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Invited Review Article

Gibberellin-regulated protein allergy: Clinical features and cross-reactivity

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ABSTRACT

Gibberellin-regulated proteins (GRPs)/GASA proteins are members of cysteine-rich antimicrobial peptide families and are conserved in a broad range of plants. Some GRPs in fruits and pollens have been identified as allergens including peach Pru p 7, Japanese apricot Pru m 7, orange Cit s 7, pomegranate Pun g 7, and cypress pollen GRP. The clinical features of fruit-derived GRP allergies frequently include systemic reactions, multiple fruit allergies regardless of plant kingdom classifications and, less frequently, cofactor-dependence. Multiple fruit allergies might be related to cross-reactivity between GRPs. Clinical cross-reactivity, at least between the four fruit-derived GRPs, has been proven. In addition, GRP allergy induces peculiar clinical symptoms, such as laryngeal tightness and facial swelling, especially eyelid edema, which was proposed to be a predictive factor for Pru p 7 allergy. Fruit-derived GRPs have an unusually high content of cysteine, resulting in high stability to heat and resistance to digestive enzymes. Therefore, GRPs are considered "true" food allergens that sensitize hosts via the gastrointestinal tract and induce severe allergic reactions. As an alternative mechanism of fruit-derived GRP allergies, cross-reactivity between fruit GRP and cypress pollen GRP, which might play a role as a sensitizer, is suspected. Taken together, these characteristics indicate GRPs are clinically relevant plant allergens.

This review article summarizes our current knowledge of the clinical features and important aspects of GRP sensitization and allergy.

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Introduction

Because of advancements in molecular biology techniques over the past few decades, many allergens have been identified and the mechanisms of fruit allergies have been elucidated. In recent years, fruit allergies have been classified into two types depending on their cross-reactivity to pollens. Fruit allergy related to pollen cross-reactivity is termed pollen-food allergy syndrome (PFAS)/pollen allergy syndrome.^{1,2} The main causative allergens are pathogenesis-related protein type 10 (PR-10) and profilin. In PFAS, the symptoms are mainly limited to the oral cavity and are relatively mild including itching, tingling, redness and swelling in the oropharyngeal area. From the perspective of clinical symptoms, PFAS has also been called oral allergy syndrome.³ In contrast, fruit allergies unrelated to pollens tend to be more severe than PFAS. Non-specific lipid transfer protein (LTP) is considered a representative allergen that causes

pollen-unrelated fruit allergies in European countries.^{4–7} However, the diagnostic concepts and procedures using these three allergen components are not sufficient, and do not cover all fruit allergies. Thus, there are still several issues to resolve before an appropriate diagnosis of fruit allergies can be achieved.

Gibberellin-regulated protein (GRP) was recently identified as a new allergen in fruit allergies.⁸ Interestingly, fruit GRPs might be a marker allergen for severe fruit allergies that cause peculiar clinical symptoms, which sometimes occur in a cofactor-dependent manner.^{9–11} They might cause also multiple fruit allergies regardless of plant kingdom classification. GRPs have some similarities to LTP with regard to the aspects mentioned above. In addition, GRP has gained increasing attention as an allergen potentially associated with the two types of fruit allergies mentioned above.^{12–14} It is thought that cross-reactivity between fruit and pollen GRPs might occur, indicating that GRP might induce PFAS.

GRP has brought a new perspective to fruit allergies and might compensate for our lack of diagnostic measures regarding fruit allergies. Although there are few reports related to GRPs, this review focuses on our current knowledge of GRP allergies.

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Gibberellin-regulated protein in plants

GRP, also known as a Snakin/GASA protein family, is a member of the cysteine-rich plant antimicrobial peptide families. The first GRP, snakin-1, was isolated from potato plants (*Solanum tuberosum*) in 1999.¹⁵ GRPs are involved in plant defense to pathogens as well as in plant growth.¹⁶ The expression of GRP is up-regulated by gibberellins, which are a class of natural plant hormones produced by plants and some fungi and bacteria.¹⁷ Gibberellin and GRP play an essential role in plant growth and developmental processes, including germination, cell division, dormancy, flowering and fruit development.¹⁸ Therefore, the concentration of GRP in plants is finely regulated by the specific tissue and developmental stage. In addition to these normal physiological processes, GRP concentration is also affected in response to abiotic stresses, such as salt, drought, cold and heat stress.¹⁸ Recently, the external application of synthetic gibberellin was introduced as an agricultural treatment to increase and improve crop production.¹⁹ Gibberellin treatment can affect the amount of GRP produced in plant-derived foods and pollens.

The process of identification as a food allergen

A new allergen, GRP, was identified during research to address clinical issues related to peach LTP, Pru p 3. In the Mediterranean area and central Europe, LTPs are a major allergen of plant-food allergies often associated with severe allergic reactions.^{4–7} Pru p 3, was confirmed to have allergenicity in the many studies and is considered a representative LTP in these countries. However, when we investigated the results of allergic tests with Pru p 3, some issues were found.

In Italy, Tuppo *et al.* had addressed discrepancies in their Pru p 3 results assessed using several diagnostic procedures.⁸ In their study, some patients developed severe allergic reactions after the ingestion of peaches, but had negative ISAC 103 results for native Pru p 3 (nPru p 3) and any other available allergens. Two of the patients also had negative results for ImmunoCAP with recombinant Pru p 3 (rPru p 3). Nevertheless, both patients had positive skin prick test (SPT) results for commercial whole peach extract (Allergopharma) and for two Pru p 3-enriched peach peel preparations (ALK and BIAL). These results indicated that several extracts purified from whole peaches and peach peel contained proteins that could induce skin allergic reactions in severe peach allergy patients, different to Pru p 3. Through this study, the comparison of allergic test results for Pru p 3 using several peach preparations led to the identification of Pru p 7.

In Japan, the low prevalence of Pru p 3 sensitization in Japanese peach patients was considered a clinical issue. Even in Japanese patients who experienced systemic allergic reactions after the ingestion of peaches, specific IgE against rPru p 3 has rarely been detected using ImmunoCAP.²⁰ This suggested there was no marker to predict severe reactions in peach allergy. We suspected there were three reasons for this low prevalence of positive IgE results for Pru p 3 in Japanese peach allergy patients: i) differences in the peach cultivars consumed between Europe and Japan; ii) immunological responses to allergens based on race; and iii) sensitization to unknown allergens. To investigate the allergenicity of Pru p 3 from peaches originating in Japan, we tried to purify Pru p 3 from a Japanese cultivar. By chance, in the course of purification of Pru p 3 from Japanese peaches, we identified a new allergen, a small protein with chromatographic similarity to Pru p 3, which corresponded to Pru p 7. Using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of peach extract under reducing conditions, a single intense band at 9 kDa, suspected to be Pru p 3, was clearly divided into two bands at molecular weights of 9 and 14 kDa under non-reducing conditions. N-terminal sequence

analysis revealed that the protein band at molecular weight 9 kDa on SDS-PAGE corresponded to a protein with 100% identity to Pru p 7, whereas the 14 kDa protein was Pru p 3.⁹

In addition, Tuppo *et al.* noted the peculiar behavior of Pru p 7 in immunological investigations. In their study, native Pru p 7 (nPru p 7) had specific IgE binding in dot blot assay.⁸ However, denatured Pru p 7 non-specifically bound to the two secondary antibodies in the immunoblotting. These results indicated that denaturation might cause the exposure of hidden parts of the molecule responsible for the non-specific binding of immunoglobulins from different sources. The authors therefore hypothesized that this behavior of Pru p 7 in immunoblotting might have misled researchers in previous studies to identify new allergens based on comparisons with fully controlled positive/negative immunoblotting results.

Combined with its overlapping migration with Pru p 3 by SDS-PAGE as mentioned above, these peculiar immunological features of Pru p 7 might explain why it has been overlooked as an allergen.

GRPs identified as allergens

To date, four food allergens from GRPs have been reported to have clinically relevant allergic activity and have been registered by the World Health Organization (WHO)-International Union of Immunology Societies (IUIS) (Fig. 1, Table 1).^{8,12–14,21–23}

GRP isolated from peach fruit, also named Peamaclein, was the first GRP to be identified as a food allergen and was assigned the designation Pru p 7.⁸ Subsequently, allergenic homologs in other fruits were described, namely Pru m 7 from Japanese apricot, Pun g 7 from pomegranate, and Cit s 7 from orange.^{21–23} In addition, a GRP homolog from cypress pollen, Cypmaclein, was recently identified as the first inhalant allergen from GRPs.¹⁴

When allergenic GRPs are compared with their homologs found in the basic local alignment search tool, their amino acid sequence identity is very broad, ranging from approximately 50%–100%. On the basis of homology searches, Pru p 7 is the most similar to Pru m 7 (100% identity), followed by cherry GRP (97%), Pun g 7 (90%), Cit s 7 (87%), black cottonwood GRP (84%), potato GRP (82%), castor bean GRP (82%), grape GRP (82%), and soybean GRP (81%) (Fig. 2). Although these GRPs, apart from Pru m 7 and cherry GRP, belong to taxonomic orders different to Pru p 7, they have a high identity—greater than 80%—with Pru p 7.

Figure 2 shows most of the amino acid positions, including the pattern of 12 cysteines, are conserved in all GRPs. The N-terminal regions of GRP from two Cupressales, Cypmaclein and the homolog from *Cryptomeria japonica* (*C. japonica*), are highly conserved.¹⁴ In contrast, this region is highly variable between Cupressales proteins and fruits, such as peach, pomegranate and orange.

Although Pru p 7 and Pru p 3 share some in biochemical, immunological and clinical similarities, they have distinct primary and secondary structures.

Molecular features of GRPs: structure and stability

GRPs are small, basic, cysteine-rich proteins with a molecular weight of approximately 7–8 kDa. GRPs are synthesized as precursors with a high molecular mass containing a signal peptide. Then, the signal peptide is removed from the mature form of approximately 7 kDa. In the mature form, two regions, a short N-terminal stretch with low sequence conservation and a highly conserved C-terminal region containing the 12-cysteine pattern characteristic of this protein family, have been detected.⁸

All GRPs are composed of 12 cysteines in the C-terminus, forming six disulfide bonds. Such an unusually high presence of cysteine provides GRPs with extraordinarily high chemical, thermal

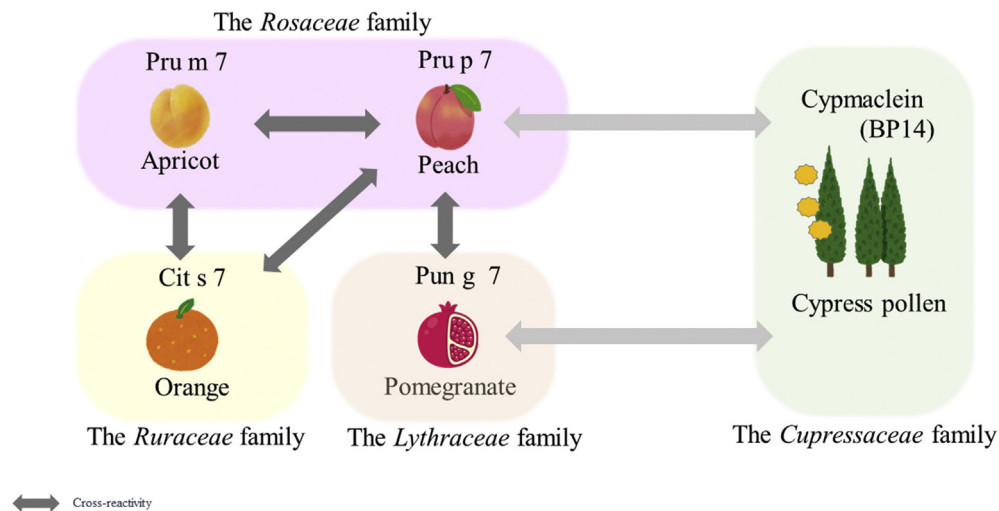


Fig. 1. Gibberellin-regulated proteins (GRPs) identified as allergens and their cross-reactivity. To date, clinical cross-reactivity between at least four fruit-derived GRPs, Pru p 7, Pru m 7, Cit s 7, and Pun g 7, have been proven. In addition, cross-reactivity between Cypmaclein and two fruit-derived GRPs, Pru p 7 and Pun g 7, has been demonstrated. Left right double arrow indicates cross-reactivity.

and proteolytic stability. Therefore, Pru p 7 is stable under heating and under simulated gastric digestion, similar to Pru p 3, which is also part of the cysteine-rich plant antimicrobial peptide family. A study conducted by Tuppo *et al.* showed that Pru p 7 was more resistant to digestion than Pru p 3 in simulated gastrointestinal digestion experiments.²⁴ Therefore, Pru p 7, similar to Pru p 3, is a suspected true food allergen with the capacity to sensitize hosts by itself via the gastrointestinal tract and induce severe reactions after ingestion. Only the heat-denatured protein was sensitive to intestinal proteases.²⁴ Similar to Pru p 3, Pru p 7 maintains its native 3D structure up to 90 °C, but it unfolds at temperatures of 100–120 °C. Tuppo *et al.* indicated that heat denaturation affected the immunological properties of the peach allergens, which partially lose their IgE-binding epitopes.²⁴ Indeed, SPTs using the prick-prick method were positive for fresh peaches and processed foods, such as canned peaches, umeboshi, and ume juice in patients with Pru p 7 allergy.²⁵

GRP is present in the pulp and peel of peaches, unlike LTP, which, in the same fruit, is distributed dominantly in the peel rather than the pulp. The existence of GRPs in the pulp and peel of peaches was supported by individual positive SPT results.²⁵ In addition, Pru p 7 and Pun g 7 protein concentration are highly variable in natural sources—they were present in high amounts or were undetectable in fruit cultivars or in different batches of the same cultivar.^{8,22}

Clinical features of food-derived GRP allergies

Because Pru p 7 was identified as the first allergen among GRPs, they have gained increasing attention as a marker allergen related to systemic reactions in fruit allergies.^{8,9} When we performed detailed investigations of GRP allergies in clinical settings, we found clinical features of GRP allergies other than severe reactions. To diagnose GRP allergies and prevent severe reactions, we should increase our knowledge of the clinical features of GRP allergies.

First, GRP allergies occur mostly in adolescents and adults and are less common in children (Table 2). Patients with a broad range of ages from 5 to 58 years had GRP allergies according to previous reports.^{8,11,21} Second, GRPs, especially Pru p 7 and Pru m 7, frequently elicit anaphylaxis.^{9,21} GRP allergies often include peculiar clinical symptoms, such as facial swelling and laryngeal tightness, which can be predictive factors for GRP allergies (Fig. 3).^{10,25}

Third, most GRP allergy patients have multiple fruit allergies, particularly Rosaceae fruit allergies, which may occur because of cross-reactivity between their GRPs.¹¹ Indeed, cross-reactivity between at least four fruit-derived GRPs, Pru p 7, Pru m 7, Cit s 7 and Pun g 7, have been proven.^{21–23} Finally, GRP allergies less frequently occur in a cofactor-dependence manner, similar to LTP allergy.¹¹ Cofactor-dependence may make the diagnosis of GRP allergies difficult, which can cause us to overlook GRP allergies.

Prevalence of GRP sensitization in fruit allergies

During investigations of sensitization to PR-10, profilin, LTP and GRP in 100 Japanese patients with fruit allergies, the prevalence of monosensitization to GRP was 13% (13/100) (Fig. 4).¹¹ Of 20 patients without sensitization to PR-10 or profilin, which are marker allergens for PFAS, the prevalence of GRP sensitization was high (65%, 13/20). In that study, the level of IgE specific to nPru p 7 as a representative for GRP was measured using enzyme-linked immunosorbent assay (ELISA). However, only one of the 20 patients had positive ImmunoCAP results for rPru p 3.

When grading the severity of clinical reactions in accordance with the recently proposed classification of food allergic and anaphylactic reactions, all 13 GRP mono-sensitized patients experienced at least grade 2 or higher systemic reactions after the ingestion of causative foods, and four patients (31%, 4/13) developed anaphylactic shock.²⁶

In addition, 10 of the 13 GRP mono-sensitized patients (76.9%) had multiple causative fruits. Peaches (92.3%) most frequently induced allergic reactions, followed by apricots (61.5%), oranges (46.2%), apples (30.8%), strawberries (30.8%), grapes (23.1%), cherries (15.4%), lemons (15.4%), and grapefruits (15.4%). These results indicated that peaches might be a representative causative fruit in GRP allergies. Additionally, the causative allergen sources might be distributed over a broad range of plant kingdom classifications and might be cross-reactive with each other. In eight of the 13 GRP-mono-sensitized patients (61.5%), exercise or aspirin intake enhanced the allergic reaction onset as cofactors.

Peach GRP, Pru p 7

Pru p 7 was identified as the first allergen in GRPs (Table 1).⁸ In 2013, Tuppo *et al.* reported the first study of Pru p 7.⁸ They enrolled

	Allergen name	Allergen Source	Plant kingdom classification	Protein name	Molecular weight determined by mass spectrometry (estimated from electrophoretic migration)	Reference (No.)
Food	Pru p 7	Peach	<i>Order: Rosales</i> <i>Family: Rosaceae</i>	Peamaclein	6910.84 Da (7–8.5 kDa)	2013 Tuppo L (8) 2014 Inomata N (9) 2019 Tuppo L (14)
	Pun g 7	Pomegranate	<i>Order: Myrtales</i> <i>Family: Lythraceae</i>	Pommaclein	(7 kDa)	2017 Tuppo L (22)
	Pru m 7	Japanese apricot	<i>Order: Rosales</i> <i>Family: Rosaceae</i>	Not applicable	6896.5 Da (7 kDa)	2017 Inomata N (21)
	Cit s 7	Orange	<i>Order: Sapindales</i> <i>Family: Rutaceae</i>	Not applicable	6941.6 Da (8 kDa)	2018 Inomata N (23)
Pollen	Cup s 7 (submitted ¹)	Cypress pollen	<i>Order: Pinopsida</i> <i>Family: Cupressaceae</i>	Cypmaclein	6821.88 Da (9.5 kDa)	2019 Tuppo L (14)

	1	10	20	30	40	50	60	Identity%
Prup 7	G S S F C D S K C G V R C S K A G Y Q E R C L K Y C G I C C E K C H C V P S G T Y G N K D E C P C Y R D L K N S K G N P K C C P							
Prum 7	G S S F C D S K C G V R C S K A G Y Q E R C L K Y C G I C C E K C H C V P S G T Y G N K D E C P C Y R D L K N S K G N P K C C P							100
Cherry	G S S F C D S K C G V R C S K A G Y K E R C L K Y C G I C C E K C H C V P S G T Y G N K D E C P C Y R D L K N S K G N P K C C P							97
Pung 7	G S S F C D S K C A V R C S K A G V Q D - - - - - - - - - - - - - - - - K D E C P C Y R D M K N S K G X P K C C X							90
Cit s 7	G S D F C D S K C A V R C S K A G R E D R C L K Y C G I C C D K C H C V P S G T Y G H K D E C P C Y R D L K N S K G K P K C C P							87
Cotton	G S D F C D S K C A V R C S K A G V Q D R C L K Y C G I C C E K C K C V P S G T F G N K H E C P C Y R D M K N S K G K S K C C P							84
Potato	G S D F C D S K C N Y R C S K A G R Q D R C L K Y C G I C C E E C H C V P S G T S G N K H E C P C Y R D M K N S K G G P K C C P							82
Castor bean	G S G F C D S K C E K R C E K A G V K D R C L K Y C G I C C E K C K C V P S G T Y G N K H E C P C Y R D M K N S K G K P K C C P							82
Grape	G S S F C D S K C A A R C S K A G M K D R C L K Y C G I C C E E C K C V P S G T Y G N K H E C P C Y K D K K N S K G Q P K C C P							82
Soybean	G S P F C D S K C A Q R C A K A G V Q D R C L R F C G I C C E K C N C V P S G T Y G N K D E C P C Y R D M K N S K G K D K C C P							81
Cypress	A Q I D C D K E C N R R C S K A - - - - - Y C G I C C E K C H C V P G T - - - - - - - - - - - - - - - -							
C.japonica	A H I D C D K E C N R R C S K A S A H D R C L K Y C G I C C E K C N C V P P G T Y G N E D S C P C Y A N L K N S K G G H K C C P							68

In 2016, eyelid edema was proposed as a clinical predictive factor related to Pru p 7 sensitization in peach allergy.¹⁰ In our previous study, 50 patients (14 men and 36 women; mean age, 31.8 years) with peach allergy were enrolled. By ELISA, 13 patients (26%) were sensitized to nPru p 7, whereas 33 patients (66%) had positive ImmunoCAP results for rPru p 1. In the 13 cases with Pru p 7-positive results, the most frequent symptoms after the ingestion of fresh peaches were oropharyngeal symptoms in eight cases (69.2%), followed by laryngeal tightness in six (46.2%), facial edema in six (46.2%), eyelid edema in six (46.2%), urticaria in five (38.5%), dyspnea in three (23.1%), nasal obstruction in three (23.1%), conjunctival injection in two (15.4%), lip edema in two (15.4%), loss of consciousness in two (15.4%) and hypotension in one (7.7%). However, in contrast to cases associated with sensitization to Pru p 1, laryngeal tightness ($p < 0.01$), facial edema ($p < 0.05$), eyelid edema ($p < 0.01$), conjunctival injection ($p < 0.05$), and urticaria ($p < 0.05$) were significantly associated with Pru p 7 sensitization.

GRP, gibberellin-regulated proteins; PR-10, pathogenesis-related protein

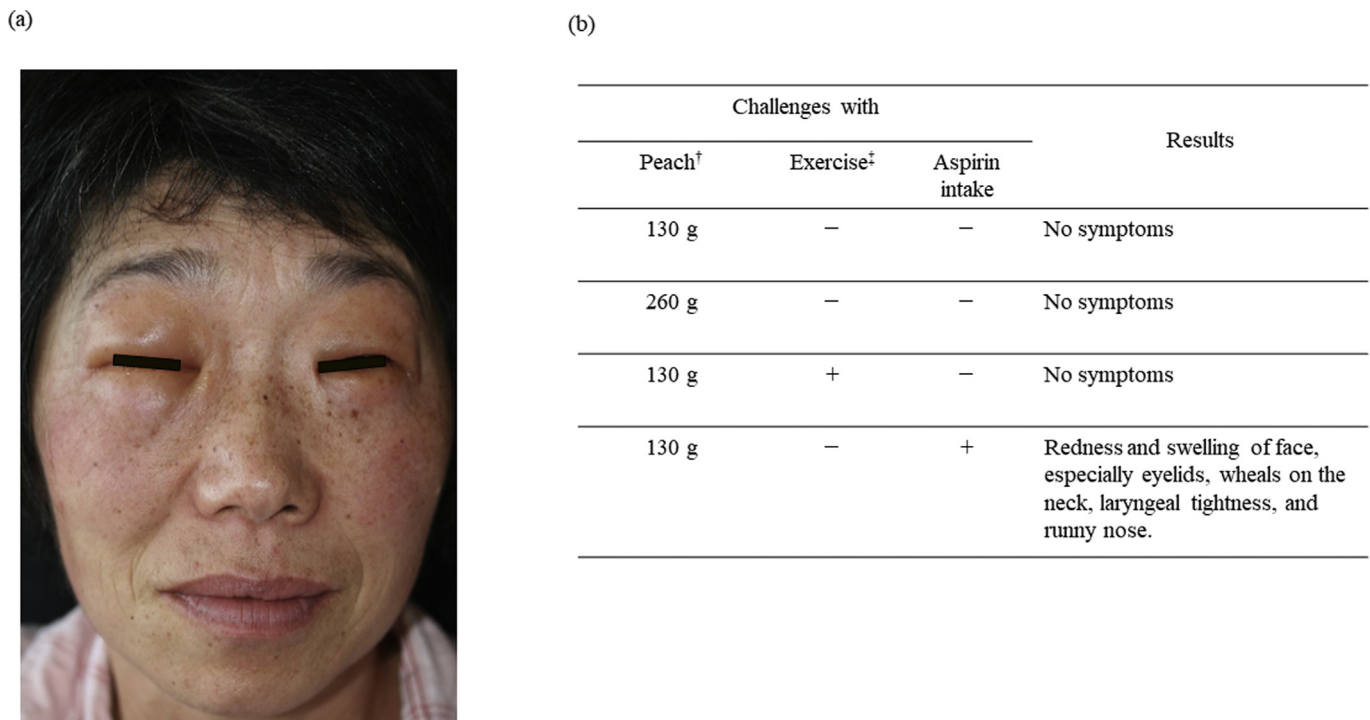


Fig. 3. (a) Prominent edema of face, especially eyelids, induced by oral challenge of 130 g peach pulp after the intake of 500 mg aspirin in a 54-year-old woman. A permission from the patient was obtained. (b) Results of oral challenge tests of peaches with exercise or intake of aspirin as a cofactor. Oral challenge of 130 g peach pulp after intake of 500 mg aspirin induced swelling of her face, wheals on her neck, oropharyngeal tightness and runny nose. [†] The weight of a whole peach is approximately 260 g. [‡] The exercise stress test was performed according to the Bruce protocol (up to stage 4) using a treadmill.

Likewise, in an Italian study, angioedema on the face was also found in all seven patients with sensitization to Pru p 7 alone.⁸ In contrast, oropharyngeal symptoms were strongly related to Pru p 1 sensitization but were significantly less common in the Pru p 7-positive group. Along with the absence of co-sensitization to representative allergen components for PFAS, namely PR-10 and profilin, facial edema and especially eyelid edema after the ingestion of peaches might be a predictive factor for sensitization to Pru p 7.

In 2019, Klingebiel *et al.* investigated the prevalence of sensitization to Pru p 7 in a multicenter study, in which they enrolled 316 patients with suspected peach allergy from many different regions throughout France.¹³ They selected ImmunoCAP with recombinant Pru p 7 (rPru p 7) for IgE measurement. Mono-sensitization to rPru p 7, without the detection of IgE specific to any of the other tested peach allergens (rPru p 1, rPru p 3, and rPru p 4), was more frequent in peach allergy patients (66/123, 54%) than in patients without peach allergy (8/48, 17%) ($p < 0.0001$). In addition, in nine peach allergy patients mono-sensitized to rPru p 7, CD203c expression was induced by rPru p 7 in basophil activation tests. Higher frequency and greater magnitude of Pru p 7 sensitization were associated with the presence of peach allergy, the clinical severity of peach-induced allergic reactions and the level of cypress pollen exposure, which was dominant in the Mediterranean region compared with non-Mediterranean regions in France.

Japanese apricot GRP, Pru m 7

Pru m 7 was identified as the first allergen in Japanese apricot allergy.²¹ Japanese apricot (*Prunus mume*), also known as Chinese plum, is closely related to the apricot (*Prunus armeniaca*) and belongs to the *Prunus* family as does the peach. Japanese apricots are commonly used in juices, sauces and as pickles in Japanese and Korean cooking. In Japan, pickled and dried Japanese apricots,

umeboshi, are very popular as a side dish. Nevertheless, few cases of Japanese apricot allergy have been reported.

In a previous study in 2017, we enrolled seven Japanese apricot allergy patients (two men and five women; age range, 14–54 years; mean age, 28.0 years).²¹ Of the seven patients, ELISA and IgE-immunoblotting using nPru m 7 showed positive reactions in six (85.7%) and seven (100%) patients, respectively. These results were consistent with basophil activation tests and SPTs using nPru m 7. In contrast, all seven patients had negative ImmunoCAP results for three peach allergen components, rPru p 1, rPru p 3, and rPru p 4. Therefore, Pru m 7 might be the major Japanese apricot allergen, at least in Japan.

In addition, cross-reactivity between GRPs was proven for the first time using Pru m 7 and Pru p 7.²¹ Indeed, all seven patients with Japanese apricot allergy experienced allergic reactions after the ingestion of peaches—100% sequence identity between Pru m 7 and Pru p 7 frequently causes cross-reactivity between peaches and Japanese apricots.

Interestingly, compared with Pru p 7 (2/12, 16.7%), Pru m 7 more frequently induced allergic reactions in a cofactor-dependent manner (7/8, 84.6%).^{11,21} We hypothesized that cofactors enhancing the absorption of allergens were more likely to be required for the onset of Japanese apricot allergy than peach allergy because of the small amount of Japanese apricot generally consumed in one serving. In cases where allergic reactions occur after a small amount of allergen source is ingested in one serving, as for Japanese apricot allergy, we should pay attention to the involvement of cofactors. The most frequent symptom of Pru m 7 allergy is facial swelling, especially of the eyelids, similar to Pru p 7 allergy.

Orange GRP, Cit s 7

Sweet orange GRP, Cit s 7, was identified as the first GRP allergen from the Rutaceae family. Prior to its identification, three proteins had been registered as orange allergens by WHO/IUIS, namely Cit s

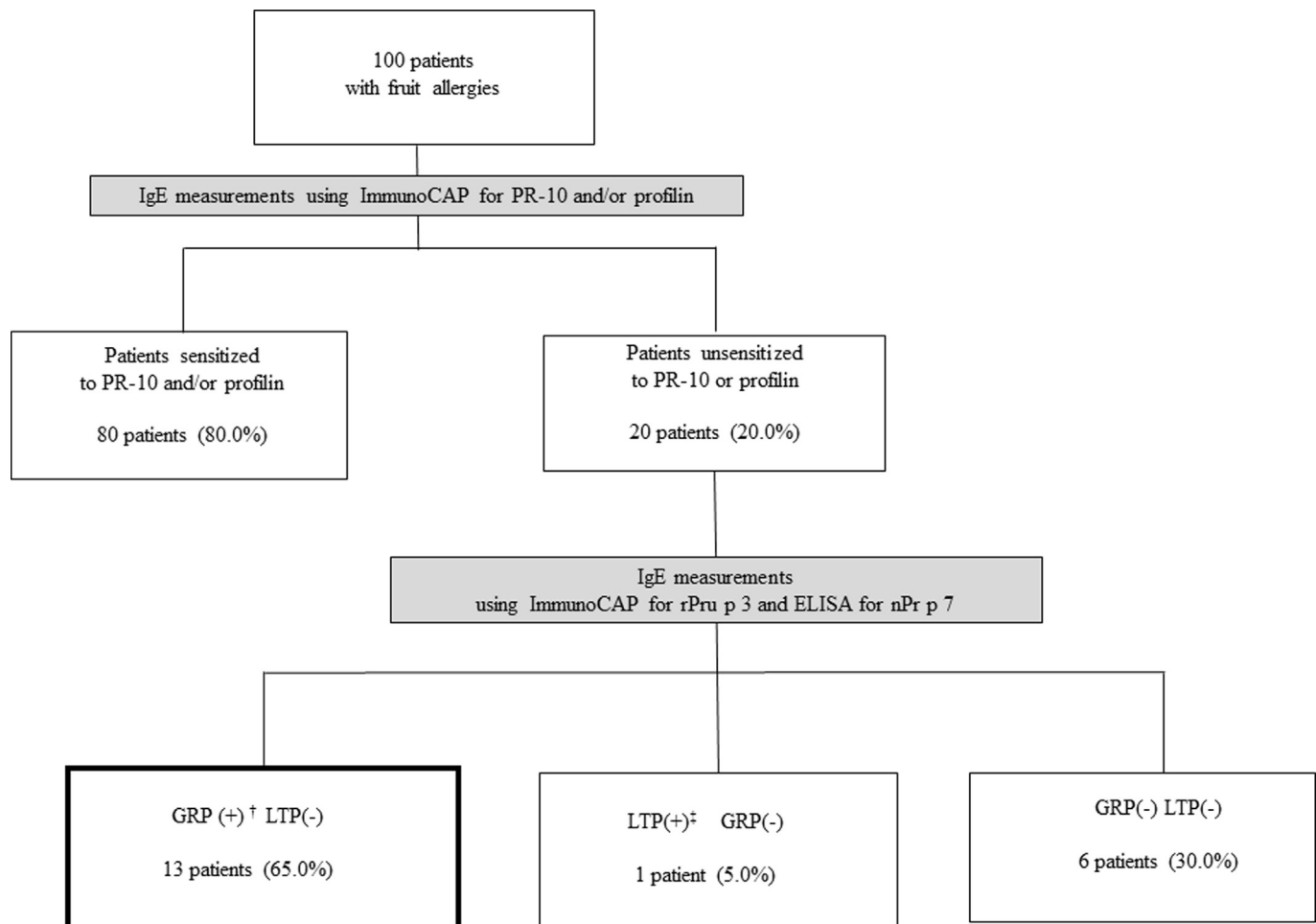


Fig. 4. The prevalence of Pru p 7-mono sensitization in Japanese patients with fruit allergies. Of 100 Japanese patients with fruit allergies, 20 patients had negative ImmunoCAP results for Bet v 1 homologs and profilin. Of these 20 patients, 13 patients had positive specific immunoglobulin E results by enzyme-linked immunoassay and/or skin prick test (SPT) examinations using purified nPru p 7 as a representative gibberellin-regulated protein (GRP). One patient was ImmunoCAP positive for nPru p 3 as a representative lipid transfer protein (LTP). †GRP-sensitized patients indicate patients with positive results for nPru p 7 in at least one allergic test. ‡LTP-sensitized patients indicate patients with positive ImmunoCAP results for Pru p 3.

1 (germin-like protein), Cit s 2 (profilin), and Cit s 3 (LTP). In Europe, most orange allergy patients were reported to be sensitized to Cit s 1 or Cit s 2.^{27–29} By contrast, in our previous study of orange allergy, the prevalence of GRP sensitization was very high (12/14, 85.7%), and we confirmed that patients with orange allergy were sensitized to Cit s 7 based on positive results for Cit s 7 in at least one of three assays (ELISA, basophil activation tests or SPTs).²³

Cit s 7 allergy seemed to be milder than Pru p 7 and Pru m 7 allergies, although the main symptoms, such as facial symptoms and oropharyngeal symptoms, are shared by all three allergy types. In two of 12 patients (16.7%) with Cit s 7 allergy, skin and mucosal symptoms were accompanied by dyspnea; however, no patient experienced anaphylactic shock.²³ The prevalence of anaphylactic shock was 15.4% (2/13) and 14.3% (1/7) in Pru p 7 and Pru m 7 allergies, respectively.¹¹ Relatively light exercise, such as jogging, walking, and cycling, was a cofactor for four patients (33.3%) (three self-reported and one by challenge testing).²³

In addition, all 12 patients with Cit s 7 allergy had allergies related to other fruits. The causative fruits included citrus fruits (lemon and grapefruit) and fruits not closely related to orange, such as peach, Japanese apricot, apple and kiwi. Furthermore, ELISA inhibition assays using sera from orange allergy patients showed cross-reactivity of Cit s 7 with Pru p 7 and Pru m 7. Interestingly, in

patients with Cit s 7 allergy, causative fruits involved in the first allergic episode were peaches (9/12, 75%). Accordingly, peach allergy patients with Pru p 7 sensitization should be evaluated for comorbidity of orange allergy related to cross-reactivity between Pru p 7 and Cit s 7.

Pomegranate GRP, Pun g 7

In 2017, Pun g 7 was identified from pomegranate, which belongs to the Lythraceae family.²² Analysis of an Italian large population (10,393 patients) by FABER test revealed that 0.5% and 0.6% of subjects were sensitized to Pun g 7 and Pru p 7, respectively.¹⁴

Cross-reactivity between fruit GRPs and cypress pollen GRP

In 2018, it was suggested that a GRP-homolog, BP-14, in cypress pollen is allergenic and potentially cross-reactive with fruit-derived GRPs. *In vitro* studies of BP-14 mainly focused on IgE cross-reactivity to two GRPs, potato GRP and Pru p 7.^{12,13} The authors of the two groups indicated that BP-14 might be involved in PFAS. The speculation of Sénéchal *et al.* was based on the finding that Pru p 7 has a >80% sequence identity with potato GRP and >95% with other fruit GRP allergens, and that there was cross reactivity between

potato GRP and cypress pollens by the immunoblotting inhibition tests.¹² However, the study lacked clinical relevance to food allergies, such as potato allergy, and did not show direct cross-reactivity of cypress pollen allergen and fruit GRPs. Klingebiel *et al.* demonstrated that the frequency and magnitude of Pru p 7 sensitization were statistically associated with the level of cypress pollen exposure in a multicenter study of 316 patients with suspected peach allergy from many different regions throughout France.¹³ They showed the cross-reactivity of Pru p 7 with cypress pollens, but not with BP-14 in patients sensitized to peach and cypress pollen.

In 2019, Tuppo *et al.* identified GRP as a new cypress pollen allergen, termed Cypmaclein.¹⁴ In their study, mass spectrometry experiments revealed that the exact molecular mass of Cypmaclein (6821.88 Da) was very close to that of Pru p 7 (6909.90 Da). Interestingly, Cypmaclein (9.5 kDa) has a slightly higher molecular weight compared with Pru p 7 (8.5 kDa) and Pun g 7 (8.5 kDa) by SDS-PAGE. This different electrophoretic behavior of GRPs suggests some structural differences between the cypress GRP and the two fruit homologs.

In addition, Tuppo *et al.* suggested that BP-14 might correspond to Cypmaclein. BP-14 derives its name from its electrophoretic migration with a molecular mass of 14 kDa, which is different to that of Cypmaclein.^{14,30} However, Sénéchal *et al.* identified a fragment of BP-14 with structural similarity to Pru p 7 by mass spectrometry after in-gel digestion.¹² Considering that electrophoretic migration can be affected by electrophoretic conditions, for instance the gradient of gels and the concentration of reducing agents, BP-14 might correspond to Cypmaclein.^{14,31}

In the study conducted by Tuppo *et al.*, two sequenced regions of Cypmaclein were identified and accounted for approximately 50% of its primary structure (Fig. 2).¹⁴ The available sequences of Cypmaclein had 94% identity with the corresponding regions of Japanese cedar, *C. japonica* (*C. japonica*), which belongs to the same plant family (the Cupressaceae family) as cypress (*Cryptomeria sempervirens*). A comparison with peach, pomegranate and orange GRP revealed a similarly high sequence identity in the region comprising residues 26–41.¹⁴ In contrast, when the first 16 residues of the N-terminal region were compared, the fruit GRP shared 50% of the residues of Cypmaclein. The sequence identity of *C. japonica* GRP (EST clone BY902962) with Pru p 7 is also not high (68%).

Furthermore, Tuppo *et al.* reported an important insight into potential cross-reactivity between fruit-derived and pollen-derived GRPs. In four patients sensitized to Pru p 7, Cypmaclein completely inhibited IgE binding to Pru p 7 and Pun g 7 spotted on a FABER biochip, indicating that the three GRPs share IgE epitopes.¹⁴ However, they noted that only a subset of patients were co-sensitized to both Pru p 7 and Pun g 7, indicating that some IgE epitopes are not shared, despite their high sequence identity.¹⁴ Therefore, Tuppo *et al.* hypothesized that epitope sharing between Pru p 7 and homologs that have low structural similarity, such as Cypmaclein, is even lower.

Conclusions

The identification of GRP has uncovered the profiles of some fruit allergies, especially severe fruit allergies and cofactor-dependent fruit allergies, which have been underestimated to date. However, clinical data regarding GRP allergies is still limited. Previous studies were conducted using small numbers of subjects from limited areas, such as Italy, Japan and France. In addition, some aspects of GRP allergies are still not well-defined. We should investigate how broad the distribution of GRPs as allergens is in plants. Moreover, we should investigate which GRPs are cross-reactive. In particular, it will be important to clarify whether

pollen-derived GRP is a primary sensitizer that causes clinical cross-reactivity to fruit-derived GRPs. A greater understanding of these mechanism might have an impact on the treatment and prevention of GRP allergies.

In addition to clinical observations, we should pay attention to the increased concentrations of GRP in plants. Gibberellin treatment is widespread in agricultural practice to control and drive plant development, including growth, fruit size, and ripening. Increased GRP content in plants influenced by external gibberellin might promote the sensitization and onset of allergic reactions.

Further studies of GRP allergies using larger cohorts in many parts of the world will provide additional information on the immunological features of plant GRPs, allowing a clearer view of the whole picture of GRP allergies.

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Conflict of interest

The author has no conflict of interest to declare.

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