

Allergy to Gibberellin-Regulated Proteins (Peamaclein) in Children

Benedetta Biagioni^a Leonardo Tomei^b Claudia Valleriani^c Giulia Liccioli^b
Simona Barni^b Lucrezia Sarti^b Francesco Citera^c Mattia Giovannini^b
Francesca Mori^b

^aAllergy Outpatient Clinic, Division of Internal Medicine, IRCCS Azienda Ospedaliera Universitaria, Bologna, Italy;

^bAllergy Unit, Department of Pediatrics, Meyer Children's University Hospital, Florence, Italy; ^cSection of Pediatrics, Department of Health Sciences, University of Florence and Meyer Children's Hospital, Florence, Italy

Keywords

Peamaclein · Gibberellin-regulated proteins · Pru p 7 ·
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Abstract

Background: Gibberellin-regulated proteins (GRPs, Peamaclein) are allergens recently identified in plant-derived food allergy (FA), and little is known about the clinical manifestations of this allergic condition in the European population, especially in children. **Objective:** Our study aimed to identify and characterize pediatric patients with pollen-FA due to GRP sensitization. **Methods:** We retrospectively analyzed the charts of patients referred to the Allergy Unit of the Meyer Children's Hospital in Florence for suspected FA. Three main eligibility criteria based on the actual knowledge of GRP allergy were used to select patients deserving further investigations: (1) systemic reactions after consumption of fruit or an unknown culprit food, (2) positive skin prick tests to both cypress pollen and Pru p 3-enriched peach peel extracts, (3) negative in vitro test results for Pru p 3 serum-specific Immunoglobulin E (sIgE). We performed the in vitro test to determine the anti-rPru p 7 (Peamaclein) sIgE levels in the selected patients. **Results:** We identified 10 pediatric patients

with Pru p 7 allergy and described their characteristics. The use of our eligibility criteria showed a high accuracy in identifying these patients: 100% of the selected patients had positive in vitro results for Pru p 7. We therefore proposed a diagnostic algorithm for Pru p 7 allergy. **Conclusion:** This is the first case series of European pediatric patients with a demonstrated Peamaclein allergy. These findings broaden our knowledge on GRP allergy in pediatric populations and could help clinicians to suspect, diagnose, and manage this recently discovered plant-derived FA. © 2021 S. Karger AG, Basel

Introduction

Food allergy (FA) is dramatically increasing worldwide, following the rise in respiratory allergy [1, 2], and affecting approximately 5% of adults and 8–10% of children [3, 4]. Fruit represents a common trigger of FA, especially in patients with pollinosis. Pollen-FA syndrome

Benedetta Biagioni and Leonardo Tomei should be considered joint first author.

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(PFAS) has a prevalence in pollen-allergic adult patients of 40–50% in Europe [5] and of 20% in the Mediterranean area (a birch-free and ragweed-free area) [6]. The major allergens implicated in plant-derived FA belong to pathogenesis-related proteins 10 (PR-10), the profilins family, and lipid transfer proteins (LTPs); the latter being very frequent in Mediterranean countries [7]. In particular, an Italian study on the adult population found that PFAS was the most frequent FA (prevalence of 55%) followed by LTP sensitization, with a pattern of allergy influenced by different pollen exposure and dietary habits depending on geographic area [8].

The presentation and severity of clinical manifestations in PFAS are extremely variable, also depending on the causative allergen [9]. Recent findings in new allergen identification, as the Gibberellin-regulated proteins (GRPs), and the increasing availability of component resolved diagnosis are refining IgE-mediated fruit allergy, leading to a deeper understanding of its pathogenesis and essential management implications [10]. GRPs are a family of basic, cysteine-rich proteins with a molecular weight of approximately 7 kDa involved in plant growth and defense against pathogens [11, 12]. In 2012, the first GRP allergen was identified after observing systemic allergic reactions induced by peach in patients with negative specific IgE results to the already known allergen families of *Rosaceae* fruits (profilin, PR-10, and LTP) [13]. In fact, despite negative specific IgE results to the available peach allergens, these patients had positive skin prick test results to commercial Pru p 3-enriched peach peel extracts. The analysis of these preparations led to the identification of a new peach allergen with a different primary structure from Pru p 3, but with heat- and digestion-stability similar to peach LTP. This new peach allergen is called Peamaclein and the World Health Organization-International Union of Immunological Societies subcommittee assigned it the designation of Pru p 7.

After these crucial findings, studies conducted in Japan showed that Pru p 7 was closely related to severe allergic reactions [14]. Other GRP allergens have been identified in apricot (Pru m 7) [15], orange (Cit s 7) [16], and pomegranate (Pun g 7) [17]. Moreover, allergic reactions against other fruits (apple, pear, cherry, strawberry, melon, watermelon, kiwi, fig, and grape) have been reported in Pru p 7-positive patients [18, 19]. Other reports of PFAS involving cypress pollen and peach/citrus sensitizations demonstrated a high cross-reactivity between Pru p 7 and BP14, a *Cupressus sempervirens* pollen protein belonging to GRPs [20–22].

Despite intense research in this field in the last decade, little is currently known about the prevalence, severity, and management of GRP allergy in Europe, especially in children. Indeed, so far only one Japanese study has investigated Pru p 7 allergy in children and adolescents [18].

Our study aimed to identify GRP-sensitized patients in a monocentric cohort of children and adolescents with fruit or nonidentified FA and to describe the characteristic of GRP-induced allergy in this pediatric population. In addition, it aimed to provide a possible valuable diagnostic algorithm to identify potential patients suffering from this condition, especially where Pru p 7 is not yet commercially available.

Methods

We retrospectively analyzed the charts of patients referred to the Allergy Unit of the Meyer Children's Hospital from January 2015 to June 2020 with a history of systemic allergic reactions after consumption of fresh fruit or an unknown culprit food and potential PFAS due to GRPs sensitization. Demographic data, clinical manifestations of FA, and allergy history were collected, together with the diagnostic workup. Anaphylaxis was diagnosed according to the clinical criteria proposed by Sampson et al. [23, 24] and the severity was assessed according to Mueller's score [25]. The patients' clinical histories of FA were also carefully evaluated to determine the possible role of cofactors such as exercise in food-dependent exercise-induced anaphylaxis (FDEIA) [26]. The time latency period accepted for a potential relationship with a food-induced reaction was 4 h after food ingestion.

All patients were evaluated with in vivo (skin prick tests [SPTs] and prick by prick) and in vitro tests. The detailed methods are fully described in the online suppl. Material (for all online suppl. material, see www.karger.com/doi/10.1159/000517413).

Patients with a clinical history and positive in vivo and in vitro results consistent with GRP allergy were evaluated for Peamaclein sensitization. Eligibility criteria for determining serum-specific Immunoglobulin E (sIgE) levels anti-Pru p 7 (Peamaclein) with the ImmunoCAP test were (1) history of systemic allergic reactions after consumption of fruit or history of suspected food-induced systemic allergic reactions with a non-clearly identified culprit food; (2) positive SPTs to both cypress pollen and Pru p 3-enriched peach peel extracts; and (3) negative anti-rPru p 3 sIgE. Conversely, all patients with fruit-related FA history with negative SPT results to cypress pollen or Pru p 3-enriched peach peel extracts, or with positive anti-rPru p 3 sIgE were excluded from further evaluations.

Results

Among all the subjects analyzed in the period, 10 patients met the eligibility criteria for further investigations for suspected Peamaclein allergy, 7 males and 3 females with a mean age of 12.89 years (standard deviation 3.07

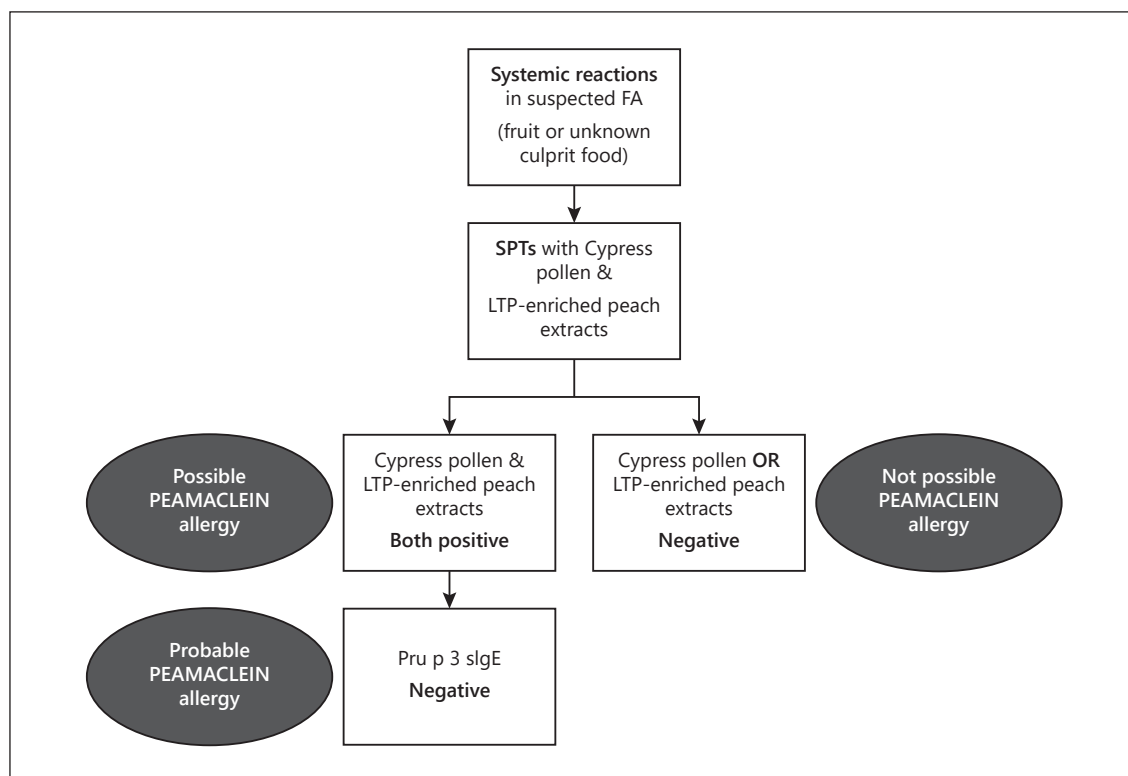


Fig. 1. Proposed diagnostic algorithm for GRP-Peamaclein allergy. GRP, Gibberellin-regulated protein.

years). Regarding their allergic background, all but one patient had allergic rhino-conjunctivitis (90%), 2 patients reported a history of atopic dermatitis (20%), and 4 suffered from allergic asthma (40%). All the data concerning their demographic data, clinical history, clinical presentation, causative foods, cofactors, and allergy test results are reported in online suppl. Table 1.

All the selected patients resulted positive to rPru p 7, with a mean value of 25.93 kUA/L (standard deviation 26.30 kUA/L). On the contrary, all resulted negative for Pru p 1, Pru p 3, and Pru p 4 except for one who was slightly positive to Pru p 1 (1.59 kUA/L). Therefore, the causative role of Peamaclein sensitization in eliciting the reported systemic allergic reactions was demonstrated in all the selected patients.

The most frequent fruits involved in allergic reactions belonged to the *Rosaceae* family: peaches in 7, apples in 2, strawberries in 2, and cherries and plums in one patient, respectively. Two patients reported clinical manifestations after the ingestion of orange and lemon, belonging to the *Rutaceae* family. Four patients had multiple causative fruits: one patient reacted to 4, 2–3, and one to 2 fruits. Of the 5 patients with a single causative fruit, in 4

cases the culprit was peach, and in the other, strawberry. In one patient, the causative food was not clearly identified.

In our case series, anaphylaxis was very common (90%), and FDEIA was reported in 4 patients (40%), with both severe and mild systemic reactions. The most frequent clinical manifestation was generalized urticaria (9 patients), followed by dyspnea (6 patients). Furthermore, 4 patients developed angioedema, 3/4 had facial swelling, and 1/4 hand and foot angioedema. In addition, 2 patients had bronchospasm, 2 gastrointestinal involvement (one had vomiting and the other one abdominal pain). Finally, one patient presented laryngeal edema, and in one case, the child suffered from syncope with loss of consciousness. Only 2 children (20%) reported oral allergy syndrome (OAS), but one showed concomitant positivity for Pru p 1 sIgE. Therefore, a causative role of PR-10 proteins cannot be excluded. The reported latency time between culprit food ingestion and clinical manifestations varied from 30 min to 3 h.

As regards the severity of signs and symptoms, 4 patients developed severe allergic reactions (Mueller's score 3 and 4), while these were milder in 6 patients

(scores 1 and 2). As previously mentioned, in 4/10, we identified physical exercise as a cofactor in the onset of anaphylaxis; other cofactors were not found. According to our proposed diagnostic algorithm (shown in Fig. 1), 100% of the selected patients showed Pru p 7 sIgE positivity.

Discussion

Fruit-derived GRP allergy has recently been investigated. Nowadays, GRPs are considered responsible for both systemic allergic reactions and PFAS. In the latter case, cross-reactivity between fruit GRP and cypress pollen GRP, which might play a role as a sensitizer, has also recently been demonstrated [21, 27, 28]. In Japan, given the low frequency of LTP sensitization, most cases of fruit allergy with a history of severe reactions have been explained thanks to the discovery of Pru p 7 [29]. In the Mediterranean area, LTPs are a leading cause of severe allergen plant-food allergies [7]. However, in some cases, particularly in those with a clinical history of severe reactions, Pru p 1, Pru p 3, and Pru p 4 are not sufficient to provide a correct diagnosis.

In a recent multicentric work conducted in Italy on cypress pollen-allergic adult patients, the detection of GRP sensitization appeared rare [30]. Nonetheless, this work analyzed a population of cypress allergic patients with pollen allergy clinical manifestations, with or without concomitant FA signs and symptoms, identifying both Peamaclein allergy and Peamaclein sensitization. However, data on clinical manifestations of FA due to Peamaclein sensitization are scarce in Europe, especially in pediatric populations.

In this manuscript, 10 patients were selected and investigated for Pru p 7 allergy. The utilization of the abovementioned eligibility criteria showed a very high accuracy in identifying Peamaclein allergic patients; in fact, 100% of the suspected patients showed Pru p 7 sIgE positivity. Therefore, we can assume that Peamaclein allergy is likely and should always be suspected in the presence of all these clinical characteristics. In agreement with published studies, 9/10 patients had a history of at least a grade 2 reaction according to Mueller's classification, making Peamaclein a marker of severe fruit allergy [15]. In our population, 9/10 patients were monosensitized to Pru p 7 and 4/9 presented allergic reactions only after the ingestion of peach, showing tolerance for other fruits. This fact is in contrast with data in literature, where adults often report reactions to sev-

eral fruits [11]. Nevertheless, our children were only studied for Pru p 7, consequently we cannot rule out that they could have positive in vitro results for other GRPs, as usually happens for LTPs, or that they could react to fruits other than peach in the future. In any case, a greater number of children need to be investigated to assess similarities and differences with adults in terms of clinical reactivity to fruit and severity of the reaction.

Moreover, in our case series of children, eyelid edema after ingestion of peach cannot be considered a predictive factor of sensitization to Pru p 7 as it is in adult studies [9]. Indeed, according to our data, only 4 children reported angioedema, and it was not isolated but part of a spectrum of other clinical manifestations. It could be speculated that these results might not be in line with data reported to date in literature either because we are describing a pediatric population and/or because this study includes a low number of patients.

The presence or the absence of cofactors in our study was not clearly associated with the severity of systemic reactions; in particular, only 1/4 patients with severe reactions had a history of physical exercise. Nonetheless, this was the only subject to experience syncope (grade 4 severity according to Mueller's score) due to eating peach 3 h before intense physical activity, which consequently could be considered as an important cofactor at least in this case.

The identification of Peamaclein as the causative food allergen in these patients represented an important step for their correct management. In fact, the similarity between the clinical presentation of LTP and GRP allergy and the similar chemical and physical characteristics of these proteins allow clinicians to make the same recommendations for both allergic conditions. In particular, to avoid the identified culprit food in both raw and processed forms, plus extremely careful assumption of food belonging to the same botanical family as the culprit, especially if known FDEIA cofactors are present, and the use of an adrenalin autoinjector in case of anaphylaxis [7]. The already known differences between LTP and GRP distribution in the different plant-derived food families and in the single fruits, respectively, suggest that for GRP-allergic patients not only *Rosaceae* but also *Rutaceae* represent major sources of allergen exposure [11] and that peeling fruits does not help avoid GRP ingestion because these proteins seem to be evenly distributed in peel and pulp [13, 22].

To the best of our knowledge, this is the first pediatric study in Europe to identify patients with possible or prob-

able allergy to Pru p 7. In addition, we propose a diagnostic algorithm for this allergic condition based on clinical manifestations and SPT results. The use of the proposed eligibility criteria has shown a high accuracy and led to the identification of 10 young patients with a confirmed Peamaclein allergy. They all received an accurate diagnostic workup and the correct identification of their allergy at a molecular level provided the basis for proper management of their FA. Thus, in the case of a systemic allergic reaction after consumption of fruit or an unknown food culprit, we suggest performing in vivo allergy tests using inhalant and food allergens, including both cypress pollen and Pru p 3-enriched peach peel extracts. In the case of a positive result for both cypress and Pru p 3-enriched peach peel extract, a sensitization to Peamaclein is possible and when further investigations give a negative in vitro result for Pru p 3 sIgE, the diagnosis of Peamaclein allergy is highly probable. In these cases, determining serum sIgE levels anti-Pru p 7 (Peamaclein) is suggested.

Besides the strengths of our work, its limitations are mainly due to its retrospective design that prevented a longitudinal follow-up of the population and a more accurate diagnostic workup in vivo which should include prick by prick with raw and processed culprit foods. Another limitation could have been the decision to only include patients with systemic reactions: this approach led to easier identification of Peamaclein allergic patients because of the chemical and physical properties of GRPs that cause systemic and frequently severe reactions, as reported in previous case series [11]. This decision prevented us from identifying GRP-allergic patients with OAS as the only clinical manifestation; however, in some reports, this eventuality appears to be rare [11, 14, 19]. On the other hand, the Italian retrospective study previously mentioned found that in an Italian adult population with cypress pollen allergy, non all GRP-sensitized subjects experience severe clinical reactions following the ingestion of offending foods [30]. It further showed that other allergens beyond GRP might be involved in the cypress PFAS/FA syndrome.

We also have to take into consideration that when OAS is the only clinical manifestation and a sensitization to panallergens belonging to PR-10 and Profilin family is also demonstrated, the clinical relevance of a concomitant Pru p 7 sensitization is difficult to assess. Finally, this study provided no information regarding the prevalence of Pru p 7 allergy because patients were recruited by retrospectively reviewing charts of children referred to our Allergy Unit.

Conclusion

This manuscript first suggests that after a detailed clinical history and simple targeted investigations, Pru p 7 positivity can be detected with high accuracy, leading to the correct explanation of severe fruit allergy cases in children. Nevertheless, more extensive studies are necessary to better clarify the phenotypical characteristics of patients with Pru p 7 allergy, as well as the role of cofactors in severe reactions, the pattern of cross-reactivity with other CGPs, and the prevalence of GRP allergy and sensitization in pediatric populations.

Statement of Ethics

Upon admission, all the children's parents signed a written informed consent to the processing of clinical data for future research studies. Patients whose parents denied consent were excluded from the study. The code of the event report issued by Meyer Children's University Hospital is IR904-21-50917.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

B.B. and L.T. collected the data, performed the investigations, analyzed the data, drafted the initial manuscript, and reviewed the manuscript. C.V. performed the investigations, analyzed the data, and reviewed the manuscript. G.L. collected the data, performed the investigations, analyzed the data, and reviewed the manuscript. S.B., L.S., and M.G. performed the investigations, analyzed the data, and reviewed the manuscript. F.C. performed the investigations, analyzed the data, and reviewed the manuscript. F.M. conceptualized, designed the work, performed the investigations, analyzed the data, drafted the initial manuscript, and reviewed the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and/or its supplementary material files. Further enquiries can be directed to the corresponding author.

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Supplementary material

Methods

All patients were evaluated during the previous diagnostic workup with Skin Prick tests (SPTs) performed on the volar surface of the forearm using a standard 1-mm tip lancet, according to the recommendations of the European Academy of Allergy and Clinical Immunology [S1,S2]. The positive and negative controls for the skin prick test were obtained using histamine (10 mg/mL; Lofarma, Milan, Italy) and normal saline respectively. Results were read after 15 min. and were considered positive if the largest allergen-induced wheal diameter was ≥ 3 mm. The standard SPT panel performed consisted of common airway allergens (mites, grass, weed, birch, hazel, olive and cypress pollens, molds, and animal epithelia) and common food allergens (milk, egg, wheat, cod, tomato, soy, peanut) (Lofarma, Milan, Italy). Profilin extract and a commercial peach extract enriched with Pru p 3 (with a declared 30 mcg/ml concentration), both produced by ALK-Abellò, Madrid, Spain, were used as well [S3]. Finally, most of the patients were also evaluated with skin Prick-by-Prick (PbP) tests with the suspected culprit raw fruit.

In patients with positive SPT results with Pru p 3-enriched peach peel extract, serum specific Immunoglobulin E (sIgE) levels of anti-peach and of anti-Pru p 1, 3, 4 were determined using the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden), according to the manufacturer's instructions with a positive cut-off point set at 0.1 kUA/L.

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Patient Number	Age (years)	Sex	Other Allergic Disease	Offending Foods	Time Latency (hour)	Symptoms	Muller Grading	Cofactors	SPTs			PbP (mm)	sIgE (kUa/L)			
									Cypress Pollen extract	Pru p 3-enriched peach peel extract	Profillin extract		Pru p 1	Pru p 3	Pru p 4	Pru p 7
1	14	M	OR, BA	Strawberry	Not known	U, DY	2	Exercise	3	5	0	Strawberry 4 Peach 5	0.01	0.09	0.01	1.9
2	15	M	-	Peach, apple, lemon, orange	1	U, DY	2	Exercise	4	4	0	Peach 7 Apple 6 Lemon 10 Orange 6	0.12	0.03	0.05	50.6
3	9	M	OR, BA	Peach	2	U	1	-	4	6	0	Peach 3	0	0.04	0.01	30.3
4	9	F	OR	Peach	3	U, AN, BS	3	-	3	5	0	Peach 6	0	0.02	0.05	35.9
5	13	F	OR,BA	Peach	3	SY, DY, V	4	Exercise	5	5	0	Peach 4	0.03	0.06	0.09	83
6	10	M	OR	Strawberry, apple, plum	0.5	U, AN, BS, OAS	3	-	3	3	0	Peach 6 Apple 2 Strawberry 4 Plum 6	1.59	0.02	0	31.8
7	12	F	OR	Not identified	2	U, AN, DY, OAS	2	-	3	6	0	Peach 3 Apple 5 Orange 4 Plum 3	0.08	0.02	0.04	15.1
8	16	M	OR,BA	Peach, cherry	1	U, DY	2	Exercise	4	5	0	Peach 4	0	0.01	0	1.78
9	8	M	OR,AD	Peach, lemon, orange	3	U, AN, LE	2	-	4	4	0	Peach 4 Lemon 4 Orange 3	0	0.02	0	2.53
10	17	M	OR,AD	Peach	0.5	U, DY, AP	3	-	5	4	0	Peach 7	0	0.02	0.03	6.37

Table 1. Demographic data, clinical characteristics and results of the allergy tests of the patients studied. AD, atopic dermatitis; AN, angioedema; AP, abdominal pain; BA, bronchial asthma; BS, bronchospasm; DY, dyspnea; LE, laryngeal oedema; OAS, oral allergy syndrome; OR, oculorhinitis; SY, syncope; U, urticaria; V, vomiting.