

Allergy and Anaphylactic Reaction to Loquat (*Eriobotrya japonica*) Are Induced by a Bet v 1 Homolog

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Fruit allergens from plants belonging to the *Rosaceae* (rose) family cross-react with pollen from plants of the *Betulaceae* (birch) family [1]. The causative allergens include Bet v 1 [2]. Typically, the primary symptoms of allergic reactions to Bet v 1 homologs are oral, although there are reports of generalized symptoms in the case of soybean allergies [3]. Loquat (*Eriobotrya japonica*), which is grown in Asia and several other locations, is also a member of the *Rosaceae* family.

Loquat allergy is diagnosed based on the clinical history and skin prick test results [4]. However, the primary allergen responsible for loquat allergy remains unidentified. In this study, we collected the serum of individuals with loquat allergy—including those who had experienced anaphylactic responses—to identify the causative allergen. This approach may lead to better prognostic and therapeutic options for the treatment of loquat allergy.

Fifteen patients with positive results in prick-prick testing with fresh loquat (wheal diameter of 3 mm or more) using a bifurcated needle (Tokyo M.I CO. Inc) were selected for this study (Supplementary Table 1). There were 13 complaints of oral symptoms induced by loquat and 2 of systemic symptoms. The titers of white birch pollen (Bet v 1) and Mal d 1–specific IgE antibodies were positive in all the patients for whom residual serum was available. Serum samples from 2 healthy volunteers without food allergy and umbilical cord blood from infants born at Fujita Medical University were used as controls.

The study was approved by the Research Ethics Committee of Fujita Medical University (Approval Number 10-216), and written informed consent was obtained from the patients and parents of patients aged under 19 years of age.

We electrophoresed the loquat extract proteins as described by Laemmli [5] using 4%-12% Bis-Tris gels (Thermo Fisher Scientific). Following SDS-PAGE, loquat-extracted proteins were transferred to an Immobilon-P polyvinylidene fluoride membrane (pore size, 0.45- μ m; Millipore) and reacted with 20-fold diluted serum. Alkaline phosphatase–labeled polyclonal goat antihuman IgE (ϵ) antibody (Kirkegaard & Perry Laboratories) and 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium phosphatase substrates (1-Component System; Kirkegaard & Perry Laboratories) were used to detect IgE antibodies bound to the antigen. Target protein analysis with a mass spectrometer (TripleTOF; AB Sciex) was performed following the method reported by Yagami et al [6]. Protein analysis was performed using ProteinPilot software version 5.0 (AB Sciex), and proteins were identified using sequence data from UniProt.

Several protein bands that reacted with patient IgE were detected by immunoblotting; these bands ranged in size from 15 kDa to 50 kDa (Figure). The bands that reacted with more than half of the samples had a molecular weight of 15 kDa (93% positive) or 17 kDa (100% positive). In the immunoblot, the 15-kDa band was thinner than the 17-kDa band.

The 15- and 17-kDa bands were identified by mass spectrometry as Mal d 1.02 (accession number Q9S7M5). Protein coverage for each band was 100% (159 aa/159 aa for 17 kDa) and 95.6% (152 aa/159 aa for 15 kDa). The N-termini of the 15-kDa bands showed complete homology with Mal d 1. However, the degree of homology of the corresponding C-termini of the 15-kDa bands indicated C-terminal deletions after the 153rd amino acid sequence. We believe that the 15-kDa proteins may be identical to the 17-kDa proteins, albeit with C-terminal deletions. The binding capacity of 15-kDa proteins may be lower than that of 17-kDa proteins because the presence of epitopes has been reported at the C-terminus of Mal d 1 [7].

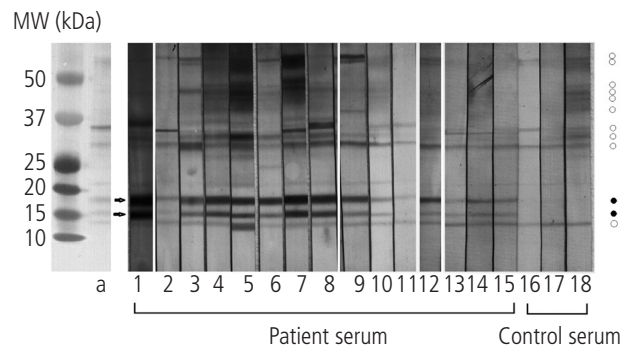


Figure. Immunoblot assay of sera from patients with confirmed loquat allergy and controls. More than half of the patients presented specific IgE-binding bands with relative molecular weights (MW) of 15 and 17 kDa (indicated by arrows and ●). The white circle indicates a specific IgE-binding band. Lane a, loquat proteins stained with amido black.

Interestingly, only 8 patients presented symptoms of apple allergy. Immunological analysis of Mal d 1 and Bet v 1 showed that diversity of allergenicity was determined mainly by the difference in allergen expression levels [8]. Bet v 1 homologs of loquat and Mal d 1 were also considered to have different expression levels. Further investigation of the differences between the properties of the Bet v 1 homolog of loquat and Mal d 1 is needed.

The limitations of this study included its small sample size, especially with respect to patients who experienced anaphylaxis. It is necessary to investigate more cases of anaphylaxis to loquat in order to determine the exact protein identities of possible allergens.

Our results indicated that the main allergen causing loquat allergy was a Bet v 1 homolog with a sequence similar to that of Mal d 1, but with a different immunoblot pattern. These findings may contribute to the development of improved prognostic and therapeutic tools for loquat allergy and loquat-related anaphylaxis.

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Conflicts of Interest

Kayoko Matsunaga belongs to an endowed department sponsored by Hoyu Co., Ltd. The remaining authors declare that they have no conflicts of interest.

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