WHY LIPID TRANSFER PROTEIN ALLERGY IS NOT A POLLEN-FOOD SYNDROME: NOVEL DATA AND LITERATURE REVIEW

Riccardo Asero MD (1), Ignazio Brusca MD (2), Lorenzo Cecchi MD (3), Patrizia Pignatti PhD (4), Valerio Pravettoni MD (5), Enrico Scala MD (6), Carina Gabriela Uasuf MD (7), Danilo Villalta MD (8).

1. Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano, Italia.
2. U.O.C. di Patologia Clinica Ospedale Buccheri La Ferla F.B.F., Palermo, Italy
3. SOS Allergy and Clinical Immunology, USL Toscana Centro, Prato, Italy
4. Allergy and Immunology Unit, Istituti Clinici Scientifici Maugeri, IRCCS Pavia, Italy.
5. UOC General Medicine Immunology and Allergology - IRCCS Foudation Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.
6. Clinical and Laboratory Molecular Allergy unit, Istituto Dermopatico dell'Immacolata, Rome, Italy.
7. Centro Malattie Allergiche "Prof Giovanni Bonsignore. Istituto per la Ricerca e l'Innovazione Biomedica, CNR, Palermo, Italy.
8. SSD di Immunologia e Allergologia, Presidio Ospedaliero S. Maria degli Angeli, Pordenone, Italia.

KEYWORDS: Food allergy, Lipid transfer protein, Pollen allergy, Cross-reactivity.

CONFLICT OF INTEREST DECLARATION: No Authors has any COI with the present manuscript to declare.

FUNDING SOURCES: The present study was not funded.

CORRESPONDENCE:

Dr. Riccardo Asero, Ambulatorio di Allergologia, Clinica San Carlo, 20037 Paderno Dugnano, Italia.
r.asero@libero.it

ABSTRACT

BACKGROUND: Based on the cross-reactivity between pollen lipid transfer proteins (LTPs) and the peach LTP, Pru p 3, it has been suggested that the pollen might initiate the LTP sensitization process.

OBJECTIVE: To establish whether LTP allergy can be considered as a pollen-food syndrome.

METHODS: The literature was reviewed and new data of component-resolved diagnosis from Italy obtained by both ISAC immunoassay and ImmunoCAP on large populations of LTP hypersensitive patients were provided and analyzed.

RESULTS: Among Pru p 3 reactors, patients positive for Art v 3 and Pla a 3 largely exceeded those sensitized to the respective major pollen allergens, Art v 1 and Pla a 1/Pla a 2. Pru p 3 reactivity remained stable around 80-90% at all ages, whereas Art v 3 and Ole e 7 recognition was missing in younger patients. Pru p 3 IgE exceeded IgE specific for pollen LTP at all ages. Inhibition studies carried out on LTP reactors showed that commercial extracts of mugwort and plane pollen were unable to inhibit significantly Pru p 3 IgE reactivity. In follow-up studies, baseline Pru p 3 IgE levels exceeded Art v 3 IgE levels in 84% of those sensitized to both allergens, and all patients positive to only one LTP allergen at baseline were sensitized to Pru p 3. Further, most of the patients who did not show any LTP reactivity at baseline became exclusive Pru p 3 reactors. On ImmunoCAP singleplex Pru p 3 IgE levels exceeded Art v 3 IgE levels in 89% of cases ($p < 0.0001$). Most literature data were in keeping with these new observations.

CONCLUSION: The evidence for LTP syndrome being a pollen-food syndrome is presently very thin. Our data do not rule out the possible sensitization to the protein, via the airways or the skin.

INTRODUCTION

A pollen food syndrome is the occurrence of a food allergy following primary sensitization to a phylogenetically conserved pollen allergen that is homologous (and hence, cross-reacting) to the relevant food allergen protein. There are several examples of pollen food syndrome in allergy. The best known is the cross-reactivity between the major birch pollen allergen Bet v 1, a PR-10, and homologous allergens in several fruits and vegetables (1). Other examples include sensitization to the pollen pan-allergen profilin which generally starts from grass pollen but can be triggered also by other sources such as birch or ragweed (2), the so-called mugwort-celery-spice syndrome, characterized by the primary sensitization to a minor mugwort allergen (3), and the food allergy to gibberellin-regulated proteins, that follows sensitization to a minor cypress pollen allergen (4). Two main in-vitro criteria must be fulfilled to define which the primary sensitizer among cross-reactive allergens is: a) IgE level to the primary sensitizer is higher than IgE to the cross-reacting allergens; and b) Cross-inhibition experiments show complete inhibition of the cross-reactive allergen when the primary sensitizer is used as an inhibitor, whereas the opposite does not happen. (1-4) This because in most cases the primary sensitizer shows a larger number of IgE reactive epitopes than the cross-reacting allergen.

Non-specific Lipid Transfer Protein (LTP) is the most frequent cause of systemic allergic reactions induced by foods in the Mediterranean area (5). Its phylogenetically conserved nature and widespread distribution in the plant kingdom potentially expose hypersensitive patients to react to several botanically unrelated plant-derived foods. The peculiar geographical distribution of this type of food allergy, which is frequent in Southern Europe, rare north of the Alps, and virtually never described outside Europe except in China (6), has prompted the search for a putative airborne “primary sensitizer” ever since. Over the years, the major candidates for the role of

"primary sensitizer" to LTP have been plane-tree, mugwort, and olive tree pollen due to their content in the three LTPs, Pla a 3, Art v 3, and Ole e 7, respectively. The presence of some degree of cross-reactivity between these pollen LTPs and Pru p 3, the peach LTP that is generally considered as the starting point for LTP sensitization, have fueled an ongoing discussion about the possible role of these pollens in the sensitization process.

The present article reviews the currently available literature regarding each single putative sensitizing pollens, and adds new data, concluding that the evidence for a pollen-derived sensitization to LTP is presently very thin.

CRITICAL REVIEW OF THE LITERATURE

Mugwort (*Artemisia vulgaris*)

The first in-vitro studies about the cross-reactivity between Art v 3 and Pru p 3 and about the hypothetical role of the former in the LTP sensitization process appeared 20 years ago. Those studies concluded that mugwort LTP shares some epitopes with the homologous peach allergen but lacks other "main ones". The inhibition assays showed an almost full inhibition of IgE binding when peach was used as an inhibitor, whereas mugwort pollen was able to inhibit only partially the IgE binding by the fruit LTP (7). Subsequently, the same authors (8), as well as others, based on other cross-inhibition experiments (9), confirmed this finding. In the study by Pastorello and co-workers (9) the absorption of sera with as few as 4 µg of Pru p 3 was sufficient to abolish IgE reactivity to Pru p 3 in a peach extract, while 40 µg of Art v 3 caused only a partial inhibition. Further, importantly no pollen (including grass, ragweed, pellitory and olive tree) at the concentrations of 0.4 and 0.04 mg were able to inhibit the IgE reactivity to Pru p 3 suggesting that Pru p 3 is the LTP showing the highest number of epitopes (Table 1).

Surprisingly enough, after two years the authors of the first two papers changed their mind stating, based on new in-vitro inhibition experiments, that Art v 3 behaves as a primary sensitizer in some patients with IgE to both Pru p 3 and Art v 3 (10). Some years later, an in-vivo and in-vitro Italian study tackled this view reporting that in Pru p 3 hypersensitive subjects skin tests with Artemisia pollen extract scored positive only in a minority of cases and that in patients co-recognizing peach and mugwort LTPs the former showed always much more intense skin reactions and elevated IgE levels than the latter (11). Later on, the possibility of primary sensitization to LTP via mugwort pollen returned into the discussion as some Chinese studies showed that in that country mugwort pollen plays a dominant role as a primary sensitizer to LTP (6,12). Further, one

Spanish study (13) showed that Artemisia LTP (Art v 3) can elicit allergic respiratory symptoms, but also stated that sensitization occurs through cross-reactivity starting from the peach. Finally, one study from Italy (14) showed that only one-fourth of 286 Art v 3 reactors recognized Art v 1, the mugwort major allergen, thus suggesting against a primary pollen sensitization. Again, in vitro inhibition experiments showed only a partial inhibition (just more than 50%) by Art v 3 over Pru p 3 IgE reactivity (14).

One consideration of pollen distribution is also worth doing. It is generally accepted that Artemisia pollen is present all over Europe (15,16), but less distributed if not virtually absent in southern areas of the continent (https://www.polleninfo.org/FI/en/current-data/pollen-load-map-of-europe.html. Accessed 30/12/2020). Thus, firstly, it seems rather odd that mugwort pollen (specifically Art v 3) may induce a primary sensitization to LTP only in the southern part of the continent. Secondly, it seems unlikely that exposure to mugwort pollen and prevalence of LTP allergy show an opposite gradient of distribution over Europe. Further, the (limited) cases of LTP hypersensitivity in northern Europe have been associated with conditions other than mugwort pollen sensitization, such as Cannabis use (17,18). In the UK as well as in Central Europe, Pru p 3 remains the key allergen in LTP hypersensitive patients (19,20). Therefore, the conclusion drawn in 2012 by Spanish authors that "mugwort sensitization results from cross-reactivity with other LTP sensitizations, rather than being a primary sensitization or a co-sensitization" (21) seems the most reasonable one.

Olive tree (*Olea europaea*)

The olive tree pollen lipid transfer protein, Ole e 7, displays a sequence identity with plant food LTPs that has been found to range between 50% (22) and 20% (23). The geographical distribution

of olive tree pollen in Europe, which is quite overlapping with that of LTP-induced food allergy, prompted to consider this plant as a possible primary source of LTP sensitization. Although the association between severe food allergy and sensitization to Ole e 7 has been described (24), two Spanish studies were unable to detect any correlation between peach and olive tree pollen in LTP hypersensitive subjects (25) and between food allergy and Ole e 7 (18), respectively. Nonetheless, recently the possibility of olive tree pollen being the primary sensitizer to LTP in regions with high exposure was put forward once more from Spanish authors based on in-vitro inhibition assays (26). Although about 80% of Ole e 7 reactors score positive to at least one plant food LTP (27), the fact remains that most Pru p 3 hypersensitive patients do not show any IgE reactivity to olive tree pollen on in-vivo testing (11).

Planetree (*Platanus acerifolia*)

Planetree pollen sensitization is frequent in Spanish food-allergic individuals (28), and the planetree pollen LTP, Pla a 3 cross-reacts to other pollen and food LTPs (21,29). Although the cross-reactivity between Pla a 3 and Pru p 3 seems bi-directional (30), specific IgE levels to Pru p 3 are generally higher than those to Pla a 3 (30). Further, also in this case, only a fraction of Pru p 3 hypersensitive patients show plane tree pollen hypersensitivity in the clinical setting (11). Finally, one Spanish study found a high prevalence of profilin sensitization in patients with plane tree pollen sensitization and food allergy (31). In the case of the plane tree, maps of pollen distribution (https://www.polleninfo.org/FI/en/current-data/pollen-load-map-of-europe.html. Accessed 30/12/2020) are consistent with the putative distribution of LTP allergy in Europe. Even though plane tree pollen is polluting virtually all European countries, including the London area where the largest case series of LTP allergy north of the Alps has been published (20), this is again not

completely in favour of the “pollen food” hypothesis for LTP allergy. LTP allergy prevalence is higher in the Mediterranean countries than in continental Europe where exposure to plane tree pollen is as high, if not higher, as in the southern areas.

Cypress (*Cupressus arizonica*)

Based on its geographic distribution, cypress pollen is another putative candidate as a primary sensitizer to lipid transfer protein (328). Nowadays we know that cypress pollen is the primary sensitizer to gibberellin-regulated protein, which is associated with systemic reactions to different fruits, particularly the peach (33,34). To our knowledge, there are no data regarding an association with food LTP hypersensitivity and besides, no LTPs have been identified in cypress pollen so far (http://www.allergen.org. Accessed 30/12/2020)

Pellitory (*Parietaria judaica*)

Despite pellitory is one of the major sources of aeroallergens in the Mediterranean areas (16) and therefore a putative sensitizer in the LTP allergy, this is not the case from both a clinical and molecular point of view. In a study on Mor m 3, the mulberry nsLTP (35), the Authors investigated the alignment of the amino acid sequences from Mor m 3 and other nsLTP (including Pru p 3, Art v 3, and Par j 2) evaluating the relevant regions showing IgE-binding activity in Pru p 3 vs other nsLTPs. Little amino acid identity was found in the sequence of the IgE-binding regions between Pru p 3 and both Art v 3 and Par j 2, suggesting that the two pollens cannot be considered responsible for the sensitization to Pru p 3.

Natural history

Another way to establish whether fruits (peach) or pollen is the "primary sensitizer" to LTP is to look both at the natural history and the epidemiological data of allergic diseases in patients included in the studies dealing with LTP allergy. Unfortunately, these aspects are not addressed in most cases. In an international study (36), apple allergy started later than pollen allergy in all 4 participating countries (Austria, Italy, Netherlands, and Spain), but while in the former three apple allergy followed the primary sensitization to birch pollen, in Spain apple allergy followed Pru p 3 hypersensitivity which in turn occurred at the same time as pollen allergy, with grass being by far the main one. Two further studies from Spain (25, 37) did not find any relationship between the prevalence of sensitization to Pru p 3 and any pollinosis.

METHODS

Five allergy units (Milan, Palermo, Pavia, Pordenone, and Rome) scattered throughout the Italian territory provided their in-vitro data obtained in 9138 allergic patients measuring IgE either by ImmunoCAP ISAC 112 or by singleplex ImmunoCAP (both Thermo Fisher Scientific, Uppsala, Sweden), between September 2015 and December 2020. All tests were performed during routine care, and the samples were anonymized, since no personal data, except for age and sex, was available. The Institutional Review Board of IDI-IRCCS confirmed that ethical approval was not required in this case (n. 493.1)

Serum IgE reactivity was analyzed using the latest commercially available ImmunoCAP-ISAC platform as per the manufacturer's instructions. In brief, ImmunoCAP-ISAC 112 slides were washed, rinsed and dried at room temperature (RT). Undiluted serum (30 µl) from each patient was pipetted on to the slide and after 120min incubation at RT in a humid chamber, slides were washed, rinsed and dried. IgE binding was detected by the addition of an anti-human secondary antibody (ThermoFisher Scientific). Slides were then washed, rinsed, dried, and stored in the dark until scanning. Images were acquired by scanning allergen biochips with a CapitalBioLuxScan™ 10K microarray scanner. IgE values are expressed as ISU arbitrary units (ISAC Standardized Units) corresponding to IgE antibody levels in the ng/ml range (detection limit: 0.01 ISU-E, values above 0,3 ISU-E were considered as positive) (38). For the follow-up studies, since in some cases the comparisons were made with versions of the ISAC test containing a lower number of LTPs, the serial evaluations were performed only for Art v 3 and Pru p 3.

Sera from Palermo were tested with the singleplex ImmunoCAP 250 following the manufacturer's instructions and the selected cutoff value was 0.1 kU/L.

Statistics

All data were analyzed with the IBM SPSS statistical package version 21 (Armonk, NY). The TD-Synergy Laboratory Information System was used to search and collect demographic information, i.e., age and gender, and clinical and laboratory data for patients who attended the outpatient Allergy clinic and underwent specific IgE testing. Categorical variables were analyzed using Pearson's χ^2 or Fisher's exact test. Differences between prevalences were evaluated using the nonparametric Mann–Whitney U-test. The degree of relationship between quantitative variables was analyzed using Spearman's correlation (r) coefficient, given the non-parametric distribution of the observed values. Separate modelling was performed for each condition including all molecules, in addition to sex and age. P values <0.05 were considered significant.

RESULTS

ISAC IMMUNOASSAY DATA

a) PREVALENCES AND IgE LEVELS

IgE levels to Pru p 3, Art v 3, Ole e 7, and Pla a 3 were measured in 2048 LTP hypersensitive patients (age 30±16, 1136 F). Among Pru p 3 reactors, the number of patients positive for Art v 3 and Pla a 3 largely exceeded that of patients sensitized to the respective major pollen allergens, Art v 1 and Pla a 1/Pla a 2, which are generally considered as markers of genuine pollen sensitization (Table 2), suggesting that both Art v 3 and Pla a 3 sensitizations were the result of a cross-reactivity in which Pru p 3 acts as the primary sensitizer.

Further, the age distribution of pollen nsLTP molecules throughout the entire population showed that the prevalence of Pru p 3 recognition remained stable around 80-90%, whereas Art v 3 and Ole e 7 recognition were missing in patients younger than two years of age, and progressively increased in older children to reach the adult level after 6 years. On the other hand, Pla a 3 was regularly recognized in about one half of the population in all age subsets observed. Overall, Pru p 3 IgE recognition exceeded, if not doubled, the IgE recognition of the pollen LTP molecules in all the age subsets considered, making it very difficult to hypothesize that the latter could act as sensitizing molecules in the Mediterranean population studied (Figure 1).

The mean levels of IgE to a series of different LTPs including also Ara h 9, Cor a 8, Jug r 3, and Tri a 14 were calculated and plotted against the presence or absence of Pru p 3 IgE reactivity. The mean specific IgE levels increased significantly in the presence of Pru p 3 reactivity in all cases except for Tri a 14 and Ole e 7, which did not change (Table 3). The linear correlation between Pru p 3 IgE

levels and IgE levels of all other LTPs studies was significant at 0.001 (2-tailed) in all cases (Spearman's rank correlation coefficient between Pru p 3 and Ara h 9: 0,781; Art v 3: 0,720; Cor a 8: 0,735; Jug r 3: 0,830; Ole e 7: 0,399; Pla a 3: 0,798).

b) INHIBITION STUDIES

IgE reactivity to Art v 3, Pla a 3, and Pru p 3 of sera from 3 patients sensitized to all three allergens were measured before and after absorption of sera with commercial extracts of Artemisia vulgaris and Platanus acerifolia (Stallergenes, Anthony, France). Inhibition < 75% of IgE reactivity was arbitrarily considered as not relevant. Results are shown in figure 2. In no case, the two commercial extracts were able to induce significant inhibition of Pru p 3 IgE reactivity, whereas this was often the case for IgE reactivity to Pla a 3 and Art v 3.

c) FOLLOW-UP DATA

IgE to Pru p 3 and Art v 3 were measured serially in 102 pediatric (age range 6mo-6 years) patients. Measurements were carried out at intervals of at least one year; 85, 11 and 6 patients had 2, 3 and 4 measurements, respectively. Based on baseline findings these patients were divided into 3 subgroups:

a) Patients who showed IgE to both LTPs at baseline (n=19)

b) Patients who showed IgE to one of the two allergens (n=60)

c) Patients who did not show IgE to any of the two allergens (n=23)

Subgroup a: In patients reactive to both LTPs, baseline Pru p 3 IgE levels exceeded Art v 3 IgE levels in 16/19 cases (84%) (median levels 3.09 vs 1.4 ISU-E, respectively). At the follow-up observations, Pru p 3 IgE levels exceeded Art v 3 IgE levels in 15/19 cases (78%), including 2/3 of those showing higher Art v 3 levels at baseline.

Subgroup b: All patients positive for only one of the two LTPs at baseline scored positive for Pru p 3 (100%). At the follow-up analyses, 27 (45%) were still monosensitized to Pru p 3 while 33 (55%) had become positive to Art v 3 also, although IgE levels to Pru p 3 exceeded Art v 3 IgE levels in 30/33 (90,9%) cases.

Subgroup c: Of 23 patients who did not show any LTP reactivity at baseline and had become LTP reactors at the first follow-up control, 13 (56,5%) were exclusive Pru p 3 reactors, 9 (39,1%) reacted to both Pru p 3 and Art v 3 (with Pru p 3 IgE exceeding Art v 3 IgE in 8 cases, while in 1 case the levels were identical), whereas the remaining patient showed elevated levels of Art v 3 IgE but no reactivity to Pru p 3.

IMMUNOCAP DATA

Data from 285 consecutive LTP-sensitized patients (mean age 38.2 years; range 2-79; 184 F) collected in Palermo were evaluated using the singleplex ImmunoCAP. Of these, 275 (96.5%) were Pru p 3 reactors, and 200 (70%) showed IgE to Art v 3. IgE reactivity to other food LTP including Ara h 9 (80.7%), Jug r 3 (82.5%), Tri a 14 (57.2%) and Cor a 8 (68.8%) are summarized in Table 4. Data from further 3,026 patients (mean age 34.1 years; range 3-74;1104 males, 1922 females), tested for Pru p3, *Parietaria judaica* and *Olea europea* extracts were also analyzed. No significant relationship between the allergens tested was found (Concordance correlation coefficient Pru p 3-*Olea europea* = 0,318; Pru p 3 -*Parietaria judaica* =0,322).

DISCUSSION

The concept of pollen-food allergy syndrome implies the primary sensitization to a seasonal aeroallergen which is followed by a food allergy caused by the homology between one or more pollen allergens with one or more food proteins. Apple or hazelnut allergy in birch pollen allergic patients represent a perfect example in this sense, and nobody could reasonably claim that apple is the primary sensitizer despite apple IgE can be detected in the majority of birch pollen-allergic patients (1, 39).

In the case of allergy to LTP, things appear completely different. Available data, including the new in vitro data that we reported here, seem to rule out the sensitization to a pollen source as the starting point of LTP syndrome unless one postulates that peach LTP allergy is the result of the sensitization to any pollen LTP among planetree, mugwort, olive tree, or pellitory all leading to the same eventual food allergy. Furthermore, the lack of cross-reactivity between Ole e 7 and/or Par j 1-2 sensitization and Pru p 3 has already been described in the literature (23), mainly due to the widely known structural difference between such LTPs. Inhibition as well as prevalence data seem to rule out this possibility. Inhibition studies have been performed with only 3 sera, but the inability of plantain or mugwort extracts to completely inhibit the Pru p 3 signal in all cases can be considered as indirect evidence that neither plantain nor mugwort act as the primary sensitizers in patients with LTP allergy. In all cases studied, pollen LTP allergens seem to show less allergenic epitopes than peach LTP, and IgE levels are in favour of peach LTP in most cases.

The peculiar geographic distribution of LTP allergy points to a local (Mediterranean) trigger. Of course, we cannot exclude *tout court* the primary airborne sensitization to a hitherto unknown pollen source although this hypothesis seems unlikely if one considers that a large proportion of LTP allergic patients score completely negative on allergic testing for all seasonal airborne

allergens and do not report any respiratory allergy. However, several data have accumulated over the years suggesting a possible direct sensitization to peach LTP via the airways (40-43) or the skin (44-47). Again, this does not explain the geographic prevalence of this allergy, although one has to consider that for instance peach fuzz is removed from the fruits to be exported in countries where peaches are not grown (40). The main producers of peaches in the world are China, Italy, Greece, Spain, and the USA (48). Interestingly, except for the USA, these countries represent the areas showing the highest prevalence of LTP allergy.

In conclusion, we believe that the data available to date, including those of the present study, point against a primary pollen sensitization in LTP allergic patients.

REFERENCES

1. Kazemi-Shirazi, Pauli G, Purohit A, Spitzauer S, Froschl R, Hoffmann-Sommergruber K, Breiteneder H, Scheiner O Kraft D, Valenta R. Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergy. J Allergy Clin Immunol 2000; 105: 116-25.
2. Ebner C, Hirschwehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, Kraft D, Scheiner O. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). J Allergy Clin Immunol 1995; 95: 962-9.
3. Borghesan F, Mistrello G, Amato S, Giuffrida MG, Villalta D, Asero R. Mugwort-fennel-allergy-syndrome associated with sensitization to an allergen homologous to Api g 5. Eur Ann Allergy Clin Immunol. 2013; 45: 130-7.
4. Sénéchal H, Keykhosravi S, Couderc R, Selva MA, Shahali Y, Aizawa T, Busnel JM, Arif R, Mercier I, Pham-Thi N, Charpin D, Poncet P. Pollen/Fruit Syndrome: Clinical Relevance of the Cypress Pollen Allergenic Gibberellin-Regulated Protein. Allergy Asthma Immunol Res. 2019; 11: 143-151.
5. Asero R, Antonicelli L, Arena A, Bommarito L, Caruso B, Crivellaro M, De Carli M, Della Torre E, Della Torre F, Heffler E, Lodi Rizzini F, Longo R, Manzotti G, Marcotulli M, Melchiorre A, Minale P, Morandi P, Moreni B, Moschella A, Murzilli F, Nebiolo F, Poppa M, Randazzo S, Rossi G, Senna GE. EpidemAAITO: features of food allergy in Italian adults attending allergy clinics: a multi-centre study
Clin Exp Allergy. 2009; 39: 547-55.

6. Gao Z-S, Yang Z-W, Wu S-D, Wang H-Y, Liu M-L, et al. Peach allergy in China: a dominant role for mugwort pollen lipid transfer protein as a primary sensitizer. J Allergy Clin Immunol 2013; 131: 224-5.
7. Diaz-Perales A, Lombardero M, Sanchez-Monge R, Garcia-Selles FJ, Pernas M, Fernandez-Rivas M, Barber D, Salcedo G. Lipid transfer proteins as potential plant panallergens: cross-reactivity among proteins of Artemisia pollen, Castanea nut a Rosaceae fruits, with different IgE-binding capacities. Clin Exp Allergy 2000; 30: 1403-10.
8. Garcia-Selles FJ, Diaz-Perales A, Sanchez-Monge R, Alcantara M, Lombardero M, Barber D, Salcedo G, Fernandez-Rivas M. Patterns of reactivity to lipid transfer proteins of plant foods and Artemisia pollen: an in-vivo study. Int Arch Allergy Immunol 2002; 128: 115-22.
9. Pastorello EA, Pravettoni V, Farioli L, Rivolta F, Conti A, Ispano M, Fortunato D, Bengtsson A, Bianchi M. Hypersensitivity to mugwort (Artemisia vulgaris) in patients with peach allergy is due to a common lipid transfer protein allergen and is often without clinical expression. J Allergy Clin Immunol 2002; 110: 310-7.
10. Lombardero M, Garcia-Selles FJ, Polo F, Jimeno L, Chamorro MJ, Garcia-Casado G, Sanchez-Monge R, Diaz-Perales A, Salcedo G, Barber D. Prevalence of sensitization to Artemisia allergens Art v 1, Art v 3, and Art v 60 kDa. Cross-reactivity among Art v 3 and other relevant lipid transfer protein allergens. Clin Exp Allergy 2004; 34: 1415-21.
11. Asero R. Co-recognition of lipid transfer protein in pollen and foods in Northern Italy: clinician's view. Eur Ann Allergy Clin Immunol 2010; 42: 205-8.
12. Ma S, Nie L, li H, Wang R, Yin J. Component-resolved diagnosis of peanut allergy and its possible origins of sensitization in China. Int Arch Allergy Immunol 2016; 169: 241-8.

13. Sanchez-Lopez J, Tordesillas L, Pascal M, Munoz-Cano R, Garrido M, Rueda M, Vilella R, Valero A, Diaz-Perales A, Picado C, Bartra J. Role of Art v 3 in pollinosis of patients allergic to Pru p 3. J Allergy Clin Immunol 2014: 133: 1018-25.

14. Scala E, Till SJ, Asero R, Abeni D, Guerra EC, Pirrotta L, Paganelli R, Pomponi D, Giani M, De Pità O, Cecchi L. Lipid transfer protein sensitization: reactivity profiles and clinical risk assessment in an Italian cohort. Allergy 2015; 70: 933-43.

15. Spieksma FThM, von Whl PG. Allergenic significance of Artemisia (Mugwort) pollen. In: D'Amato G, Spieksma FThM, Bonini S, Eds. Allergenic pollen and pollinosis in Europe. Oxford, Blackwell Science. 1991; 121-124

16. D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, Liccardi G, Popov T, van Cauwenberge P. Allergenic pollen and pollen allergy in Europe. Allergy 2007; 62: 976-90.

17. Ebo DG, Swerts S, Sabato V, Hagendorens MM, Bridts CH, Jorens PG, De Clerck LS. New food allergies in a European non-Mediterranean region: is Cannabis sativa to blame? Int Arch Allergy Immunol 2013; 161: 220-8.

18. Faber MA, Decuyper II, Van Gasse AL, Sabato V, Hagendorens MM, Ebo DG. Letter to the Authors Concerning the Published Manuscript by Rial and Sastre: Food Allergies Caused by Allergenic Lipid Transfer Proteins: What Is Behind the Geographic Restriction? Curr Allergy Asthma Rep 2018; 18: 70.

19. Mothes-Luksch N, Raith M, Stingl G, Focke-Tejkl M, Razzazi-Fazeli E, Zieglmayer R, Wöhrl S, Swoboda I. Pru p 3, a marker allergen for lipid transfer protein sensitization also in Central Europe. Allergy 2017; 72: 1415-1418.

20. Skypala IJ, Cecchi L, Shamji MH, Scala E, Till S. Lipid Transfer Protein allergy in the United Kingdom: Characterization and comparison with a matched Italian cohort. Allergy 2019; 74: 1340-1351.
21. Pascal M, Muñoz-Cano R, Reina Z, Palacín A, Vilella R, Picado C, Juan M, Sánchez-López J, Rueda M, Salcedo G, Valero A, Yagüe J, Bartra J. Lipid transfer protein syndrome: clinical pattern, cofactor effect and profile of molecular sensitization to plant-foods and pollens. Clin Exp Allergy 2012; 42: 1529-39.
22. Salcedo G, Sanchez-Monge R, Diaz-Perales A, Garcia-Casado G, Barber D. Plant non-specific lipid transfer proteins as food and pollen allergens. Clin Exp Allergy 2004; 34: 1336-41.
23. Tordesillas L, Sirvent S, Díaz-Perales A, Villalba M, Cuesta Herranz J, Rodríguez R, Salcedo G. Plant lipid transfer protein allergens: no cross-reactivity between those from foods and olive and Parietaria pollen. Int Arch Allergy Immunol 2011; 156: 291-6.
24. Florido Lopez JF, Quiralte Enriquez J, Arias de Saavedra Alías JM, Saenz de San Pedro B, Martin Casañez E. An allergen from Olea europaea pollen (Ole e 7) is associated with plant-derived food anaphylaxis. Allergy 2002; 57 Suppl 71:53-9.
25. Barber D, de la Torre F, Feo F, Florido F, Guardia P, Moreno C, Quiralte J, Lombardero M, Villalba M, Salcedo G, Rodríguez R. Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. Allergy 2008; 63: 1550-8.
26. Oeo-Santos C, Navas A, Benedé S, Ruíz-León B, Díaz-Perales A, Vogel L, Moreno-Aguilar C, Jurado A, Villalba M, Barderas R. New insights into the sensitization to nonspecific lipid transfer proteins from pollen and food: New role of allergen Ole e 7. Allergy 2020; 75: 798-807

27. Scala E, Abeni D, Pomponi D, Paganelli R, Locanto M, Giani M, Cecchi L, Asero R. Ole e 1, Ole e 7, and Ole e 9: Identifying distinct clinical subsets of olive tree-allergic patients. J Allergy Clin Immunol. 2016; 137: 629-631.

28. Lauer I, Miguel-Moncin MS, Abel T, Foetisch K, Hartz C, Fortunato D, Cistero-Bahima A, Vieths S, Scheurer S. Identification of a plane pollen lipid transfer protein (Pla a 3) and its immunological relation to the peach lipid transfer protein, Pru p 3. Clin Exp Allergy 2007; 37: 261-9.

29. Scala E, Cecchi L, Abeni D, Guerra EC, Pirrotta L, Locanto M, Giani M, Asero R. Pla a 2 and Pla a 3 reactivities identify plane tree-allergic patients with respiratory symptoms or food allergy. Allergy 2017; 72: 671-674.

30. Wangorsch A, Larsson H, Messmer M, García-Moral A, Lauer I, Wolfheimer S, Schülke S, Bartra J, Vieths S, Lidholm J, Scheurer S. Molecular cloning of plane pollen allergen Pla a 3 and its utility as diagnostic marker for peach associated plane pollen allergy. Clin Exp Allergy 2016; 46: 764-74.

31. Enrique E, Alonso R, Bartolomé B, San Miguel-Moncín M, Bartra J, Fernández-Parra B, Tella R, Asturias JA, Ibarrola I, Martínez A, Cisteró-Bahíma A. IgE reactivity to profilin in Platanus acerifolia pollen-sensitized subjects with plant-derived food allergy. J Investig Allergol Clin Immunol 2004; 14: 335-42.

32. Sánchez-López J, Asturias JA, Enrique E, Suárez-Cervera M, Bartra J. Cupressus arizonica pollen: a new pollen involved in the lipid transfer protein syndrome? J Investig Allergol Clin Immunol 2011; 21: 522-6.

33. Klingebiel C, Chantran Y, Arif-Lusson R, Ehrenberg AE, Östling J, Poisson A, Liabeuf V, Agabriel C, Birnbaum J, et al. Clin Exp Allergy. Pru p 7 sensitization is a predominant cause of severe, cypress pollen-associated peach allergy 2019; 49: 526-536.

34. Asero R, Abbadessa S, Aruanno A, Barilaro G, Barzaghi C, Bignardi D, Bilò MB, et al. Detection of Gibberellin-Regulated Protein (Peamaclein) Sensitization among Italian Cypress Pollen-Sensitized Patients. J Investig Allergol Clin Immunol. 2020; doi: 10.18176/jiaci.0542.

35. Ciardiello MA, Palazzo P, Bernardi ML, Carratore V, Giangrieco I, Longo V, Melis M, Tamburrini M, Zennaro D, Mari A, Colombo P. Biochemical, immunological and clinical characterization of a cross-reactive non-specific lipid transfer protein 1 from mulberry. Allergy 2010; 65: 597-605.

36. Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E, Asero R, van Leeuwen A, Bohle B, et al. Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. J Allergy Clin Immunol 2006; 118: 481-8.

37. Barber D, de la Torre F, Lombardero M, Antépara I, Colas C, Dávila I, Tabar AI, Vidal C, Villalba M, Salcedo G, Rodríguez R. Component-resolved diagnosis of pollen allergy based on skin testing with profilin, polcalcin and lipid transfer protein pan-allergens. Clin Exp Allergy. 2009; 39: 1764-73.

38. Harwanegg C, Laffer S, Hiller R, Mueller MW, Kraft D, Spitzauer S, Valenta R. Microarrayed Recombinant Allergens for Diagnosis of Allergy. Clin Exp Allergy 2003; 33: 7-13.

39. Bircher AJ, Van Melle G, Haller E, Curty B, Frei PC. IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy. Clin Exp Allergy 1994; 24: 967-74.

40. Asero R, Mistrello G, Amato S, Roncarolo D, Martinelli A, Zaccarini M. Peach fuzz contains large amounts of lipid transfer protein: is this the cause of the high prevalence of sensitization to LTP in Mediterranean countries? Eur Ann Allergy Clin Immunol 2006; 38: 118-21.

41. Borghesan F, Mistrello G, Roncarolo D, Amato S, Plebani M, Asero R. Respiratory allergy to lipid transfer protein. Int Arch Allergy Immunol 2008; 147: 161-5.

42. García BE, Lombardero M, Echechipía S, Olaguibel JM, Díaz-Perales A, Sánchez-Monge R, Barber D, Salcedo G, Tabar AI. Respiratory allergy to peach leaves and lipid-transfer proteins. Clin Exp Allergy. 2004; 34: 291-5.

43. Pérez-Calderón R, Gonzalo-Garijo MÁ, Rodríguez-Velasco FJ, Sánchez-Vega S, Bartolomé-Zavala B. Occupational respiratory allergy in peach crop workers. Allergy 2017; 72: 1556-1564.

44. Asero R. Peach-induced contact urticaria is associated with lipid transfer protein sensitization. Int Arch Allergy Immunol. 2011; 154: 345-8.

45. Metz-Favre C, Pauli G, Bessot JC, De Blay F. Molecular allergology in practice: an unusual case of LTP allergy. Eur Ann Allergy Clin Immunol 2011; 43: 193-5.

46. Ebo DG, Swerts S, Sabato V, Hagendorens MM, Bridts CH, Jorens PG, De Clerck LS. New food allergies in a European non-Mediterranean region: is Cannabis sativa to blame? Int Arch Allergy Immunol 2013; 161: 220-8.

47. Gandolfo-Cano M, Bartra J, González-Mancebo E, Feo-Brito F, Gómez E, Bartolomé B, Muñoz-García E, Sanz Marcto A, Vivanco F, Cuesta-Herranz J, Pastor-Vargas C.Molecular characterization of contact urticaria in patients with melon allergy. Br J Dermatol 2014; 170: 651-6.

48. Production of peaches and nectarines in 2018; Crops/Regions/World/Production Quantity (from picklists)". United Nations, Food and Agricultural Organization, Statistics Division (FAOSTAT). 2019.

TABLE 1: Amino acid sequence identity (%), identical positions and similar positions of LTP from different pollen sources vs Pru p 3 (IUIS data)

POLLEN	nsLTP	IDENTITY	IDENTICAL POSITIONS	SIMILAR POSITIONS
Platanus orientalis	Pla or 3	46,6%	55	20
Platanus acerifolia	Pla a 3	45,7%	54	21
Artemisia vulgaris	Art v 3	40,5%	47	25
Ambrosia artemisifolia	Amb a 6	26,7%	32	25
Parietaria judaica	Par j 2	18,8%	25	25
Parietaria judaica	Par j 1	14,8%	26	20
Olea europea	Ole e 7	4.3%	4	7

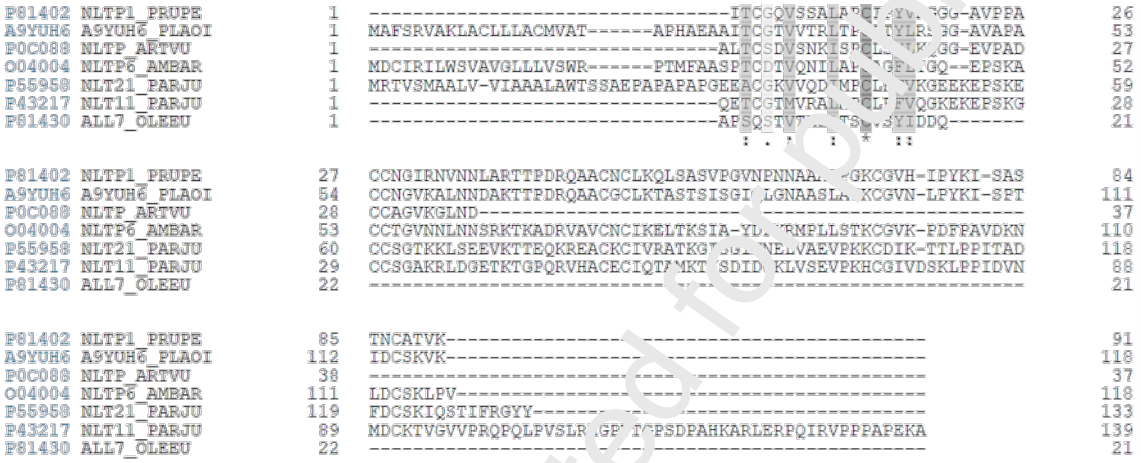

Table 2. The proportion of patients positive for Art v 1, Art v 3, Ole e 1, Ole e 7, and Pla a 1-3 among patients not showing or showing IgE reactivity to Pru p 3.

	Pru p 3^{neg} *(466)*	**Pru p 3^{pos}** *(1582)*	
	% within the respective subset		
Art v 1	*21,9%*	*11,1%*	*
Art v 3	*21,2%*	*57,0%*	*
Ole e 1	*44,6%*	*34,6%*	*
Ole e 7	*31,1%*	*24,5%*	*
Pla a 1	*4,3%*	*5,4%*	
Pla a 2	*28,3%*	*31,0%*	
Pla a 3	*26,2%*	*69,7%*	*
			* *<0.01*

The comparisons were carried out by the z test. Tests are adjusted for all pairwise comparisons within a row of each innermost subtable using Bonferroni's correction

Table 3: Comparison between the mean IgE levels to several LTPs in the presence or the absence of Pru p 3 sensitization.

	Pru p 3^{neg} -	Pru p 3^{pos} (3,79±7,59 ISU)	
	IgE Mean±Standard Deviation		
Ara h 9	0,14±0,61	1,55±3,21	*
Art v 3	0,33±2,08	1,4±3,38	*
Cor a 8	0,06±0,32	1,11±2,98	*
Jug r 3	0,27±1,11	2,28±4,1	
Ole e 7	1,87±9,08	0,99±5,98	
Pla a 3	0,42±1,74	1,72±3,53	*
Tria14	0,15±1,47	0,45±2,47	
		* <0.01	

Figure 1: (A) Prevalence of IgE recognition of several LTPs in pediatric patients at different ages. (B) Major pollen allergens Ole e 1, Pla a 1, Pla a 2, and Art v 1 trend of IgE prevalence in the same population.

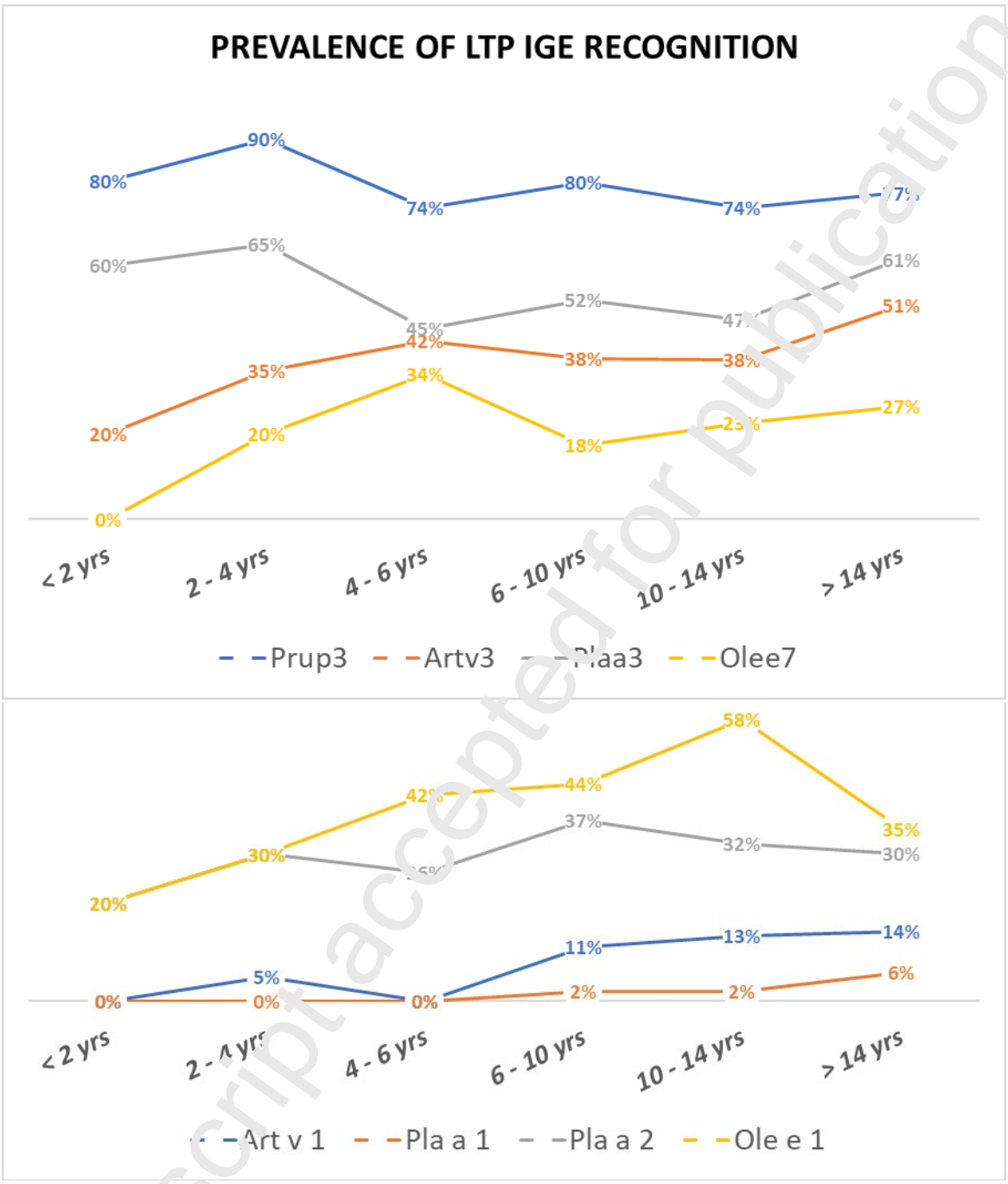

Figure 2: Inhibition of IgE reactivity to Pru p 3, Art v 3 and Pla a 3 by commercial mugwort and plane tree extracts

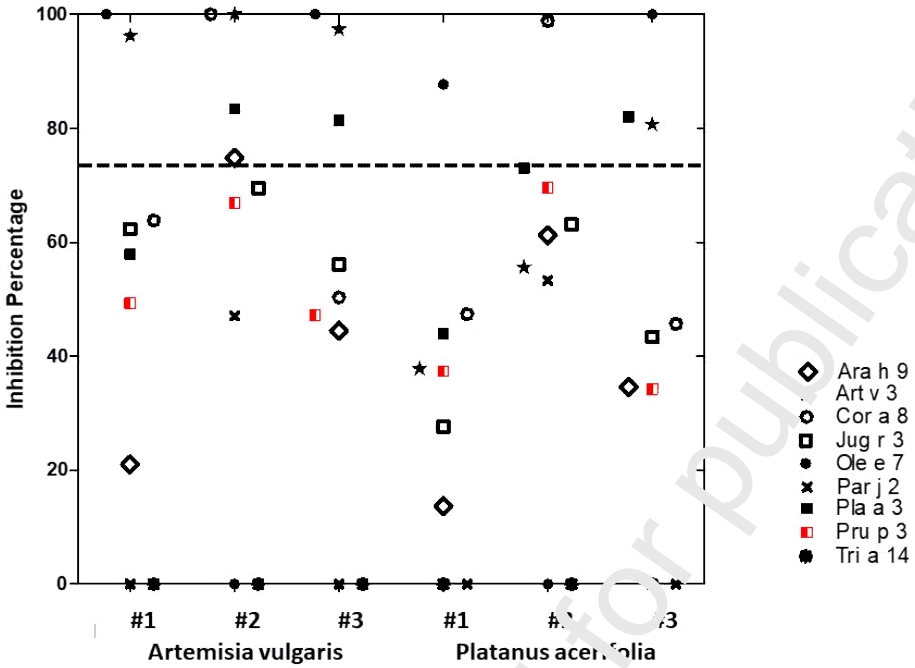

Table 4: Serological data of 285 LTP sensitized subjects. The table shows the values (and percentages) of those patients who had specific IgE levels towards the nsLTPs evaluated by ImmunoCAP with values lower than those found for Prup3.

IgE level			n. positive patients (%)	χ^2	*sig*
Pru p 3 (6,06±11,86)	>	**Art v 3** (2,25±6,62)	253 (89,4%)	357,202	P < 0,000
	>	**Jug r 3** (4,36±12,80)	244 (86,22%)	301,098	
	>	**Tri a 14** (1,56±4,38)	256 (90,46%)	383,046	
	>	**Ara h 9** (4,08±10,36)	242 (85,51%)	308,388	
	>	**Cor a 8** (2,00±5,63)	251 (88,69%)	357,255	