ORIGINAL ARTICLE

Sensitization to Gibberellin-Regulated Protein (Peamaclein) Among Italian Cypress Pollen–Sensitized Patients

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Abstract

Background: Peach gibberellin-regulated protein (peamaclein) has recently emerged as a relevant food allergen in cypress pollen–hypersensitive patients.
Objective: We investigated monosensitization to peamaclein among Italian cypress pollen–allergic patients.
Patients: A total of 835 cypress pollen–hypersensitive patients from 28 Italian allergy centers underwent a thorough work-up to determine food-allergic reactions and performed skin prick testing with a commercial peach extract containing peamaclein. IgE to rPru p 3 was measured in peach reactors, and those with negative results were enrolled as potentially monosensitized to peamaclein. IgE reactivity to rPru p 7 was evaluated using immunoblot and an experimental ImmunoCAP with rPru p 7.
Results: Skin prick tests were positive to peach in 163 patients (19.5%); however, 127 (77.9%) were excluded because they reacted to Pru p 3. Twenty-four patients (14.7%) corresponding to 2.8% of the entire study population) were considered potentially monosensitized to peamaclein. No geographic preference was observed. Seventeen of the 24 patients (70.8%) had a history of food allergy, mainly to peach (n=15). Additional offending foods included other Rosaceae, citrus fruits, fig, melon, tree nuts, and kiwi. On peach immunoblot, only 3 of 18 putative peamaclein–allergic patients reacted to a band at about 7 kDa; an additional 4 patients reacted at about 50-60 kDa. Ten of 18 patients (56%) had a positive result for Pru p 7 on ImmunoCAP.
Conclusion: Allergy and sensitization to peamaclein seem rare in Italy. Most patients react to peach, although other Rosaceae fruits and several citrus fruits may also be offending foods. Peach and cypress pollen probably also share cross-reacting allergens other than peamaclein.

Key words: Food allergy. Pollen-food syndrome. Peamaclein. Peach. Cypress pollen allergy.

Resumen

Antecedentes: La proteína del melocotón regulada por giberelina (peamacleina) ha sido descrita recientemente con alérgeno alimentario en los pacientes con alergia al polen de ciprés.
Objetivo: Determinar la presencia de monosensibilización a peamacleina en los pacientes italianos con alergia al polen de ciprés.
Pacientes: Se estudiaron 835 pacientes italianos con alergia al polen de ciprés, provenientes de 28 centros hospitalarios. En todos ellos se realizó historia clínica dirigida a detectar alergia alimentaria así como *prick test* con extractos comerciales de melocotón que contenían peamacleína. En los pacientes sensibilizados a melocotón se determinó IgE específica a Pru p 3 y aquellos con resultado negativo se clasificaron como potencialmente monosensibilizados a peamacleina. Se realizó determinación de IgE específica a Pru p 7 mediante immunoblot e ImmunoCAP con Pru p 7.
Resultados: El *prick test* con melocotón fue positivo en 163 pacientes (19,5%), pero 127 de estos pacientes fueron excluidos por estar sensibilizados a Pru p 3. 24 pacientes (14,7%), que correspondían al 2,8% de la población global, fueron considerados como potencialmente monosensibilizados a peamacleína. La distribución de estos pacientes no seguía ningún patrón geográfico. 17/24 (70,8%) tenían historia de alergia alimentaria, en la mayoría de los casos a melocotón (n=15). Los pacientes también referían síntomas con otros alimentos como otras frutas rosáceas, cítricos, higo, melón, frutos secos y kiwi. Solo 3/18 pacientes presentaban en el *immunoblot* una banda de alrededor de 7 kDa; otros 4 pacientes reconocían una banda de 50-60 kDa. 10/18 presentaron positividad en el ImmunoCAP a Pru p 7.
Conclusión: En Italia, la alergia o sensibilización a peamacleina es baja. La mayor parte de los pacientes reaccionan con el melocotón, aunque otras frutas rosáceas y cítricos también desencadenan síntomas. El melocotón y el polen de ciprés comparten otros alérgenos diferentes a la peamacleina que producen reactividad cruzada.

Palabras clave: Alergia alimentaria. Síndrome polen-alimento. Peamacleína. Melocotón. Alergia a polen de ciprés.

Introduction

Plant-derived foods are by far the most frequent cause of food allergy in adults. In the Mediterranean area, and specifically in Italy, where, in contrast with English-speaking and northern European countries, peanut allergy is rare, lipid transfer protein (LTP) is the most relevant food allergen [1]. Interest has grown in a novel family of allergens in plant-derived foods, namely the gibberellin-regulated proteins (GRPs), which include peamaclein in peach. This new food allergen was first detected about 10 years ago with the observation of systemic allergic reactions induced by Rosaceae fruits (in most cases peach) in patients who did not show IgE reactivity to any member of the then known allergen families represented in peach, namely, PR-10, profilin, and LTP [2-4]. Interestingly, affected patients showed strong reactivity on skin prick testing (SPT) not only with the fresh offending fruit, but also with commercial peach extracts [3,4], suggesting IgE-mediated reactivity to a heat- and pepsin-stable allergen [5]. In fact, peamaclein was first isolated within the LTP peak of peach skin and subsequently also in peach pulp, thus explaining why the protein is present in Pru p 3–enriched peach extracts for SPT. GRPs are small basic proteins with a molecular weight of 7 kDa and a structure characterized by 12 cysteines and 6 disulfide bridges, which confer the typical resistance to chemical/physical treatments. The purified protein is denatured at 100°C for 10 minutes [6]. GRPs are antimicrobial peptides expressed by plants upon stimuli by biotic and abiotic cues. Since then, several studies, mostly from Japan and France (both countries where Cupressaceae pollen allergy is common, at least in specific areas) confirmed the identity of this protein and led to the conclusion that GRP allergy is possibly a novel form of pollen-food allergy syndrome in which pollen appeared to act as the primary sensitizer [7-10]. In fact, the cypress pollen

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GRP was recently identified [9,11], and the homologous cross-reacting peach allergen (currently known as Pru p 7) has been sequenced and cloned. This protein is not yet commercially available for the in vitro diagnosis of GRP-induced pollen-food allergy syndrome. Other foods have been reported to be potential inducers of allergic reactions in patients who are hypersensitive to this allergen family, including Japanese apricot [12], orange [13], and pomegranate [14] (Pru m 7, Cit s 7, and Pun g 7, respectively). Furthermore, clinical reactivity to apple, melon, watermelon, and strawberry [2], as well as to exotic fruits, kiwi, tomato, fig, carrot, grapes, coconut, and celeriac [15], has been reported in Pru p 7–hypersensitive patients, making GRP a potential novel plant food panallergen.

Little is known about the prevalence of peamaclein sensitization among patients sensitized to cypress pollen, the prevalence of clinically relevant food allergy among those sensitized, and whether foods other than those reported above represent a risk for patients reacting against members of this protein family. Exposure to cypress pollen varies widely throughout Italy, reaching its maximum in central regions such as Tuscany and Lazio, although sensitization to cypress pollen can be detected throughout the country. We carried out a large clinical survey across all regions of Italy to investigate the frequency of sensitization to peamaclein among cypress pollen–allergic patients and its clinical relevance in our cypress-rich country.

Patients and Methods

Participating Centers and Patients

Twenty-eight outpatient allergy clinics from throughout Italy took part in the study. The initial study population comprised all patients who presented spontaneously at the participating centers between the beginning of January and the end of June 2019 reporting a history suggesting pollen allergy. Before undergoing the diagnostic procedure (see below), the patients were thoroughly interviewed about their clinical respiratory symptoms (seasonality, severity, presence, or absence of asthma) and about recent or past adverse reactions induced by foods. A possible allergic reaction was defined as a history of oral allergy syndrome, severe gastroenteritis, urticaria/angioedema, and/or anaphylaxis following the ingestion of a specific food.

Diagnostic Procedure

The detection of potential GRP reactors followed a 3-step procedure:

a) Detection of cypress hypersensitivity

All patients underwent SPT with a series of commercial pollen extracts including grass, mugwort, ragweed, pellitory, plantain, birch, plane, olive, and cypress (*Cupressus arizonica*), as well as house dust mite, *Alternaria*, and cat and dog dander. Participating centers were free to use the commercial SPT preparations that they used in their routine activity; the manufacturers included Allergopharma, ALK, and Lofarma. Skin tests were carried out following established methods [16]; readings were taken after 15 minutes, and skin responses were considered positive in the presence of a wheal and flare reaction exceeding 3 mm in diameter. Histamine 10 mg/mL and saline were used as positive and negative controls, respectively. In patients showing skin reactivity to cypress, IgE to Cup a 1 was measured using ImmunoCAP (Thermo Fisher Scientific). Values >0.10 kU_A/L were regarded as positive.

b) Detection of peach sensitization

All cypress-hypersensitive patients underwent SPT with a commercial peach extract enriched in LTP (ALK-Abelló, LTP 30 µg/mL; or Lofarma, LTP 50 µg/mL). Again, the participating centers were free to use the diagnostic extract in use during their routine activity. The ALK extract is known to lack labile allergens (such as PR-10 and profilin) and to contain stable allergens such as LTP [17] and peamaclein [3]. In 2011, this extract led to the detection of the first case of exercise-induced anaphylaxis induced by an allergen that was subsequently identified as GRP [4]. In a recent comparative study carried out on >200 patients, the Lofarma peach extract was shown to behave in the same way as the ALK extract [18]. In patients showing skin reactivity to peach extract, IgE to rPru p 3 was determined using ImmunoCAP, as described above.

c) Detection of potential GRP reactors

Cypress-hypersensitive patients showing positive SPT results to peach extract but no reactivity to Pru p 3 were considered potentially sensitized to Pru p 7. The serum of these potential GRP reactors left after the in vitro tests reported above was used for an immunoblot analysis with peach extract (at the Lofarma laboratories) and for detection of IgE to rPru p 7 with an experimental ImmunoCAP assay (at the Thermo Fisher Scientific laboratories). Recombinant Pru p 7 was produced, characterized, and functionally assessed as described elsewhere [15]. Both immunoblot and rPru p 7 ImmunoCAP were carried out complementarily.

Immunoblot Analysis

a) Peach peel extract

Peach was extracted as previously described by Bjorksten et al [19]. Protein content was measured according to Bradford [20] using a commercial BioRad Protein Assay Dye Reagent (Bio-Rad) and bovine serum albumin as the reference standard. Before use, the protein concentration of the peach extract was adjusted to 1 mg/mL.

b) Immunoblot

Patients' IgE reactivity to peach peel extract was assessed using immunoblot analysis under reducing conditions. The extract was mixed with Tricine sample buffer (Invitrogen) and 5% ß-mercaptoethanol and denatured by heating at 100°C for 5 minutes. Electrophoresis of the extract (25 µg/lane) was carried out in a 16% polyacrylamide precast gel (Novex Tricine, Invitrogen) at 180 mA for 1 hour. The resolved proteins were transferred onto a nitrocellulose membrane for 1 hour according to Towbin et al [21]. The membrane was saturated with 0.1 mol/L of Tris-buffered saline containing 5% fat-free milk powder (saturation buffer) and incubated for 16 hours at 4°C with serum (700 µL of serum and 700 µL of saturation buffer). After 3 washes, bound IgE antibody was detected using peroxidase-conjugated antihuman IgE goat IgG antibodies (BiosPacific, diluted 1:10000 in saturation buffer) and an ECL Western blotting kit (Amersham).

The presence of peamaclein in the peach peel extract used to carry out the immunoblot analyses was ascertained using direct ELISA with a pool of rPru p 7+/rPru p 3– sera and a negative control serum pool. IgE levels were expressed as optical density (OD). Bromelain-based immunoblot was used to rule out IgE reactivity to cross-reactive carbohydrate determinants (CCDs)

Ethics

All investigations were carried out according to the principles of the Declaration of Helsinki. All patients gave their written informed consent for the use of their clinical data in an anonymous form. Cypress reactors who were hypersensitive to peach also gave their written informed consent for the use of their anonymized leftover serum for research purposes. Since the study was carried out within the routine activity of the participating centers, formal approval by an external ethics committee was not required.

Results

Patients

The final study population included 835 cypress pollen–hypersensitive patients diagnosed based on a positive SPT result. Their mean age was 35.3 years (range, 3-86 years), and the study group included 452 females and 383 males. Most patients (751/835 [90%]) were considered to have cypress pollen allergy by their physicians based on typical respiratory symptoms of rhinoconjunctivitis with or without asthma in the cypress pollen season (ranging between December and April

Table. Clinical Features and In Vitro Findings in 24 Putative Peamaclein Reactors

	Sex	Age	Cup a 1, kU_A/L	Cypress allergy	Other allergies	Bet v 1/ Phl p12	Pru p 7, kU_A/L	Food allergy	Offending foods
1	F	35	7.61	Yes	G, D	Neg/Neg	0.63	No	
2	F	24	ND	Yes	D	ND/ND	Neg	Urt	Peach
3	M	39	ND	Yes	P	ND/ND	ND	No	
4	M	29	21.5	Yes	P, D	Neg/Neg	Neg	No	
5	F	26	0.69	Yes	P, D	Neg/Neg	Neg	Urt	Strawberry, blackberry, raspberry
6	F	54	10.5	Yes	No	ND/ND	4.95	OAS	Peach
7	M	39	5.35	Yes	B, G, P, D	Neg/0.39	Neg	No	
8	M	31	5.01	Yes	G	Neg/Neg	1.13	Urt, OAS	Peach, orange, lemon
9	F	52	6.88	Yes	B, P, O, D	ND/ND	Neg	OAS	Walnut, hazelnut, kiwi
10	F	46	9.1	Yes	No	Neg/Neg	0.92	Urt	Peach
11	M	21	12.2	Yes	G	Neg/Neg	13.14	FDEIA	Peach
12	M	17	> 100	Yes	G, M, O	Neg/2.5	ND	OAS	Peach, melon
13	F	57	18.7	Yes	O	ND/ND	26	OAS, Urt	Peach, fig, plum jam, orange marmalade
14	M	44	39.6	Yes	No	ND/ND	Neg	No	
15	M	22	74.5	Yes	B, G, P	ND/ND	15.49	Urt	Peach, apple
16	F	49	Neg	No	G, P	Neg/Neg	Neg	OAS	Peach
17	M	24	5.2	Yes	G, P, O, D	ND/Neg	ND	OAS	Peach
18	M	23	0.80	Yes	G, P, cat	ND/Neg	ND	No	
19	F	55	0.79	Yes	G, M, D, cat	Neg/Neg	ND	Urt	Peach
20	F	44	2.74	Yes	G, P, O	Neg/Neg	ND	Urt	Peach
21	M	12	ND	Yes	P, D	ND/ND	3.64	OAS, Urt	Peach, lemon, grapefruit
22	F	32	0.1	Yes	B, P	ND/ND	0.13	No	
23	M	16	7.0	Yes	G, P	ND/ND	0.98	OAS	Peach
24	F	47	ND	Yes	G	ND/ND	Neg	OAS	Peach, plum

Abbreviations: B, birch; cat, Cat dander; D, house dust mite; FDEIA, food-dependent exercise-induced anaphylaxis; G, grass M, mugwort; Neg, negative; ND, not done; O, olive; OAS, oral allergy syndrome; P, pellitory; Urt, urticaria/angioedema.

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depending on the area of Italy), while 84 were considered to be sensitized but clinically nonallergic to cypress pollen. Most patients were also sensitized to other airborne allergens; only 68/835 (8%) were monosensitized to cypress pollen. IgE to Cup a 1 was measured in 620 patients and ranged between <0.1 and >100 kU_A/L (median, 6.07 kU_A/L). Sera from 3 patients did not show IgE to Cup a 1 despite a positive SPT result with cypress pollen extract.

In total, 163 (19.5%) cypress reactors had a positive result in SPT with 1 of the commercial peach extracts and were therefore considered sensitized to stable peach allergens. Of these, 127 (77.9%) had a positive result for rPru p 3 on ImmunoCAP, 24 (14.7%) were rPru p 3-negative, 3 yielded borderline results (Pru p 3 IgE values between 0.1 and 0.35 kU/L), and 9 were not tested. Thus, 24 patients were eventually considered to be potentially monosensitized to peamaclein (2.8% of cypress-hypersensitive patients), and their sera were used for subsequent analyses.

The geographical distribution of peamaclein hypersensitivity was investigated by dividing the country into 3 areas: north (including the whole Po valley and Genoa), center (including Tuscany, Marche, and the region of Rome), and south (including Naples and Sicily). The prevalence of potential peamaclein reactors was 5/258 (1.9%), 15/482 (3.1%), and 4/95 (4.2%) in the 3 areas, respectively, and no significant differences were detected.

Clinical Features of Putative Peamaclein Reactors

Putative peamaclein reactivity was not associated with a higher Cup a 1 IgE level; in this subset, IgE to Cup a 1 ranged between 0.1 and >100 kU_A/L (Table). Of 24 putative peamaclein reactors, 3 were monosensitized to cypress pollen, whereas 21 were sensitized to other airborne allergens including pollen from grass (n=12), wall pellitory (n=12), birch (n=5), olive (n=8), mugwort (n=4), house dust mite (n=11), and cat dander (n=3). Seventeen of 24 (71%) had a history of food allergy (Table), which, in most cases (n=15), was associated with the ingestion of peach. Additional reported offending foods included other Rosaceae such as apple, plum, and berries (3 cases), citrus fruits (3 cases), fig, melon, tree nuts, and kiwi. Five patients had a history of food allergy, although in vitro tests did not confirm rPru p 7 IgE reactivity (Table).

Seven of 24 patients (29%) did not report any food-induced adverse reaction. Interestingly, most of these patients had no or only low levels of IgE to rPru p 7.

Immunoblot Analysis

Peach immunoblot analysis was carried out on sera from 31 patients (18 putative peamaclein reactors [ie, patients with positive results to peach SPT but negative results to Pru p 3 ImmunoCAP] and 13 cypress-allergic controls [patients sensitized to cypress pollen but with negative results in peach SPT]). Owing to a serum shortage, 6 of the 24 putative peamaclein reactors were not tested using immunoblot analysis. The serum of only 3/18 putative peamaclein reactors yielded a very faint band at about 7 kDa in some cases despite high levels of IgE to Pru p 7 (2 of these patients are shown in Figure 1; 3 patients are shown in Figure 2) (Table). Four sera produced a band at about 50-60 kDa (Figure 1); the lack of response to bromelain excluded IgE reactivity to CCDs. The immunoblot analysis yielded negative results for the remaining 11 patients and all controls. In order to exclude the possibility that the reducing condition of the immunoblot analysis could have altered peamaclein IgE reactivity, the analysis was repeated under nonreducing conditions using the serum of a

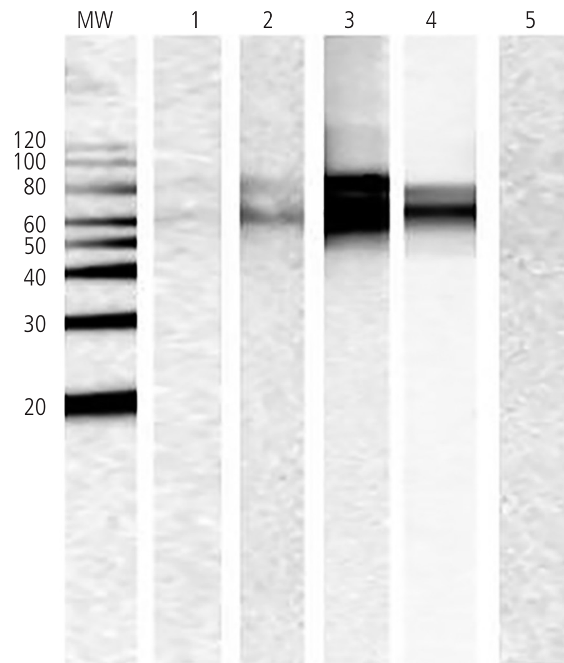

Figure 1. Peach immunoblot analysis of the 4 sera showing IgE reactivity at about 50-60 kDa.

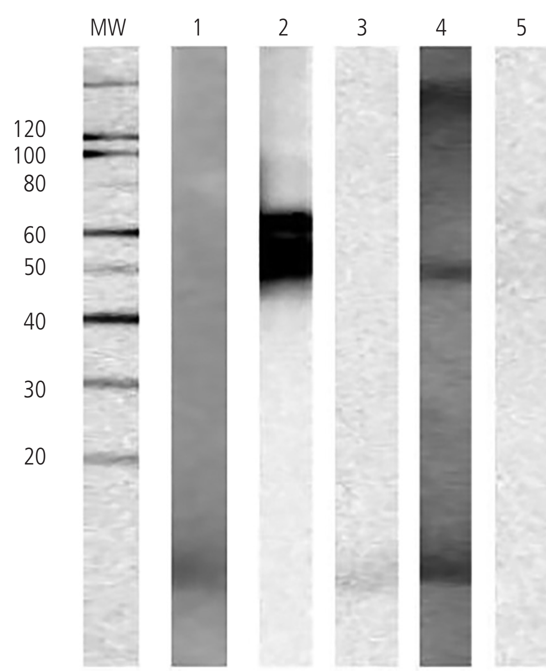

Figure 2. Peach immunoblot analysis of sera from 3 potential peamaclein reactors: Lane 1, Serum from patient # 4 in table 1; Lane 2, #15; Lane 3, #2; Lane 4, pool of LTP reactors; Lane 5, negative control serum.

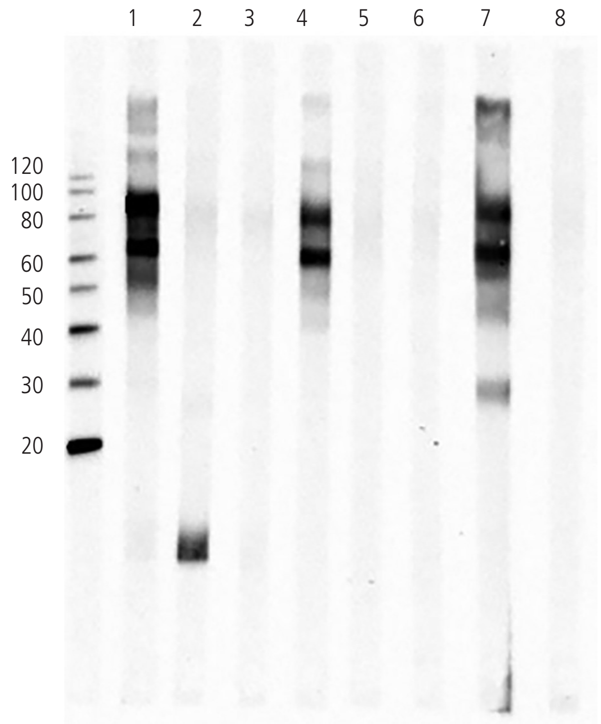

Figure 3. Peach immunoblot analysis of sera from patients reacting to lipid transfer protein, peamaclein, and a 50- to 60-kDa allergen carried out under reducing and nonreducing conditions. Lanes 1, 4, and 7, Patient # in the Table; Lanes 2 and 5, Lipid transfer protein reactor serum pool; Lanes 3, 6, and 8, Negative control serum; Lanes 1, 3, and 5, peach pulp and peel, reducing conditions; Lanes 2, 4, and 6, peach pulp and peel, nonreducing conditions; Lanes 7 and 8, peach pulp, reducing conditions.

strong Pru p 7 reactor (patient #15, Table); however, again, no reactivity was detected at about 10 kDa (Figure 3).

Detection of IgE to rPru p 7

IgE to rPru p 7 was measured in serum from 27 patients: 18 putative peamaclein reactors and 9 cypress-allergic controls. Specific rPru p 7 IgE was detected in serum from 10/18 potential reactors (56%) vs 0/9 controls (0%). Among patients with positive results, specific IgE levels ranged between 0.13 and 26 kU_A/L.

Detection of Peamaclein in the Commercial Peach SPT Used in the Study

The presence of peamaclein in the Lofarma peach extract for SPT was recently demonstrated [18] and further confirmed by direct ELISA using pools of sera from Pru p 7 reactors and control sera. Results were expressed as optical units. The peamaclein reactors' serum pool yielded an IgE reactivity level that, while low in absolute terms, was 8 times higher than the mean level (expressed as optical density [OD]) obtained with control sera (0.240 vs 0.028).

Detection of Peamaclein in the Peach Extract Used for Immunoblot Analysis

In view of the disappointing results in the immunoblot experiments, the presence of peamaclein in the peach peel extract used to carry out the immunoblot analyses was ascertained using direct ELISA with a pool of rPru p 7+/rPru p 3– sera and a negative control serum pool. IgE levels were 0.206 vs 0.042, respectively, thus confirming the presence of peamaclein in the peach extract.

Discussion

Plant food allergy due to sensitization to GRP has been considered to be pollen-food allergy syndrome in which cypress pollen might act as the primary sensitizer [7-10]. Ours is the first study to try to detect the rate of GRP hypersensitivity among Italian cypress-hypersensitive individuals. To this end, more than 800 cypress-hypersensitive individuals underwent SPT with commercial peach extracts containing exclusively stable allergens surviving extraction procedures. Of these, 19.5% tested positive, but 77.9% reacted to LTP (Pru p 3). Of course, we cannot exclude the possibility that some were cosensitized to LTP and peamaclein, although the lack of funding for this study prevented us from investigating all the sera from LTP reactors. In any case, the fact remains that only 24 patients (2.8% of cypress-hypersensitive persons) fulfilled all 3 predefined criteria for identifying patients potentially monosensitized to peamaclein (ie, cypress pollen hypersensitivity + positive SPT with commercial peach extract + negative rPru p 3 ImmunoCAP). Owing to a shortage, serum from 6 of 24 patients could not be assessed using the Pru p 7 ImmunoCAP. Of the remaining 18, only 10 were eventually Pru p 7–positive, thus indicating that hypersensitivity to peamaclein is probably rare, and much less common than hypersensitivity to LTP, at least in Italy [1]. In view of a Spanish multicenter study showing the potential relevance of thaumatin-like proteins (TLPs) in plant food cross-reactivity [22] and considering that Italian cypress pollen contains Cup a 3, a TLP [23], we verified whether some of the 8 putative GRP reactors scoring negative for Pru p 7 were in effect TLP reactors. To this end, we analyzed their sera on the novel ALEX-2 platform, which includes Mal d 2, the apple TLP. No serum reacted to this allergen (data not shown).

Interestingly, several facts seem to suggest that diagnosing peamaclein hypersensitivity may be somewhat complicated, at least today. The ImmunoCAP assay detects specific IgE with very high sensitivity, and the rPru p 7 ImmunoCAP test used in this study (currently unavailable for routine use) is an optimal approach for detecting IgE to this protein, which is attached to the solid phase when in the native state. Peamaclein appears to be very scarce in the food source (peach extract) that was used for the immunoblot experiments, as only a minority of sera produced a minimally appreciable band at about 7 kDa. Furthermore, the ELISA experiments showed only very low IgE reactivity, even when the sera of strong rPru p 7 reactors were used. Since one possible explanation for the low sensitivity of the immunoblot could be that it was performed under reducing conditions, thus potentially negatively affecting the IgE binding ability of certain allergens, the analysis was repeated under nonreducing conditions using the serum of a strong peamaclein reactor. Unfortunately, the results were equally negative, thus ruling out such a possibility. Therefore, it is not surprising that fewer Pru p 7 reactors were identified

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with the immunoblot assay than with the Pru p 7 ImmunoCAP test. The scarcity of peamaclein in the food source was also observed in one of the commercial peach extracts for SPT used in this study, although it was able to detect the putative peamaclein reactors in vivo (probably due to the much higher sensitivity of this method). The possible scarcity of peamaclein in the food sources might theoretically depend on the limited use of gibberellin as an agricultural additive in Italy. In fact, it was recently reported that, in view of the defensive properties of these proteins, synthetic gibberellin can be externally applied to crops during harvesting [24,25], and this could affect the level of GRP produced in plant-derived foods, thus potentially increasing allergenicity.

Interestingly, the sera of about only 50% of patients putatively monosensitized to peamaclein contained detectable amounts of IgE to rPru p 7 on ImmunoCAP. We cannot exclude the presence of peach allergens other than Pru p 1, Pru p 2, Pru p 3, Pru p 4, and Pru p 7. In fact, some sera appeared to react against hitherto unknown proteins at 50-60 kDa, and several other patients showed evident skin reactivity to the commercial peach extracts used for SPT in the absence of any reactivity to peach on in vitro tests. Similarly, we cannot rule out the possibility that different isoforms of Pru p 7 exist. None of the sera producing a 50- to 60-kDa band on peach immunoblot recognized bromelain, suggesting that they did not react to CCDs, which, on the other hand, do not produce skin reactions on SPT. Theoretically, the allergen recognized might be a polygalacturonase (molecular weight of around 50-60 kDa), an enzyme involved in pectin degradation whose expression is enhanced by ethylene and is normally expressed by the fruit during the ripening process. Already in 2002, Kondo et al [26] reported cross-reactivity between Japanese cedar pollen and tomato fruit, an observation that was indirectly confirmed more than a decade later in a study on immunotherapy with Japanese cedar pollen [27]. The cross-reactivity was shown to be induced by polygalacturonase. Recently, the observation has been extended to American mountain cedar [28], and a cross-reactive polygalacturonase was reported in papaya pollen and fruit [29]. Notably, the polygalacturonase protein family also includes the cypress pollen allergen Cup s 2/Cup a 2 (source Allergome), and homologous allergens are also found in olive pollen (Ole e 14) [30] and *Salsola kali* [31], which are involved in pollen/pollen cross-reactivity.

The spectrum of offending foods reported by the peamaclein-hypersensitive patients in our study was dominated by peach but also included apple, plum, orange, grapefruit, lemon, fig, and melon. Orange, apple, kiwi, fig, and melon have already been reported to be offending foods in GRP-allergic patients [2,7,9,12,13,25], whereas plum, grapefruit, and lemon are newcomers in this sense, although it is important to remember that plum belongs to the Rosaceae family and that grapefruit and lemon are citrus fruits. The reactivity of 1 patient to plum jam and orange marmalade suggests that the culprit allergen is heat-stable. While the number of patients is limited, it is quite interesting to note that no patient with a history of food allergy experienced anaphylaxis, except one who reported an exercise-induced episode (Table). This observation contrasts with those reported in other countries, where the prevalence of severe reactions among peamaclein-hypersensitive individuals seems high [15,25]. It remains to be established whether this depends on the paucity of the allergen protein in the food source in Italy, on the low level of specific IgE of the study patients, or on other factors. In contrast with published studies on the subject [7,15,32], our study suggests that hypersensitivity to peamaclein may be symptomless or associated with oral allergy syndrome, which can therefore be included in the list of allergic reactions induced by GRPs. This difference might also depend on the selection criteria applied in the present study. Looking for peach sensitization in a large population of cypress pollen–hypersensitive patients irrespective of their clinical history of food allergy may lead to results that are completely different from those obtained when patients are selected based on fruit allergy.

In summary, this study suggests that, in keeping with findings reported elsewhere [33,34], allergy and sensitization to foods secondary to cypress pollen allergy are probably rare phenomena and that most affected patients react to peach, although other Rosaceae fruits and several citrus fruits may also be offending foods. Performing SPT with commercial peach extract is currently the only way to detect potential peamaclein reactors, and this will continue to be the case until reliable in vitro tests are commercially available. Finally, our study suggests that the list of potentially cross-reacting peach and cypress pollen allergens is probably incomplete.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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