

Detection of Gibberellin-Regulated Protein (Peamaclein) Sensitization among Italian Cypress Pollen-Sensitized Patients

Short title: Peamaclein allergy in Italy

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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 looked for mono-sensitization to peamaclein among Italian cypress-pollen allergic patients.

Material and methods: 835 cypress pollen hypersensitive patients from 28 Italian allergy centers underwent thorough interview for food-allergic reactions, and SPT with a commercial peach extracts containing peamaclein. In peach reactors, IgE to rPru p 3 was measured, and those scoring negative were enrolled as potentially mono-sensitized to peamaclein. IgE reactivity to rPru p 7 was evaluated by immunoblot and by an experimental ImmunoCAP with rPru p 7.

Results: Peach SPT scored positive in 163 (19.5%) patients but 127 (77.9%) were excluded because Pru p 3 reactors. Twenty-four (14.7%, corresponding to 2.8% of the entire study population) were considered as potentially mono-sensitized to peamaclein. Their distribution did not show any geographic preference. Seventeen/24 (70.8%) had a history of food allergy, in most cases (n=15) to peach. Other offending foods included other Rosaceae, citrus fruits, fig, melon, tree nuts, and kiwi. On peach immunoblot, only 3/18 putative peamaclein allergic subjects reacted to a band at about 7kDa; 4 other patients reacted at about 50-60 kDa. Ten/18 (56%) scored positive for Pru p 7 on ImmunoCAP.

Conclusion: Peamaclein allergy and sensitization prevalence seem rare in Italy. Most patients react to peach, albeit other Rosaceae fruits and several citrus fruits may also act as offending foods. Peach and cypress pollen probably share also 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.

Material y métodos: Se estudiaron 835 pacientes italianos con alergia al polen de ciprés, provenientes de 28 centros hospitalarios. Entodosellos 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 inmunoblot 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 peamacleina. 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 inmunoblot 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. Peamacleina. Melocotón. Alergia al 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, as a difference from Anglo-Saxon and northern European countries, peanut allergy is rare, lipid transfer protein (LTP) represents the most relevant food allergen [1]. In recent years, a novel family of allergens in plant-derived foods, namely the gibberellin-regulated protein (GRP), known as peamaclein in peach, has received some attention. The detection of this new food allergen was first made about ten years ago with the observation of systemic allergic reactions induced by Rosaceae fruits (in most cases peaches) 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, these patients showed a strong reactivity on skin prick testing not only with the fresh offending fruit but also with the 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, which explains why the protein is present in Pru p 3-enriched peach extracts for SPT. GRP is a small basic protein with the 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 min [6]. GRPs are antimicrobial peptides expressed by plants upon stimuli by biotic and abiotic cues. Since then, several studies, mostly from Japan and France (notably, two countries where Cupressaceae pollen allergy is common, at least in certain 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 primary sensitizer [7-10]. In effect, the cypress pollen gibberellin-regulated protein has been recently identified [9, 11]; the homologous peach cross-reacting allergen has been sequenced and cloned and is currently known as Pru p 7. 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 hypersensitive to this family of allergens, including Japanese apricot [12], orange [13], and pomegranate [14], the names of these allergens being Pru m 7, Cit s 7, and Pun g 7, respectively. Further, clinical reactivity against 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 currently known about the prevalence of peamaclein sensitization among patients sensitized to cypress pollen, about the prevalence of clinically relevant food allergy among those sensitized, and if foods other than those reported above may represent a risk for patients reacting against members of this protein family. The exposure to cypress pollen is very variable throughout Italy, reaching its maximum in the Central regions such as Tuscany or Lazio, but sensitization to cypress pollen can be detected in the entire country. We carried out a large clinical survey across all regions of Italy to investigate the frequency of

peamacleinsensitization among cypress pollen allergic patients, and its clinical relevance in our cypress-rich country.

Material and methods

Participating Centers and patients.

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

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*) pollen, as well as house dust mite, *Alternaria* and cat and dog dander. Participating centers were left free to employ the commercial SPT preparations that they used in their routine activity; producers included Allergopharma (Reinbeck, Germany), ALK (Horsholm, Denmark), and Lofarma (Milano, Italy). 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 were measured by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). 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-Abellò, Madrid, Spain, LTP 30 µg/mL; or Lofarma, Milan Italy, LTP 50 µg/mL). Again, the single participating centers were left free to employ 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 would have been 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 were measured by ImmunoCAP as described above.

c) Detection of potential GRPreactors

Cypress hypersensitive patients showing positive SPT with peach extract but no reactivity to Pru p 3 were considered as potentially sensitized to Pru p 7. The serum of these potential GRP reactors left after the in-vitro tests reported above was employed to perform both an immunoblot analysis with peach extract (at Lofarma labs, Italy) and the detection of IgE to rPru p 7 using an experimental ImmunoCAP assay (at ThermoFisher Scientific labs, Sweden). Recombinant Pru p 7 was produced, characterized, and functionally assessed as described [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[19]. Protein content was measured according to Bradford [20] using a commercial BioRad Protein Assay Dye Reagent (Bio-Rad, Milan, Italy) and BSA 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 by immunoblot analysis under reducing conditions. The extract was mixed with Tricine sample buffer (Invitrogen, Milan, Italy) and 5% beta-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 (Tricine, Novex, Invitrogen, Milan, Italy) at 180 mA for 1 h. The resolved proteins were transferred for 1 h onto a nitrocellulose membrane according to Towbin et al. [21]. The membrane was saturated with 0.1 mol/L Tris-buffered saline containing 5% fat-free milk powder (saturation buffer) and incubated for 16 h at 4°C with serum (700 µL of the serum and 700 µL of saturation buffer). After 3 washings, bound IgE antibody was detected by peroxidase-conjugated anti-

human IgE goat IgG antibodies (Biospecific, Emeryville, CA, USA diluted 1:10000 in saturation buffer) and using an ECL Western blotting kit (Amersham, Milan, Italy).

The presence of peamaclein in the peach peel extract used to carry out the immunoblot analyses was ascertained by a direct ELISA using 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 immunoblot was used to rule out an 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 informed written consent to the use of their clinical data in an anonymous form. Cypress reactors showing peach hypersensitivity gave also informed written consent to the use of their anonymized leftover serum for research purposes. Since the study was carried out within the routine activity of all participating centers, a formal approval by an external ethical committee was not required.

Results

Patients

The final study population included 835 cypress pollen-hypersensitive patients diagnosed by positive SPT. 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 as having cypress pollen allergy by their caring doctors based on typical respiratory symptoms of rhino-conjunctivitis with or without asthma in the specific cypress pollen season (ranging between December and April in the different parts of Italy), while 84 subjects were considered as sensitized but clinically non-allergic to cypress pollen. Most patients were sensitized to other airborne allergens also; only 68/835 (8%) were mono-sensitized to cypress pollen. IgE to Cup a 1 were measured in 620 patients and ranged between < 0.1 and > 100 kU_A/L (median 6.07 kU_A/L). Sera from three patients did not show IgE to Cup a 1 despite a positive SPT with cypress pollen extract.

In total, 163 (19,5%) cypress reactors scored positive on SPT with one of the commercial peach extracts and were therefore considered as sensitized to stable peach allergens. Of these, 127 (77,9%) scored positive for rPru p 3 on ImmunoCAP analysis, 24 (14,7%) were rPru p 3-negative, 3 gave borderline results (Pru p 3 IgE values between 0.1 e 0.35 kU/L) and 9 were not tested. Thus, the subjects considered as potentially mono-sensitized to peamaclein were 24 patients (2.8% of cypress-hypersensitive subjects), and their sera were used for subsequent analyses.

The geographical distribution of peamaclein hypersensitivity was investigated by dividing the country into three 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 three areas, respectively, and no significant difference was detected.

Clinical features of putative peamaclein reactors

Presumptive peamaclein reactivity was not associated with higher Cup a 1 IgE level; in this subset IgE to Cup a 1 ranged between 0,1 and >100 kU_A/L (Table 1). Of 24 putative peamaclein reactors, three were mono-sensitized to Cypress pollen, whereas 21 were sensitized to other airborne allergens including grass (n= 12), wall pellitory (n= 12), birch (n= 5), olive (n= 8) and mugwort (n= 4) pollen, house dust mite (n= 11), and cat dander (n= 3). Seventeen of 24 (71%) had a history of food allergy (Table 1), in most cases (n=15) associated with the ingestion of peach. Other reported offending foods included other Rosaceae such as apple, plum and different 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 1).

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

Immunoblot analysis

Peach immunoblot analysis was carried out on sera from 31 subjects (18 putative peamaclein reactors [i.e., patients scoring positive on peach SPT but negative on Pru p 3 ImmunoCAP] and 13 cypress-allergic controls [i.e., patients sensitized to cypress pollen but negative on peach SPT]). Due to serum shortage, 6 of the 24 putative peamaclein reactors were not tested by immunoblot analysis. The serum of only 3/18 putative peamaclein reactors showed a very faint band at about 7kDa (2 of these patients are shown in figure 1; 3 patients are shown in figure 2) in some cases despite high levels of IgE to Pru p 7 (table 1). Four sera produced a band at about 50-60 kDa (figure 1); the lack of response to bromela in excluded an IgE reactivity to cross-reactive carbohydrate determinants (CCDs). The immunoblot analysis scored negative for the remaining 11 patients and all controls. In order to exclude that the reducing condition of the immunoblot analysis could have altered peamaclein IgE reactivity, the immunoblot analysis was repeated under non-reducing conditions using the serum of a strong Pru p 7 reactor (patient #15, table 1), but 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 subjects: 18 putative peamaclein reactors and 9 cypress-allergic controls. Specific rPru p 7 IgE were detected in serum from 10/18 potential reactors (56%) vs 0/9 (0%) controls. Among patients scoring positive, 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 Lofarmapeach extract for SPT was demonstrated recently [18] and further shown by direct ELISA using pools of sera from Pru p 7-reactors and control sera. Results were expressed as optical units. Peamaclein reactors' serum pool produced an IgE reactivity that, albeit low in absolute terms, was 8 times higher than the mean level obtained with control sera (0.240 OD vs 0.028 OD).

Detection of peamaclein in the peach extract used for immunoblot analysis

In view of the disappointing results of the immunoblot experiments, the presence of peamaclein in the peach peel extract used to carry out the immunoblot analyses was ascertained by a direct ELISA using a pool of rPru p 7+/rPru p 3- sera and a negative control serum pool. IgE levels, expressed as optical density (OD) 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 as a pollen-food allergy syndrome in which cypress pollen might act as the primary sensitizer [7-10]. This is the first study that tried to detect the rate of GRP hypersensitivity among Italian cypress-hypersensitive individuals. To this end, more than 800 subjects hypersensitive to cypress pollen underwent SPT with commercial peach extracts containing exclusively stable allergens surviving extraction procedures. Of these, 19.5% tested positive but 77,9% of them were LTP (Pru p 3) reactors. Of course, we cannot exclude that some were co-sensitized to LTP and peamaclein, but the lack of funding for this study did not allow us to investigate all the sera from LTP reactors also. However, the fact remains that only 24 patients (2.8% of cypress-hypersensitive subjects) fulfilled all 3 pre-defined criteria to identify patients potentially mono-sensitized to peamaclein (i.e., cypress pollen hypersensitivity + positive SPT with commercial peach extract + negative rPru p 3 ImmunoCAP). Due to serum shortage, 6/24 patients could not undergo the Pru p 7 ImmunoCAP. Of the remaining 18 only 10 eventually scored as Pru p 7-positive. This shows that hypersensitivity to peamaclein is probably rare, 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 (TLP) 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 presumptive 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 that includes Mal d 2, the apple TLP. No serum reacted to this allergen (data not shown).

Another relevant point is that several facts seem to suggest that diagnosing peamaclein hypersensitivity may be quite 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 (which, importantly, is currently missing for routine use) is optimal to detect IgE to this protein, which is attached to the solid phase in a native state. Peamaclein appears to be very scarce in the food source (the peach extract) that was used for the immunoblot experiments as only a minority of sera produced a minimally appreciable band at about 7kDa. Further, the ELISA experiments showed only very low IgE reactivity even using the sera of strong rPru p 7 reactors. Since one possible explanation for the low sensitivity of the immunoblot could be that it was performed under reducing conditions which may to some extent negatively affect the IgE binding ability of certain allergens, the analysis was repeated under non-reducing conditions using the serum of a strong peamaclein reactor. Unfortunately, the results were equally negative thus ruling out such possibility. In the light of these observations, it is therefore not surprising that fewer Pru p 7 reactors were identified with the immunoblot assay than with the Pru p 7 ImmunoCAP test. The scarcity of peamaclein in the food source was noticed also in one of the commercial peach extracts for SPT used in this study, although (probably due to the much higher sensitivity of this method) it was able to detect the presumptive peamaclein reactors in vivo. The possible scarcity of peamaclein in the food sources might theoretically depend on the limited use of gibberellin as an agricultural additive in our Country. In effect, it has been recently reported that, in view of the defensive properties of these proteins, synthetic gibberellin can be externally applied to crops in harvesting methodology [24,25], and this could affect the level of GRP produced in plant-derived foods, thus potentially conditioning an increase in allergenicity.

Interestingly, the sera of only about 50% of patients putatively mono-sensitized to peamaclein contained detectable amounts of IgE to rPru p 7 on ImmunoCAP. We cannot exclude the existence 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 an evident skin reactivity against the commercial peach extracts used for SPT in the absence of any reactivity to peach on in vitro tests. We also cannot rule out the possibility that different isoforms of Pru p 7 may exist. Regarding the sera producing a 50-60 kDa band on peach immunoblot, none of them recognized bromela in, suggesting that they did not react to cross-reactive carbohydrate determinants (CCD) which, on the other hand, do not produce any skin reaction on SPT. Theoretically, the recognized allergen might be a polygalacturonase (m.w. about 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, *J Investig Allergol Clin Immunol* 2022; Vol. 32(1) doi: 10.18176/jiaci.0542

Kondo et al. [26] showed the cross reactivity between Japanese cedar pollen and tomato fruit, an observation that was indirectly confirmed more than a decade later in a study of immunotherapy with Japanese cedar pollen[27]. The cross reactivity was shown to be borne by polygalacturonase. Recently, the observation has been extended to the American mountain cedar [28], and a cross-reactive polygalacturonase was shown in papaya pollen and fruit as well [29]. Notably, the polygalacturonase protein family includes also the cypress pollen allergen Cup s 2/Cup a 2(source Allergome), and homologous allergensexist also in olive pollen (Ole e 14)[30], and *Salsola kali*[31]being involved in pollen/pollen cross-reactivity.

The spectrum of offending foods reported by our peamaclein-hypersensitive patients was dominated by peach but included also apple, plum, orange, grapefruit, lemon, fig, and melon. Orange, apple, kiwi, fig, and melon have been already reported as offending foods in GRP-allergic patients [2, 7, 9, 12, 13, and 25], whereas plum, grapefruit, and lemon, are newcomers in this sense although the first belongs to the Rosaceae family and grapefruit and lemon are citrus fruits. The reactivity of one patient to plum jam and orange marmalade suggests that the culprit allergen is heat stable. Though 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 1). This observation contrasts with those coming from other countries, where the prevalence of severe reactions among peamaclein-hypersensitive individuals seems high [15,25]. Whether this depends on the paucity of the allergen protein in the food source in Italy, on the low level of specific IgE of our patients,or on other factors remains to be established.As a difference from published studies on the subject [7,15,32], our study suggests that peamaclein hypersensitivity may be symptomless or associated with oral allergy syndrome, which can be therefore included in the list of allergic reactions induced by gibberellin-regulated proteins.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 irrespectively from their clinical history of food allergy may lead to completely different results fromselecting patients based on fruit allergy.

In summary, this study suggests that, in keeping with previous studies [33,34], allergy and sensitization to foods secondary to cypress pollen allergy are probably rare phenomena and that most of such patients react to peach, albeit other Rosaceae fruits and several citrus fruits may act as offending foods as well. Performing SPT with commercial peach extract is currently the only means to detect potential peamaclein reactors, and this will be the case until reliable in-vitro tests will appear on the market. Finally, our study suggests that the list of potentially cross-reacting peach and cypress pollen allergens is probably incomplete.

Conflict of interest

All co-authors don't have any COI to declare regarding the present study.

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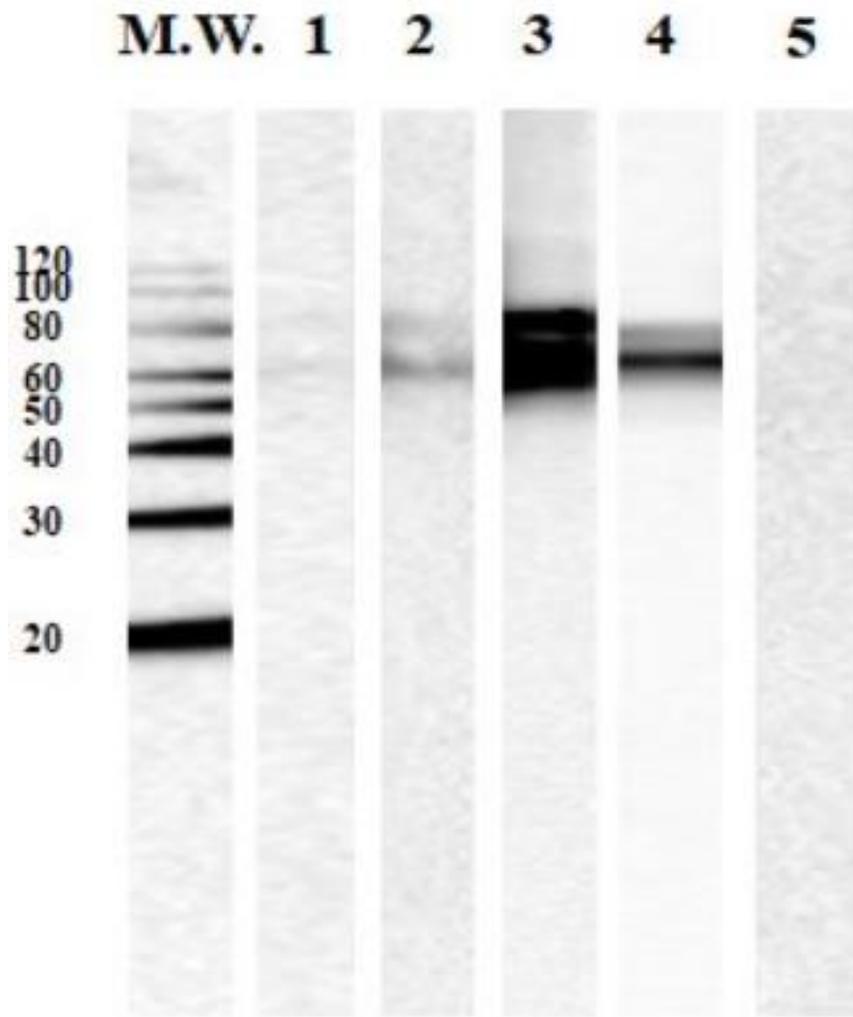
Accepted Article

	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	Y	G,D	Neg/Neg	0,63	No	
2	F	24	nd	Y	D	nd/nd	Neg	Urt	peach
3	M	39	nd	Y	P	nd/nd	nd	No	
4	M	29	21,5	Y	P,D	Neg/Neg	Neg	No	
5	F	26	0,69	Y	P,D	Neg/Neg	Neg	Urt	strawberry, blackberry, raspberry
6	F	54	10,5	Y	no	nd/nd	4,95	OAS	peach
7	M	39	5,35	Y	B,G,P,D	Neg/0.39	Neg	No	
8	M	31	5,01	Y	G	Neg/Neg	1,13	Urt, OAS	peach, orange, lemon
9	F	52	6,88	Y	B,P,O,D	nd/nd	Neg	OAS	walnut, hazelnut, kiwi
10	F	46	9,1	Y	no	Neg/Neg	0,92	Urt	peach
11	M	21	12,2	Y	G	Neg/Neg	13,14	FDEIA	peach
12	M	17	> 100	Y	G,M,O	Neg/2.5	nd	OAS	peach, melon
13	F	57	18,7	Y	O	nd/nd	26	OAS, Urt	peach, fig, plum jam, orange marmalade
14	M	44	39,6	Y	no	nd/nd	Neg	No	
15	M	22	74,5	Y	B,G,P	nd/nd	15,49	Urt	peach, apple
16	F	49	Neg	N	G,P	Neg/Neg	Neg	OAS	peach
17	M	24	5.2	Y	G,P,O,D	nd/Neg	nd	OAS	peach
18	M	23	0.80	Y	G,P,Cat	nd/Neg	nd	No	
19	F	55	0,79	Y	G,M,D,Cat	Neg/Neg	nd	Urt	peach
20	F	44	2,74	Y	G,P,O	Neg/Neg	nd	Urt	peach
21	M	12	nd	Y	PD	nd/nd	3,64	OAS, Urt	peach, lemon, grapefruit
22	F	32	0,1	Y	B,P	nd/nd	0,13	No	
23	M	16	7,0	Y	G,P	nd/nd	0,98	OAS	peach
24	F	47	nd	Y	G	nd/nd	neg	OAS	peach, plum

Table 1. Clinical features and in vitro findings in 24 presumptive peamaclein reactors.

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

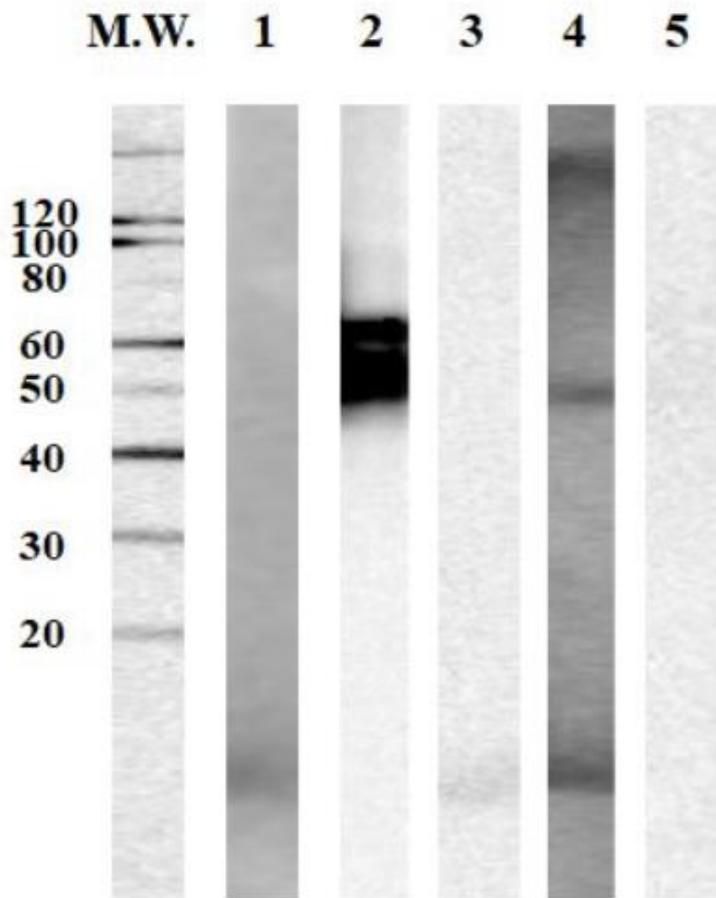
Figure 1.



Legend to figure 1: Peach immunoblot analysis of the four sera showing IgE reactivity at about 50-60 kDa.

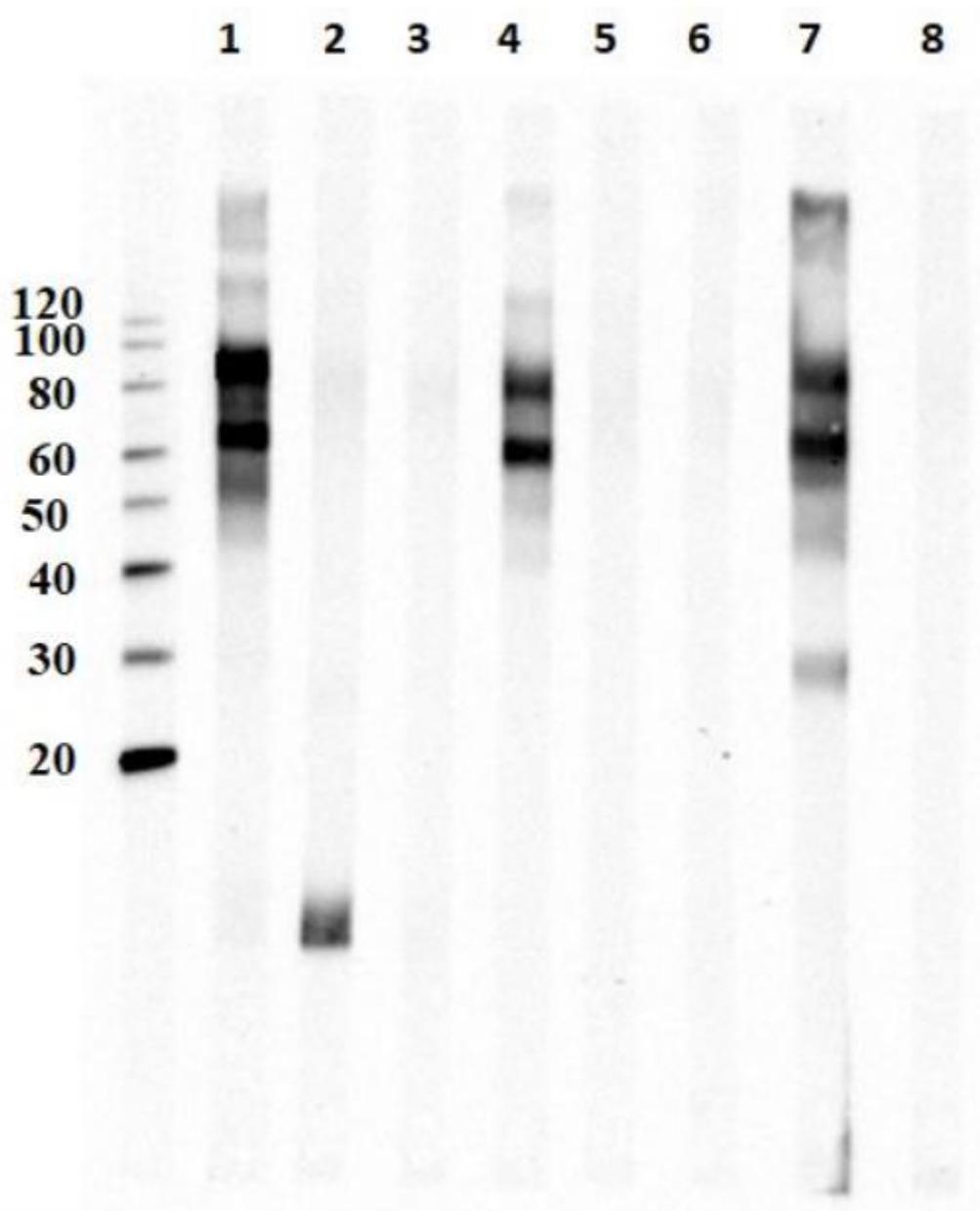
Lane 1: Serum from patients # 7 in table 1; Lane 2: #13; Lane 3: # 15; Lane 4: # 23; Lane 5: Negative control serum.

Figure 2.



Legend to figure 2: Peach immunoblot analysis of sera from 3 potential peamaclein reactors: Lane 1: Serum from patients # 4 in table 1; lane 2: #15; Lane 3: #2; lane 4: a pool of LTP reactors; Lane 5: Negative control serum.

Figure 3.



Legend to figure 3: Peach immunoblot analysis of sera from patients reacting to Lipid transfer protein, Peamaclein, and a 50-60 kDa allergen carried out under reducing and non-reducing conditions. Lanes 1, 4 & 7: Patient # 15 in table 1; Lane 2 & 5: LTP reactors serum pool; Lane 3, 6 & 8: Negative control serum. Lanes 1, 3, & 5: Peach pulp and peel, reducing conditions. Lanes 2, 4 & 6; Peach pulp and peel, non-reducing conditions. Lanes 7 & 8: peach pulp, reducing conditions.