Introduction

Non-specific lipid transfer proteins (LTP) are panallergens present in plant-foods, being the most relevant allergens of Rosacea family fruits in the Mediterranean area (1,2).

These ubiquitous proteins are highly conserved and widely distributed in the plant kingdom, sharing a moderate-to-high homologous molecular structure, which put the patients allergic to LTP at risk of developing allergic reactions after the ingestion of an array of botanically unrelated foods, including fruits, tree nuts, seeds, vegetables and cereals (1,3–7).

Despite the fact that milk and egg proteins are the main causes of food allergy in childhood, fruits and vegetables have been recognized as emergent allergens in pediatrics (8).

Since LTP may cause severe systemic reactions it is essential to better understand this allergy in childhood.

Even though multiple studies on LTP have been published in the last years, so far, not much is known about LTP allergy in children, especially regarding daily practice, since only a few case series focused on pediatric ages have been published (9–13). A recent study has shown that fresh fruits are the 5th cause of anaphylaxis in children, with tree nuts being the 2nd, both potentially caused by LTP in this region (14).

Our aim was to characterize a series of children with allergy to LTP, in order to better understand its characteristics.

Methods

We performed a retrospective analysis of medical records from patients under 18 years-old with confirmed LTP allergy (2013-2019).

Diagnosis was established based on a convincing history of immediate allergic reactions to plant-foods (i.e. repeated symptoms to LTP containing foods on several occasions) supported by positive skin prick tests (SPT) (defined as the mean diameter of the wheal ≥ 3 mm than negative control (15)) to LTP extract (Roxall, Bilbao, Spain) and positive specific IgE (sIgE) to LTP allergens (Pru p 3, Cor a 8 and/or Jug r 3) determined by ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden**;** cut-off: ≥0.35 kUA/L) and/or ImmunoCAP^{TM} ISAC microarray (ThermoFisher, Uppsala, Sweden; cut-off: ≥0.3 ISAC Standardised Unit, ISU, as per manufacturer's recommendation). sIgE to Pru p 3 were determined in all children. Since sIgE to Cor a 8 and Jug r 3 were not available in our center from the beginning of the study, they were not determined in all. ImmunoCAP^{TM} ISAC microarray 112 (ThermoFisher, Uppsala, Sweden) was performed in 9 children, providing information about the referred LTP allergens.

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SPT with airborne allergens were performed in all patients. The SPT was conducted using a standard allergen extract panel and comprised histamine and saline respectively as positive and negative controls. For children under 6 years-old, the panel tested included: *Dermatophagoides pteronyssinus*; *Dermatophagoides farinae*; *Lepidoglyphus destructor*; cat; dog; olive tree; grass pollen mix; parietaria and *Alternaria alternata*. For children with ≥ 6 years-old, the panel tested included: *Dermatophagoides pteronyssinus*; *Dermatophagoides farinae*; *Lepidoglyphus destructor*; *Tyrophagus putrescentiae*; cat; dog; birch; plane tree; olive tree; grass pollen mix; *Cynodon*; mugwort; parietaria; plantago; ambrosia; *Cladosporium*; *Alternaria alternata*; *Aspergillus fumigatus*.

The decision of performing SPT to other food extracts available at our center (24 fruits, 8 tree nuts, peanut, soy and 4 seeds extracts) was made considering the child's age and collaboration, regardless of food tolerance.

The co-sensitization to other relevant proteins in fruit and tree nuts allergy, namely profilins, PR-10 and storage proteins, was evaluated, according to the severity of the reaction, the food trigger and the laboratory availability. Co-sensitization to profilins was evaluated in all patients, by SPT to profilin (n=19) and/or ImmunoCAP^{TM} ISAC microarray 112 (n=9). Among the PR-10 family, *Bet v 1* was the protein tested in all patients, by ImmunoCAP (n=17) or ImmunoCAP^{TM} ISAC microarray 112 (n=9). Storage proteins were tested in 14 patients, according to the suspected food. In case of doubt (p.e. mixed unidentified tree nuts), all the three families were tested (Ara h 1, Ara h 2 and Ara h 3).

Clinical manifestations were classified as local (oral allergy syndrome [OAS], contact urticaria) or systemic (urticaria, anaphylaxis). Severe reaction was defined as the occurrence of anaphylaxis. The presence of cofactors (exercise and NSAIDs) and food tolerance were investigated. Food tolerance was defined as no reactivity to food in patients' usual diet.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 24 software (SPSS Inc, Chicago, Illinois). Descriptive statistics was performed. Categorical variables are presented as frequencies and percentages, and continuous variables as mean ± standard deviation (SD) or median with minimum and maximum values in brackets. Spearman's correlation, T-student test and Mann-Whitney tests were used. A 2-tailed p value < 0.05 was considered statistically significant.

Results

Twenty-six children were selected, from a universe of 281 followed in our department for suspected food allergy; 50% were female, median age of first symptoms 10 (1-17) years-old. Age at first symptoms was 0 to 2 years-old (n=3), 3 to 5 years-old (n=5), 6 to 11 years-old (n=6) and 12 to 17 years-old (n=12). The median time for diagnosis after the initial episode was of 4 (0-8) years. Sixty-one percent were atopic, 58% had pollen sensitization, 54% rhinitis, 27% asthma, 19% atopic dermatitis, 1 patient had another food allergy (cow's milk) and 1 patient had eosinophilic esophagitis. The characteristics of the children included are detailed in Table I. Different food triggers were identified, with 69% of patients reporting reactions to more than one food. Fruits were involved in 69% (n=18), tree nuts in 50% (n=13), peanut in 8% (n=2), sesame in 4% (n=1). Peach was the most frequent trigger (62%, n=16). Symptoms both with fruits and tree nuts occurred in 27% (n=7).

Fifth-eight percent (n=15) had exclusively systemic symptoms, 15% (n=4) exclusively local symptoms, and 27% (n=7) had both. Symptoms included urticaria in 58% (n=15), anaphylaxis in 46% (n=12) and OAS in 42% (n=11) of the patients.

Demographic and clinical differences between patients reporting reactions exclusively to either fruits or tree nuts/peanut/seed were evaluated. Patients exclusively allergic to fruits were 70% female, median age of first symptoms 4 (1-6) years and anaphylaxis occurred in 36%. Patients exclusively allergic to tree nuts/peanut/seed were 60% male, median age of first symptoms 12 (2-17) years and anaphylaxis occurred in 53%. Differences were also evaluated between patients allergic to either one food or multiple foods. Patients allergic to only one food were 54% male, median age of first symptoms 9 years (1;17) and anaphylaxis occurred in 39%. Patients allergic to multiple foods were 53% female, median age of first symptoms 11 (1-17) years and anaphylaxis occurred in 54%. There were no statistical differences between the different groups.

Cofactors were involved in 27% (n=7) of the patients: exercise in all and NSAIDs in 1 patient. In 2 patients, cofactors were essential to the occurrence of reactions (anaphylaxis); in 5 patients, the cofactors elicited more severe reactions [anaphylaxis in patients with urticaria and/or OAS (n=1), urticaria in patients with OAS (n=4)].

Regarding SPT, patients had a mean wheal of 7.1 mm (SD 3.81) to peach LTP, with a different spectrum of positivity to fruits and tree nuts, as detailed in Table II. Thirty-eight percent (n=10) tolerated fruits/ tree nuts for which SPT were positive.

Results were concordant for both sIgE determination methods (ImmunoCAP™ and ImmunoCAP™ ISAC microarray 112). sIgE to Pru p 3 was positive in all patients (26/26), sIgE to Cor a 8 in 35% (8/23) and sIgE to Jug r 3 in 73% (8/11), regardless of the trigger (fruits/tree nuts) (Table III).

Since sIgE to Cor a 8 and sIgE to Jug r 3 are proteins from tree nuts sources (hazelnut and walnut, respectively), the presence of sIgE to these allergens were evaluated in children with tree nuts as food triggers: sIgE to Cor a 8 was positive in 38% (5/13) and sIgE to Jug r 3 in 67% (4/6).

Co-sensitization to other panallergens was documented in 15% (n=4): 2 patients to profilins (positive profilin in SPT), 2 to PR-10 (Bet v 1). A broad spectrum of clinical manifestations occurred in these patients, ranging from OAS to anaphylaxis. Sensitization to storage proteins was not found.

A broad spectrum of fruit tolerance was found, with 92% of the patients showing tolerance to at least one fruit from Rosacea family without peel. Apple tolerance (a staple food in Portuguese diet) was present in 50%, unknown in 27% and absent in 23%. SPT to apple were positive in 4 (21%) patients (none of them tolerated the apple).

During follow-up, 12% (n=3) patients reported reactions to new LTP containing foods, with different timings since the occurrence of the first reaction (<1 year, 2 years, 8 years).

There was no association between the occurrence of severe reactions and pollen sensitization, comorbidities [rhinitis, asthma, atopic dermatitis], type of trigger (fruits/tree nuts), number of food triggers, mean wheal of SPT to peach LTP, the number of positive SPT to fruits or tree nuts or ImmunoCAP™ determination of sIgE to Pru p 3 or sIgE to Cor a 8 (Table IV, V).

Discussion

We present a pediatric series with documented LTP allergy, focusing on it in clinical practice. Considering all children referred to our department with suspected food allergy, 9% of them were diagnosed with LTP allergy, a low but not irrelevant percentage.

In our study, LTP allergy seems to be similar in terms of frequency between females and males, with more than a half reporting the first reaction before age 12. Twelve percent had their first reaction before age 3, which reinforces the importance of considering this diagnosis in infants and toddlers, as suggested by other authors (16).

Since LTP are widespread in the plant kingdom, it is not surprising that most children had more than one food trigger. Food triggers in LTP allergy are ubiquitous but probably less considered by the general population, since they are not "classical" allergens in children. This underestimation can make their recognition harder when reactions occur, explaining the delay in diagnosis verified in our study.

Clinical manifestations and severity of LTP hypersensitivity varied in our children, as described for adults (1–4,9,17). It is important to highlight that, although urticaria was the most frequent symptom, severe reactions were also common, with anaphylaxis occurring in almost half of the patients. Cofactors were present in more than one quarter of the children and the majority had more severe reactions in their presence; in some children, cofactors were essential for reactions to occur and were associated with anaphylaxis. This evidence supports the importance of cofactors as severe reaction inductors in LTP allergic children, as in adults (18–20).

The presence of risk factors for severe reactions was investigated. No associations were found with pollen sensitization, comorbidities, types/number of food triggers and co-sensitization, mean wheal of SPT to LTP extract, level of ImmunoCAP™ sIgE either to Pru p 3 or Cor a 8, the latest in agreement to November et al (9). Other authors reported different results in adults, establishing an association between higher levels of IgE to Pru p 3 and systemic reactions with fruits from Rosacea family (21).

Co-sensitization to profilins and PR-10 was found in a low number of patients, with a diverse spectrum of clinical presentations, raising doubts about their clinical relevance. On the other hand, co-sensitization to storage proteins was not found, despite the fact that tree nuts were

involved as exclusive triggers in one third of the patients, supporting the clinical relevance of LTP sensitization.

No association was found between tolerance to fruits from Rosacea family, mean wheal SPT with peach LTP extract, number of positive SPT to fruits and level of sIgE to Pru p 3. It is important to highlight that SPT to food extracts were not reliable methods to confirm clinical reactivity, since 38% of children had positive SPT to foods subsequently tolerated.

Food avoidance is the mainstay of treatment for LTP allergy and should be guided by clinical reactivity and not sensitization. As proposed by Asero et al (3), all children were prompted to maintain the ingestion of tolerated foods without peel and avoiding the presence of cofactors. The purpose of this approach was the maintenance of dietary natural tolerance and the ingestion of important nutritious foods, as fruit and vegetables. In our sample, most children tolerated fruits from Rosacea family without peel, as expected since LTP are present mainly in the fruit peel (22,23).

Awareness of possible accidental-allergic reactions and the ability to correctly identify and adequately treat them is of extreme importance in these patients; children and their caregivers should be exhaustively educated about potential elicitors, timely reaction recognition, adequate treatment and the role of cofactors in LTP allergy. Adrenaline auto-injectors were prescribed in all children with systemic reactions to LTP containing foods with or without cofactors and postponed in those who had only local reactions.

Twelve percent of children reported reactions to new LTP containing foods, with different timing considering the occurrence of first reaction. This should alert clinicians that LTP allergy may progress in number of eliciting foods.

We would like to highlight some aspects considered in our work that may not be consensual. We established LTP allergy diagnosis based on a convincing clinical history of immediate allergic reactions to plant-foods, defined as repeated symptoms to LTP containing foods on several occasions, supported by positive SPT to peach LTP extract and positive sIgE to LTP allergens (Pru p 3, Cor a 8 and/or Jug r 3).

We acknowledge that, based on the current evidence, doubts exist about the role of component resolved diagnosis when it comes to distinguish allergy from sensitization and

possible food tolerances (16). However, we considered that the presence of a convincing clinical history, with reproducible reaction not or poorly explained by other plant food allergens, were the key in distinguishing sensitization from allergy.

Based on these considerations, we assumed that the combination of clinical history, results from *in vivo* and *in vitro* tests, in the absence of other plant food-allergens that could explain clinical manifestations, were enough to establish the diagnosis of LTP allergy and food challenges were protracted.

Nevertheless, as mentioned before, in a low number of patients, co-sensitization to profilins and PR-10 was found, raising doubts about the role of each allergen and their clinical relevance. We decided to assume a diagnosis of LTP allergy also in these patients, since all of them had systemic reactions, more usually associated in our country to LTP. However, we recognize that these interpretations can be a limitation in our study.

The retrospective nature of our study and limited number of patients are also limitations, reinforcing the importance of more studies in this area.

Conclusion

In conclusion, allergy to LTP can occur since childhood, even before school-age. Clinical manifestations of LTP allergy may vary, but the occurrence of anaphylaxis is common, forcing it to be recognized as a potentially severe allergy in pediatrics. Cofactors may be essential to reaction occurrence and relate to more severe occurrences. No other risk factors to severe reactions were documented in our study. SPT to food extracts were not a reliable method to confirm clinical reactivity. Food avoidance is the mainstay of treatment however the ingestion of tolerated foods without peel should be maintained. More than 10% of the patients had subsequent reactions with new LTP-containing foods, reinforcing that follow-up is essential.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

None

Informed Consent and Ethics committee approval

This project was conducted with the approval of Ethics Committee of the Research in our center. Informed consent was obtained from the legal responsible of each child.

Author contribution

JBL and ARF designed the study. CV, ARP and MJS collected data. CS performed the statistical analysis. JBL wrote the manuscript which was reviewed by ARF.

References

1. Van Winkle RC, Chang C. The biochemical basis and clinical evidence of food allergy due to lipid transfer proteins: A comprehensive review. Clin Rev Allergy Immunol. 2014;46(3):211–24.
2. Asero R. In patients with LTP syndrome food-specific IgE show a predictable hierarchical order. Eur Ann Allergy Clin Immunol. 2014;46(4):142–6.
3. Asero R, Piantanida M, Pravettoni V. Allergy to LTP: To eat or not to eat sensitizing foods? A follow-up study. Eur Ann Allergy Clin Immunol. 2018;50(4):156–62.
4. Asero R, Mistrello G, Roncarolo D, Amato S. Relationship between peach lipid transfer protein specific IgE levels and hypersensitivity to non-Rosaceae vegetable foods in patients allergic to lipid transfer protein. Ann Allergy, Asthma Immunol. 2004;92(2):268–72.
5. Richard C, Leduc V, Battais F. Plant lipid transfer proteins (LTPs): Biochemical aspect in panallergen - Structural and functional features, and allergenicity. Eur Ann Allergy Clin Immunol. 2007;39(3):76–84.
6. Calamelli E, Liotti L, Beghetti I, Piccinno V, Serra L, Bottau P. Component-resolved diagnosis in food allergies. Medicina (Kaunas). 2019;55(8):498.
7. Azofra J, Berroa F, Gastaminza G, Saiz N, Garrido PM, Vela C, et al. Lipid transfer protein syndrome in a non-mediterranean area. Int Arch Allergy Immunol. 2016;169(3):181–8.
8. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: A meta-analysis. J Allergy Clin Immunol. 2007;120(3):638–46.
9. Novembre E, Mori F, Contestabile S, Rossi ME, Pucci N. Correlation of anti-pru p 3 IgE levels with severity of peach allergy reactions in children. Ann Allergy, Asthma Immunol. 2012;108(4):271–4.
10. Pascal M, Muñoz-Cano R, Reina J, Palacín A, Vilella R, Picado C, et al. Lipid transfer protein syndrome: Clinical pattern, cofactor effect and profile of molecular sensitization to plant-foods and pollens. Clin Exp Allergy. 2012;42(10):1529–39.
11. Fernández-Rivas M, González-Mancebo E, Rodríguez-Pérez R, Benito C, Sánchez-Monge R, Salcedo G, et al. Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. J Allergy Clin Immunol. 2003;112(4):789–95.
12. Boyano-Martínez T, Pedrosa M, Belver T, Quirce S, García-Ara C. Peach allergy in spanish children: Tolerance to the pulp and molecular sensitization profile. Pediatr Allergy Immunol. 2013;24(2):168–72.
13. Ciprandi G, De Amici M, Di Martino ML, Barocci F, Comite P. The impact of age on Pru p 3 IgE production in Italy. Asia Pac Allergy. 2017;7(1):42–7.
14. Gaspar A, Santos N, Faria E, Câmara R, Rodrigues-Alves R, Carrapatoso I, Gomes E, Pereira AM, Carneiro-Leão L, Morais-Almeida M, Delgado L, Pedro E, Branco-Ferreira M. Anaphylaxis: a decade of a nationwide allergy society registry. J Investig Allergol Clin Immunol. 2020 Jul 30;0.
15. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, et al. The skin prick test - European standards. Clin Transl Allergy. 2013;3(1):1–10.
16. Pascal M, Vazquez-Ortiz M, Folque MM, Jimenez-Feijoo R, Lozano J, Dominguez O, et al. Asymptomatic LTP sensitisation is common in plant-food allergic children from the Northeast of Spain. Allergol Immunopathol (Madr). 2016;44(4):351–8.

17. Asero R, Piantanida M, Pinter E, Pravettoni V. The clinical relevance of lipid transfer protein. Clin Exp Allergy. 2018;48(1):6–12.
18. Versluis A, Van Os-Medendorp H, Kruizinga AG, Marty Blom W, Houben GF, Knulst AC. Cofactors in allergic reactions to food: Physical exercise and alcohol are the most important. Immun Inflamm Dis. 2016;4(4):392–400.
19. Cardona V, Luengo O, Garriga T, Izquierdo A. Co-factor-enhanced food allergy. Allergy. 2012; 67(10):1316-8.
20. Niggemann B, Beyer K. Factors augmenting allergic reactions. Allergy. 2014;69(12):1582–7.
21. Rossi RE, Monasterolo G, Canonica GW, Passalacqua G. Systemic reactions to peach are associated with high levels of specific IgE to Pru p 3. Allergy. 2009;64(12):1795–6.
22. Hauser M, Roulias A, Ferreira F, Egger M. Panallergens and their impact on the allergic patient. Allergy, Asthma Clin Immunol. 2010;6(1):1–14.
23. Egger M, Hauser M, Mari A, Ferreira F, Gadermaier G. The role of lipid transfer proteins in allergic diseases. Curr Allergy Asthma Rep. 2010;10(5):326–35.