extract of patients' sera. Both mugwort and kidney beans presented reactive proteins with a molecular weight of ~60 kDa and 10–15 kDa. The ~26 kDa protein in mugwort and 30 kDa protein in kidney beans might consist of the same components. Immunoblot-inhibition assay further suggested the cross-reactivity between mugwort and kidney beans. All of these findings implicated the 26–30 kDa, 10–15 kDa, and 60 kDa proteins as potential causative agents of the cross-reactivity between mugwort and kidney beans.

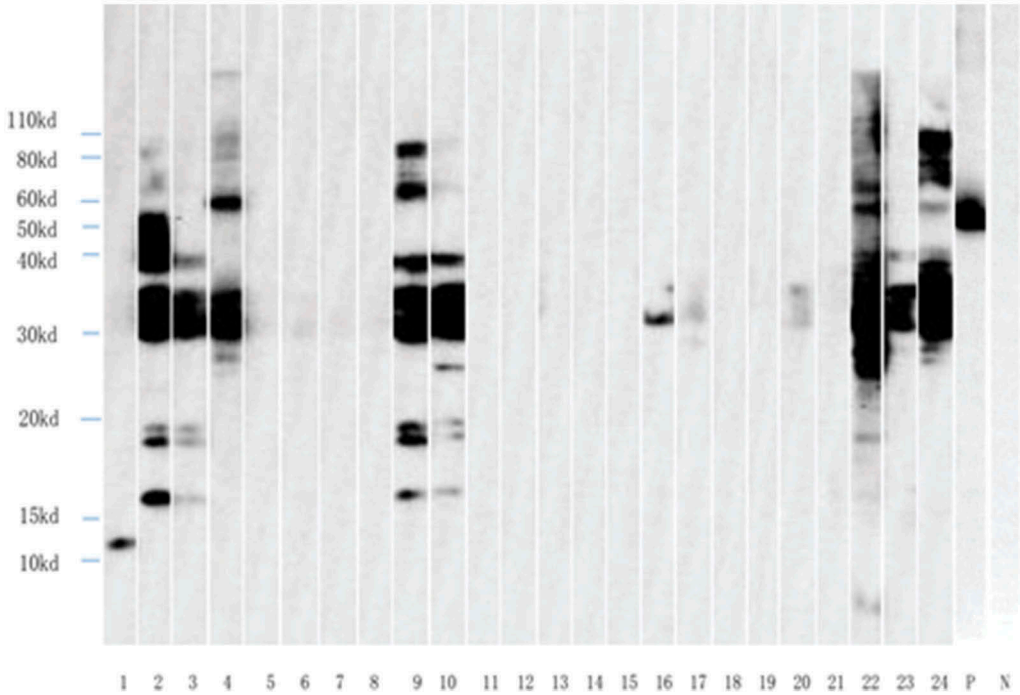


Figure 2. Binding of patients’ IgE antibodies to kidney bean seed extract was examined using immunoblotting. Each numbered lane represents a single patient. Lane P and N are positive and negative control, respectively.

Table 3. Molecular weight (MW) and percentage of positive staining of kidney bean seed proteins in immunoblotting.

MW (seed)	Number	Percentage
≈30 kDa	10	41.7%
≈60 kDa	5	20.8%
15–20 kDa	5	20.8%
10–15 kDa	1	4.2%

Total percentage of positivity to kidney bean seed extract is 54.2%.

These findings are clinically important, because kidney beans are one of the most common foods in China, and in kidney beans, few allergens have been found to induce allergic diseases. No reports about the cross-reactivity between mugwort and kidney bean have been published; therefore, this study evaluated for the first time such cross-reactivity in clinical patients.

Few allergens in kidney beans have been identified, despite the entire spectrum of allergens has yet to be studied. Among the few identified allergens, lectin, a protein component with a molecular weight of 31 kDa, is a potent allergen in kidney beans to induce severe anaphylaxis (Kasera et al. 2013, 2011; Rouge et al. 2011). Pha v3, which is a non-specific lipid transfer protein, was also identified as a 10-kDa allergen (Pastorello et al. 2010; Zoccatelli et al. 2010). Storage proteins like phaseolin (47.5 kDa) (Kumar et al. 2014), class I chitinase (31–32 kDa) (Blanco et al. 1999), and alpha-amylase inhibitor

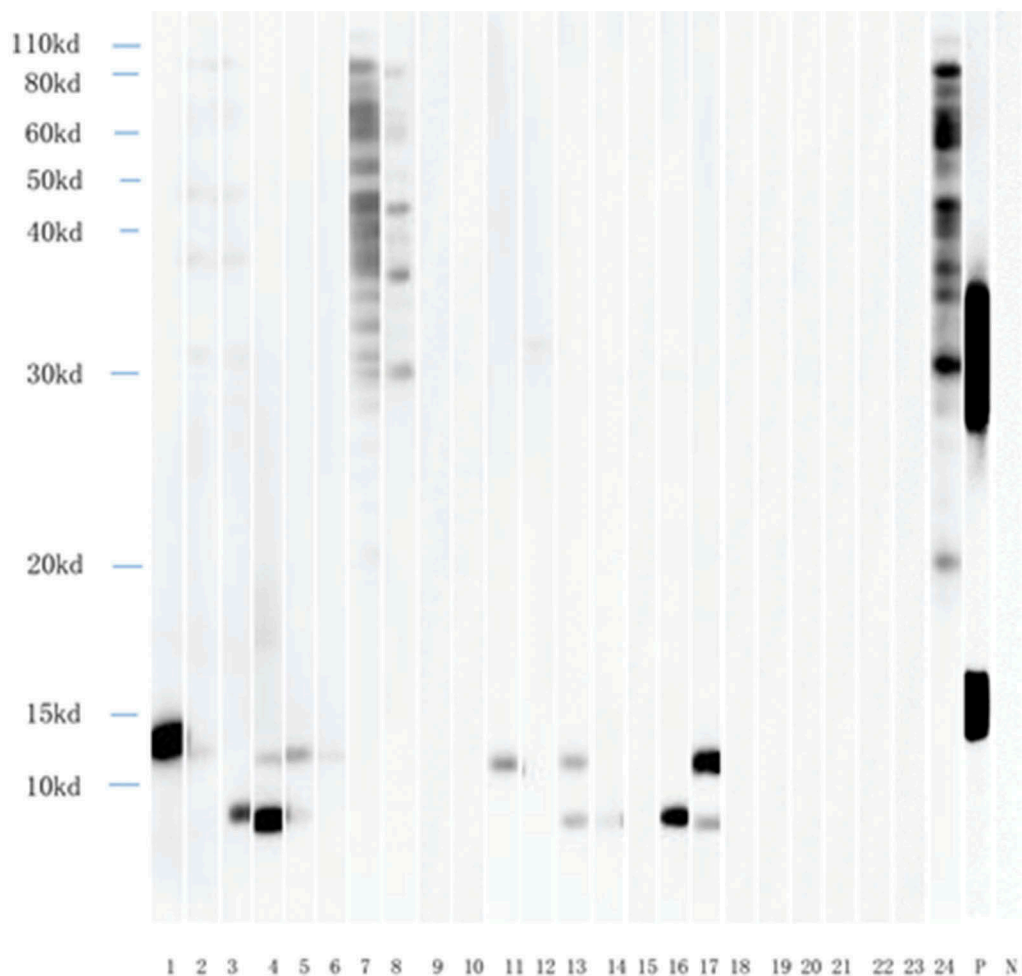


Figure 3. Binding of patients’ IgE antibodies to kidney bean pod extract was examined using immunoblotting. Each numbered lane represents a single patient. Lane P and N are positive and negative control, respectively.

Table 4. Molecular weight (MW) and percentage of positive staining of kidney bean pod proteins in immunoblotting.

MW (pod)	Number	Percentage
10–15 kDa	12	50%
30–80 kDa	5	20.8%
≈20 kDa	1	4.2%

Total percentage of positivity to kidney bean pod extract was 66.7%.
Total percentage of positivity to kidney bean seed and pod extracts was 83.3%.

(32–40 kDa) (Brain-Isasi et al. 2017) can all exhibit allergenic properties. All these published allergens have low to moderate molecular weight. In this study, we could also identify potential allergens in kidney bean seed and pod with molecular weight comparable to those of the published. Of interest, the present work demonstrated scattered binding of patients’ IgE antibodies to 30–80 kDa protein of kidney bean pod extract.

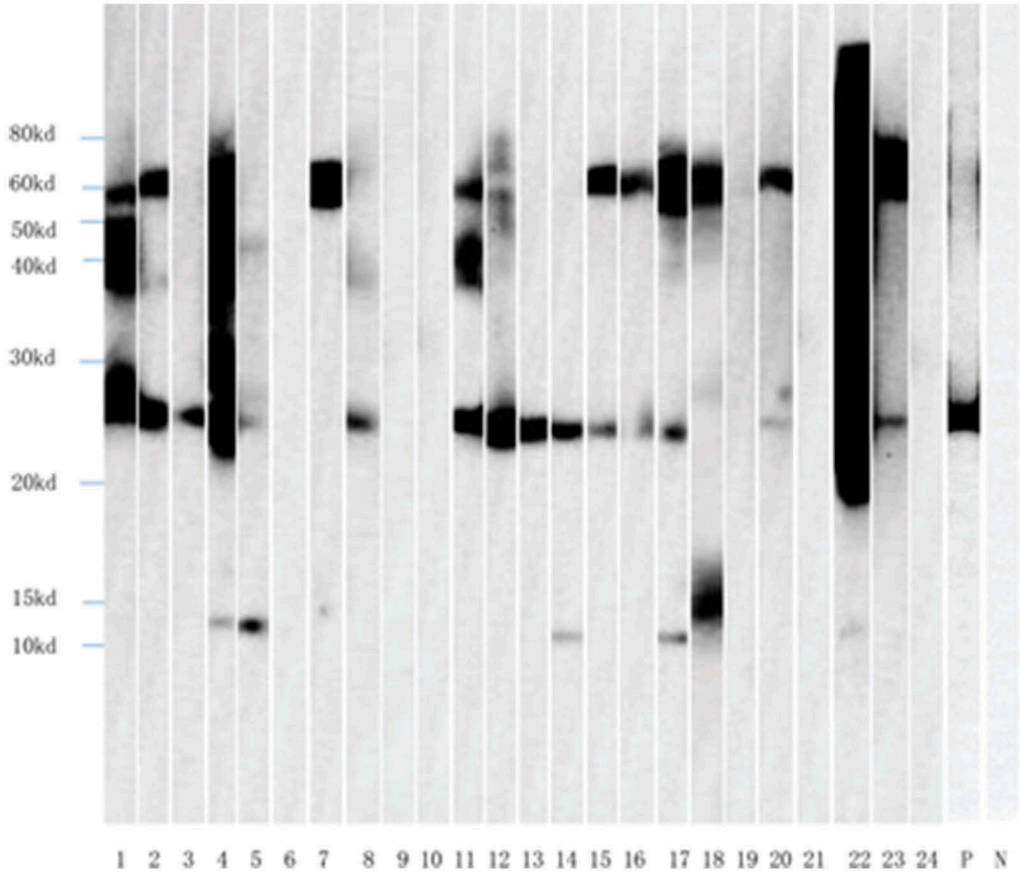


Figure 4. The cross-reactivity between kidney bean seed and mugwort was examined using immuno-blot-inhibition assay. Immunoblot showed that the kidney bean seed extract could inhibit the specific binding of patients' IgE antibodies to mugwort extract.

Table 5. Inhibition of kidney bean seed to mugwort.

MW (kidney bean seed inhibition of mugwort)	Number	Percentage
10–15 kDa	4	16.7%
≈60 kDa	2	8.3%
≈26 kDa	1	4.2%

Inhibition rate is 29.2%.

No study about the antigenicity assessment of high MW protein (≥ 60 kDa) in kidney beans has so far been published to our best knowledge.

In patients with pollen-induced respiratory allergy, cross-reactivity between pollens and foods may induce food allergies with symptoms ranging from oral allergy syndrome to severe anaphylaxis. Cross-reactions between mugwort–celery, mugwort–spice, mugwort–peach, mugwort–chamomile, and mugwort–mustard have all been identified (Popescu 2015). In the cross-reactions between mugwort–peach, it has been suggested that mugwort

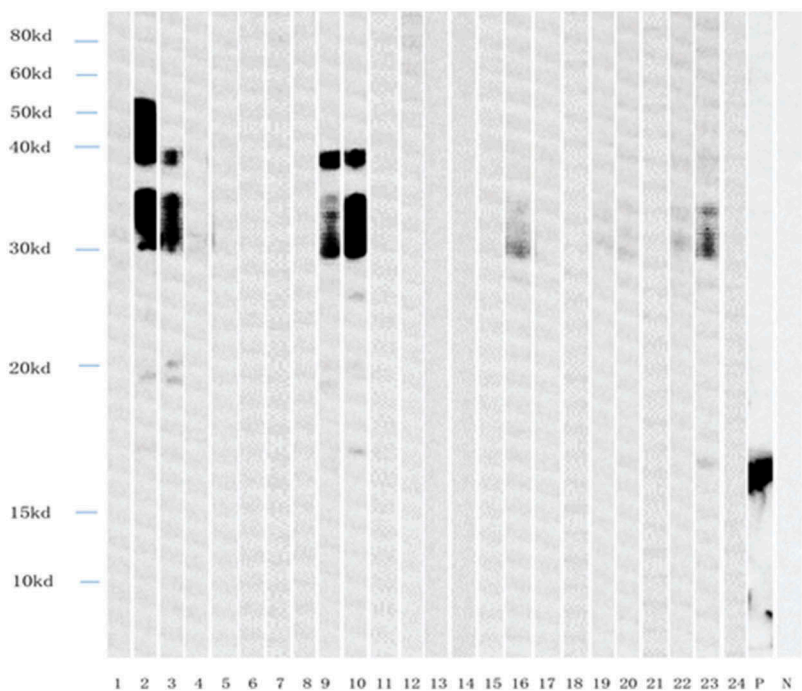


Figure 5. The cross-reactivity between kidney bean seed and mugwort was examined using immuno-blot-inhibition assay. Immunoblot showed that the mugwort extract could inhibit the specific binding of patients’ IgE antibodies to kidney bean seed extract.

Table 6. Inhibition of mugwort to kidney bean seed.

MW (Mugwort inhibition of kidney bean seed)	Number	Percentage
≈30 kDa	4	16.7%
≈60 kDa	3	12.5%
15–20 kDa	3	12.5%
10–15 kDa	1	4.2%

Inhibition rate is 33.3%.

lipid transfer protein (Art v 3) has an important role in the pollinosis of patients allergic to peach lipid transfer protein (Pru p3) (Sanchez-Lopez et al. 2014). However, no research has been published on the cross-reactivity between mugwort and kidney beans. Herein our immunoblot-inhibition assay clearly indicated for the first time the cross-reactivity between mugwort and kidney bean seeds.

The findings of this study would improve the current understanding on the allergenicity of kidney beans, and would provide invaluable insights into the refinement of treatment strategy for anaphylaxis. Nevertheless, the identity and thermal stability of allergens from kidney beans have yet to be assessed. Besides, despite kidney bean seeds were demonstrated to be cross-reactive with mugwort, the cross-reactivity between mugwort and kidney bean pods has remained to be delineated. The findings would also be validated on an independent patient cohort.

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