

## **Loquat (*Eriobotrya japonica*) allergy, including anaphylaxis, is induced by a Bet v1 homolog**

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Fruit allergens from plants belonging to the *Rosaceae* (rose) family show cross-reactivity with pollen from plants of the *Betulaceae* (birch) family [1]. The causative allergens include Bet v1 [2]. Typically, the primary symptoms of allergic reactions to Bet v1 homologs are of an oral nature, although there are reports of generalized symptoms in the case of soybean allergies [3]. Loquat (*Eriobotrya japonica*) grown in Asia and several other locations is also a member of the *Rosaceae* family.

Several cases of loquat allergy diagnosed based on clinical history and skin prick tests have been reported [4]. However, the primary allergen responsible for loquat allergy remains unidentified. In this study, we collected the serum of individuals with loquat allergy to identify the causative allergen in loquat allergy, including in anaphylactic responses. This may lead to better prognostic and therapeutic options for the treatment of loquat allergy.

Fifteen patients positive for prick-prick test with fresh loquat (wheal diameter of 3 mm or more) using a bifurcated needle® (Tokyo M.I CO. Inc, Tokyo, Japan) were selected for this study (Supplementary Table 1). There were 13 complaints of oral symptoms induced by loquats and two of systemic symptoms. The titers of white birch pollen-, Bet v1-, and Mal d1-specific IgE antibodies were positive in all the subjects for whom residual serum was available. Serum samples from two 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 the patients 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, Waltham, MA, USA). Following SDS-PAGE, loquat-extracted proteins were transferred to an Immobilon-P® polyvinylidene fluoride membrane (pore size, 0.45- $\mu$ m; Millipore, Bedford, MA, USA) and were reacted with 20-fold diluted serum. Alkaline phosphatase-labeled goat-poly reserve anti-human IgE ( $\epsilon$ ) antibody (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) 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, Framingham, MA, USA) 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 IgEs from patients were detected by immunoblotting, and these bands ranged in size from 15 to 50 kDa (Figure 1). The bands that reacted with more than half of 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 a.a./159 a.a. for 17 kDa) and 95.6% (152 a.a./159 a.a. 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 15 kDa bands indicated C-terminal deletions after the 153th amino acid sequence. We considered that the 15 kDa proteins may be identical to the 17 kDa proteins, albeit with C-terminal deletions. Possibilities of lower binding capacity of 15 kDa proteins than 17 kDa proteins are considered because the presence of

epitopes has been reported at the C-terminus of Mal d1 [7].

Interestingly, only eight patients presented symptoms of apple allergy. Immunological analysis of Mal d 1 and Bet v1 showed that diversity of allergenicity was mainly determined by the difference in allergen expression levels [8]. Bet v1 homologs of loquats and Mal d1 were also considered to have different expression levels. Further investigation of differences in the properties between the Bet v1 homolog of loquat and Mal d1 is needed.

The limitations of this study included small sample size, especially in regard to anaphylaxis cases. A larger number of loquat anaphylaxis cases may be required to determine the exact protein identities of possible allergens.

Our results indicated that the main allergen causing loquat allergy was a Bet v1 homolog with a sequence similar to that of Mal d1, 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 Hoya Co., Ltd.

The remaining authors have no conflicts of interest.

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