**Clinical severity of LTP syndrome is associated with an expanded IgE repertoire, FDEIA, FDHIH, and LTP mono reactivity.**

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**Abstract**

**Background**: LTP allergy is often a challenge for clinicians. We evaluated a multiplex diagnostic approach with diverse cofactors to stratify LTP syndrome risk.

**Methods**: Of the 1,831 participants screened with 'Allergy Explorer-ALEX-2', 426 had reactions to at least one LTP. Data was gathered and recorded via an electronic database.

**Results**: Reactivity to peach Pru p 3 was found in 77% of individuals with LTP allergy. Higher levels of specific IgE and concurrent sensitization to more than 5 molecules (50% of all LTP-sensitised participants, 62% of symptomatic cases) were significantly associated with an increased risk of severe reactions (p=0.001). Several cofactors, either alone or in combination, also influenced patients' clinical outcomes. Some cofactors increased the risk of severe reactions, such as mono reactivity to LTP in 44.6% of cases (p=0.001), FDEIA in 10.8% of patients (p=0.001), and FDNIH in 11.5% (p=0.005). On the other hand, reactivity to PR10 (24.2%; p=0.001), profilin hypersensitivity (10.3%; p=0.001), and/or atopic dermatitis (16.7%; p=0.001) had a mitigating effect on symptom severity.

**Conclusions**: Clinical severity of LTP syndrome is associated with an expanded IgE repertoire in terms of the number of LTP components recognized and increased IgE levels in individual molecules. Ara h 9, Cor a 8, and Mal d 3 showed the strongest association with clinical severity. In addition, several cofactors may either exacerbate (FDEIA, FDHIH, and LTP monoreactivity) or

ameliorate (atopic dermatitis and co-sensitization to profilin and/or PR10) individual patient outcomes. These factors may be utilized for the daily clinical management of LTP syndrome.

**Keywords.**

Lipid transfer protein, Profilin, PR10, Macroarray, IgE, allergen, Atopic Dermatitis.

**Impact Statement**

- **LTP syndrome severity:** Increased IgE repertoire and specific IgE for Ara h 9, Cor a 8, and Mal d 3.
- **Cofactors:** Exacerbate (FDEIA, FDNIH, LTP monoreactivity) or ameliorate (atopic dermatitis, profilin/PR10).

**Abbreviation**

ALEX:

OAS: oral allergic syndrome

BA: bronchial asthma

FDEIA: Food-Dependent Exercise-Induced Anaphylaxis

FDNIH: Food-dependent NSAID-induced hypersensitivity

NSAIDs: Non-steroidal anti-inflammatory drugs

## 1 | Introduction

### 1.1 | Background/rationale

The nonspecific lipid transfer proteins (LTPs), first described nearly 50 years ago (1), play a significant role as a food and environmental allergen, especially in regions with high consumption of plant foods. Initially, this problem was thought to be primarily limited to the Mediterranean region (2), but numerous studies carried out over the past two decades, including a recent one (3), have shown that LTP allergies are widespread (4) including in Northern Europe (5)(6), China (7), and North America (8). In addition, interesting differences in epitope recognition have been found in different populations, suggesting that individual, environmental, or dietary factors may influence the interaction with these molecules (9).

LTPs are small, heat-stable proteins, found in various plant foods such as fruits, vegetables, nuts, and seeds, that, although homologous, have a variable degree of structural identity influenced by plant relationships (10). Consequently, IgE antibody recognition to one LTP molecule does not necessarily imply reactivity to all other members of this protein family (11). There are three primary epitopes on the surface of LTPs, and Pru p 3, the peach LTP, is considered the molecule that identifies the most patients (12).

Evidence of the widespread occurrence of LTP IgE reactivity beyond the Mediterranean region has also led to interesting studies on the variability of clinical outcomes in LTP-hypersensitive patients, ranging from the complete absence of symptoms (13) to extremely severe reactions (14), and on the identification of the primary sensitizers. These may include mugwort pollen Art v 3, as observed in a subset of Chinese patients, Can s 3 from marijuana, even through passive smoke exposure, as hypothesized in Northern Europe (6), or Pru p 3 from peach via the oral sensitisation route in the Mediterranean basin (15).

The influence of different cofactors on the clinical outcomes of LTP allergic patients is the subject of an ongoing debate, and the exact mechanisms, either in a protective sense, where they may reduce or eliminate the risk of adverse reactions, or in an aggravating sense, leading to severe reactions including anaphylaxis, are not yet fully understood. To date, factors that have been shown to increase the severity of allergic reactions include physical exercise (FDEIA) or the use of NSAIDs (FDNIH) in conjunction with the consumption of LTP-containing foods. FDEIA is a well-documented phenomenon in which physical exercise exacerbates the severity of allergic reactions to LTP-containing foods, particularly in the Mediterranean region (16). NSAIDs are another cofactor that may exacerbate the severity of allergic reactions in individuals with LTP allergy (17).

It is generally believed that this effect is related to the increased absorption of allergenic molecules that are digestion-resistant and heat-stable.

In a previous paper, by employing a completely distinct method for the assessment of specific IgE antibodies, we also identified another significant aggravating factor characterized by the presence of hypersensitivity to more than 5 LTPs among those tested (18), although this finding was not confirmed in a study performed in the United Kingdom (19). On the other hand, the presence of atopic dermatitis in LTP-sensitized patients is associated with a significantly lower frequency of severe reactive episodes than in the general allergic population (20). In addition, both singleplex (21) and multiplex approaches have shown that the detection of hypersensitivity to panallergens such as Profilins and/or PR10s is associated with a more favourable outcome than in individuals allergic to LTPs alone (18,22).

### 1.2 | Objectives

This study aimed to investigate an unselected population collected from a single Centre in central Italy characterized by IgE reactivity to at least one out of 15 LTPs for:

- Patient's individual LTP molecular recognition profile
- Clinical outcomes, ranging from tolerance to anaphylaxis
- External factors that may have influenced the observed reactivity, assessed individually or in combination.

## 2 | Methods

### 2.1 | Study design

We conducted a cross-sectional clinical survey of lipid transfer protein allergy to evaluate the role of various cofactors that might have a mitigating or exacerbating effect on clinical outcomes. These cofactors included exercise after food intake, NSAID administration before LTP source intake, sensitization to panallergens other than LTP, and the presence of comorbidities such as atopic dermatitis, rhino-conjunctivitis, and bronchial asthma. We examined the individual role of each factor and their potential interactions. In addition, we assessed the factors that might be relevant in "tolerant" participants and examined the differences between responders with mild symptoms and those with severe reactions.

## 2.2 | Setting

The study was conducted on 1,831 unselected participants born in central or southern Italy who presented to the allergy outpatient clinic of IDI-IRCCS in Rome with a history of adverse reactions to food, allergic rhinitis, bronchial asthma, and/or atopic eczema. The IDI-IRCCS is a national reference centre for skin diseases. The data collection period was from January 2021 to December 2022, and demographic information, as well as clinical data on food-related reactions, respiratory symptoms, and dermatological symptoms, were collected in a customized electronic database.

## 2.3 | Participants

The main criterion for enrollment into the study was the detection of sensitization to at least one of the LTPs present on the IgE macroarray. The diagnosis of tolerance was based on a history of regular intake of LTP-containing foods without any problems.

## 2.4 | Variables

The patient-reported allergic symptoms induced by LTPs encompassed a range of severity from mild manifestations, including isolated gastrointestinal reactions or oral allergy syndrome, to moderate to severe reactions, such as urticaria/angioedema or anaphylaxis. The occurrence of severe food reactions (anaphylaxis) was always documented by emergency department reports that recorded the use of epinephrine, H1 antihistamines, and steroids to treat symptoms. The group of participants who did not report adverse reactions despite following LTP ingestion was categorized as "tolerant".

Further categorizations were based on the presence of upper or lower airways symptoms, atopic dermatitis, monoreactivity to LTP, co-sensitization to other allergens (i.e. PR -10, Profilin, Polcalcin, and seed storage protein [SSP]), food-dependent exercise-induced anaphylaxis (FDEIA) or food-dependent NSAID-induced hypersensitivity (FDNIH), and the number of LTP molecules detected in each patient.

Because of the observational nature of the study, no randomization procedure was performed at enrollment.

## 2.5 | Data sources/ measurement

Serum IgE reactivity was analysed using the Allergen ExplorerALEX® (Macroarray Diagnostics, Vienna, Austria), where different allergens and extracts are spotted onto a nitrocellulose membrane in a cartridge chip, including a broad spectrum of LTP molecules: nAct d 10 - 9kD from

kiwi (*Actinidia deliciosa*); rApi g 2 - 9kD from celery (*Apium graveolens*); rApi g 6 - 7kD from celery (*Apium graveolens*); rAra h 9 - 9kD from peanut (*Arachis hypogaea*); rArt v 3 - 9kD from mugwort (*Artemisia vulgaris*); rCan s 3 - 9kD from hemp (*Cannabis sativa*); rCor a 8 - 9kD from hazelnut (*Corylus avellana*); rJug r 3 - 9kD from walnut (*Juglans regia*); rMal d 3 - 9kD from apple (*Malus domestica*); rPla a 3 - 9kD from London plane tree (*Platanus acerifolia*); rPru p 3 - 9kD from peach (*Prunus persica*); nSola l 6 - 7kD from tomato (*Solanum lycopersicum*); rTri a 14 - 9kD from wheat (*Triticum aestivum*); nVit v 1 - 9kD from grape (*Vitis vinifera*); rZea m 14 - 9kD from corn (*Zea mays*). Fra a 1+3 and Ole e $7^{RUO}$ were excluded from the evaluation. The former is a mixture of Fra a 1 and Fra a 3, making it unable to accurately distinguish Fra a 3 (LTP) reactive patients from Fra a 1 (PR10) allergic patients. The latter is still an experimental molecule in this array. The chip was incubated with 0.5 mL of a 1:5 dilution of the patient's serum, containing a CCD inhibitor under agitation. After two hours of incubation, the chip was washed three times, and a pre-titred dilution of anti-human IgE labelled with alkaline phosphatase was added and incubated for 30 min. After another cycle of extensive washing, the enzyme-substrate was added, and after eight minutes, the reaction was stopped by the addition of 100 µL of ALEX Stop Solution. The membranes were dried, and a charge-coupled device camera measured the intensity of the colour reaction for each allergen spot. The dedicated software digitalized the images and prepared a report listing the allergens and components and their score in $kU_A/L$. Finally, systematic variations in signal levels between lots were normalized by heterologous calibration against an IgE reference curve. A curve fit was calculated, and the resulting equation was applied to transform arbitrary intensity units into quantitative units. Lot-specific calibration parameters are encoded in the barcode. The measuring range of ALEX-specific IgE is 0.3-50kUA/L.

**2.6 | Bias**

The diagnosis of LTP allergy was not confirmed through blinded or open oral food challenges.

**2.7 | Quantitative variables**

Quantitative measurements of specific IgE towards the single LTPs under investigation were compared among the various clinical subsets of patients, between males and females, and across different age groups.

### 2.9 | Statistical methods

All data were analyzed using the SPSS/PC + statistical package for statistical evaluation (IBM SPSS, version 29, Chicago, IL). The TD-Synergy Laboratory Information System was used to search and collect demographic (age and gender), clinical, and laboratory data for patients who attended the outpatient Allergy clinic and underwent specific IgE testing. In univariate analysis, the non-parametric Mann-Whitney U-test (two groups) was first used to compare continuous IgE values in males, females, and subjects with or without a given clinical involvement. Subsequently, each variable of interest was dichotomized as negative or positive to examine the proportion of subjects with symptoms in the two resulting groups.

Pearson's $\chi^2$ test or Fisher's exact test (used for two-by-two contingency tables with less than 50 cases) were used to assess if paired observations on two variables expressed in a contingency table, were independent of each other.

Receiver operating characteristic (ROC) analysis was performed to test results concerning Pru p 3 reactivity or severity of symptoms and was presented as the area under the curve with a 95% confidence interval (95% CI).

We performed multiple logistic regression for the clinical variables with dichotomous scores (present, absent) to see whether the association between clinical symptoms and different allergens reactivity was present after simultaneously adjusting for the other variables of interest.

The degree of relationship between the quantitative variables studied was analyzed using Spearman's non-parametric correlation (ρ) test, given the skewed distribution of the observed values. A very high positive correlation was defined as a Spearman's correlation coefficient of 0.90 to 1.00, 0.70 to 0.90 for high, 0.50 to 0.70 for moderate, 0.30 to 0.50 for low, and 0.00 to 0.30 for negligible. A value of $p<0.05$ was considered statistically significant.

To provide a visual representation of the distribution of the different molecules in panallergen families, we have produced Venn diagrams using the VennMaster 0.38 package (23).

### 3.10| Ethical issues

The study was approved by the Ethical Committee of IDI-IRCCS (IDI-IRCCS CE | 495-17). Data collection was conducted anonymously, utilizing only information obtained from routine specialist surveys. Recruited patients provided informed consent for the utilization of their clinical data in an anonymous format.

## 4 | Results

### 4.1 | Participants

A total of 426 individuals reactive to nsLTPs (239 females and 187 males; mean age 34±16 years, range 2-74 years) were included in the study. Among the selected patients, 144 (34%) had a history of local reactions (oral allergy syndrome), while 202 reported moderate (65.8%) to severe (34.2%) reactions. Eighty participants (half of whom were females) reported tolerance to LTP-containing foods.

### 4.2 | Sequence identity and IgE recognition

Reactivity to Pru p 3 was detected in most patients (77%), followed by Mal d 3 and Zea m 14, which tested positive in 60% of cases (Figure 1 | A). Ara h 9 and Pla a 3 reactivity was also detected in over 50% of cases, while the two LTP2s included in the chip (Api g 6 and Sola l 6) scored positive in only a small percentage of patients. Reactivity to Tri a 14 from wheat was found in about 6% of the study participants.

We then cross-referenced the sequences of the 15 studied LTPs with all the LTPs currently listed on the World Health Organization and International Union of Immunological Societies (WHO/IUIS) website (http://www.allergen.org/index.php). It is well-established that cross-reactivity between homologous molecules is primarily determined by the percentage of amino acid identity observed by structural comparisons. IgE cross-reactivity is highly unlikely when the structural identity is below 50%, while it is highly probable when the identity exceeds 70%. (24). Accordingly, Act d 10 from kiwi fruit exhibits significant structural similarity only with Act c 10 but not with any other LTP (Figure 1.repository). This suggests that the simultaneous recognition of Act d 10 and, for example, Pru p 3 in patients who are reactive to both molecules might be the result of distinct molecular detections. As expected, Pru p 3 demonstrated high sequence identity primarily with taxonomically related molecules, such as Pyr c 3 from pear, Pru d 3 from European plum, Pru av 3 from sweet cherry, Pru ar 3 from apricot, Mor n 3 from mulberry, and Mal d 3 from apple. Interestingly, the two LTP2s on the chip (Api g 6 and Sola l 6) also displayed significant sequence identity with the other available LTP2 for comparison, such as Ara h 16 from peanut (Figure 1.repository).

When comparing the percentage of molecules detected in each patient subset (e.g., the 326 Pru p 3 reactors, Figure 2.repository) with the percentage of structural identity between that molecule and all other tested LTPs, we observed significant heterogeneity in recognition. In some cases, the two percentages were in complete agreement, but in several instances, discrepancies were noted.

For instance, while Mal d 3 and Pru p 3 exhibited a similar profile of molecular recognition as predicted, the IgE detection of Api g 6, Sola l 6, and Tri a 14 was much higher than expected based on structural identity. This discrepancy may be attributed to the influence of the large number of Pru p 3 reactors present in these patient subsets. This is supported by the observation that in several cases (e.g., Can s 3, Jug r 3, Vit v 1, Ara h 9, Act d 10), approximately 90% of the reactors were Pru p 3 positive.

In line with the structural relationships, the bivariate analysis of the reciprocal relationships of IgE mutual co-recognition confirmed that the strongest positive association was observed among molecules with the highest amino acid sequence identities. For example, strong associations were found between Mal d 3 and Ara h 9, Cor a 8, Jug r 3, Pla a 3, and Pru p 3. A moderate correlation was observed in approximately half of the cases (Figure 1B).

**4.3 | Descriptive data**

Females exhibited significantly lower IgE levels towards Jug r 3 from walnut and Vit v 1 from grapes compared to males (Table 1). Interestingly, patients with moderate to severe symptoms had significantly higher IgE levels specific for Ara h 9 ($p<0.001$), Cor a 8 ($p<0.01$), Jug r 3 ($p<0.001$), Mal d 3 ($p<0.0001$), Pru p 3 ($p<0.0001$), and Vit v 1 ($p<0.01$) than patients with mild symptoms, and accordingly, participants who tolerated LTP sources showed significantly lower IgE values towards these molecules than “non-tolerant” patients (Table 1). Likewise, individuals who tested positive for more than 5 molecules (50% of all LTP-sensitized participants, 62% of symptomatic cases) exhibited significantly higher IgE levels for the respective LTPs than patients with fewer than 5 molecules.

Seventy-six participants (17.8%) showed reactivity to a single molecule, with 46.1% reacting to Pru p 3, while 193 participants (45.3%) showed sensitization to up to 4 molecules. The cut-off of 4.5 molecules was determined based on an ROC analysis, which demonstrated the optimal combination of sensitivity and specificity for a severe reaction. Notably, considering the archetypal LTP, Pru p 3, as a reference, 55.8% of cases with reactivity to fewer than 5 molecules were positive for Pru p 3. In contrast, the pattern of molecular recognition in participants with more than 5 reactivities was more diverse (see Figure 2).

Eighty individuals (18.8%) who exhibited reactivity to LTPs did not report clinical symptoms when consuming LTP sources, while 202 participants reported severe reactions. Interestingly, although there were no significant differences in anaphylactic events between males and females, the number of females who reported generalized urticaria after consuming LTP-containing foods was

significantly higher (85 *vs* 48, respectively, p<0.01). One hundred ninety-three participants (58.9%) reported respiratory symptoms and 88 (20.7%) of them reported bronchial asthma.

#### 4.3.1 | Cofactors

Forty-six participants (10.8%) had a history of FDEIA, 53 patients (12.4%) had taken an anti-inflammatory drug in combination with the ingestion of LTP-containing food (FDNIH), and 98 individuals (23%) had atopic dermatitis. When considering FDNIH and AD, females were less prevalent than males in the AD group (19.1% *vs* 27.5%, P= 0.039), but more prevalent in the FDNIH group (16.2% *vs* 7.4%, P=0.006).

Notably, while subjects with FDEIA and/or FDNIH were mostly not concurrently reactive to PR10 or profilins, nearly all subjects with AD in our LTP-reactive cohort were sensitized to PR10 and/or SSP (Figure 3). Almost all participants associated with FDEIA or FDNIH exhibited reactivity to more than 5 LTP molecules.

#### 4.3.2 | Cosensitization

By examining the molecular sensitization profile of the study population, we found that 234 participants (54.9%) were solely sensitized to LTPs, while the remaining individuals were also sensitized to other panallergens: 118 (27.7%) to PR10, 56 (13.1%) to profilin, and 23 (5.4%) to polcalcin.

LTP mono-reactors outnumbered patients sensitized to other panallergens in women (148 *vs* 91), while the opposite trend was observed in men (86 *vs* 101). As a result, the frequency of LTP mono-reactivity in females was significantly higher (61.9% *vs* 45.8%, respectively, p<0.05).

One hundred sixty-two patients (38%) did not report any cofactor associated with adverse reactions to LTPs. However, no differences were observed in sex, age, symptoms severity, and molecular recognition profile of the individual LTPs studied among these patients.

### 4.4 | Clinical outcome data

#### 4.4.1 | Clinical symptoms and molecular profile

When examining individual LTPs, we observed an increased frequency of reactivity with each step indicating a worsening in clinical severity upon ingestion of LTP-containing food. As shown in Table 2, the linear-by-linear association was highly statistically significant, except for the $LTP_2$s (Api g 6 and Sola l 6) and Tri a 14. The prevalence of every single LTP reactivity was particularly pronounced in patients with urticaria/angioedema or anaphylaxis compared to those with oral

allergy syndrome (i.e. the reactivity to Mal d 3 was observed in 88% of patients experiencing anaphylaxis, whereas it was present in only 46% of those diagnosed with OAS). When considering molecules associated with a high risk of severe reactions, it is important to note that the absence of specific IgE reactivity is highly indicative of tolerance to that specific source. Specifically, Ara h 9, Cor a 8, and Mal d 3, which showed the strongest linear-by-linear association, were rarely positive among tolerant subjects (15%, 9%, and 20%, respectively, Table 2).

#### 4.4.2 | Clinical symptoms and cofactors

We then examined the association of several cofactors of interest with the clinical outcome in the 346 symptomatic patients, categorized as "mild" (oral allergic syndrome) and "severe" (urticaria/angioedema and anaphylaxis). As presented in Table 3, the recognition of more than 5 LTPs was significantly associated with an increased risk of severe reactions, as well as mono-reactivity to LTP or the presence of other cofactors such as exercise or NSAID intake. If the 5 positive molecules were Ara h 9, Cor a 8, Zea m 14, Mal d 3, and Pru p 3, the risk of severe and/or anaphylactic reactions was even higher ($\chi 2$= 19.196, $p<0.0001$; OR= 7.272 (2.4-21.9)). Conversely, sensitization to PR10, Profilin, or the presence of atopic dermatitis (both moderate and severe) showed an inverse association and may therefore be considered a protective factor in LTP allergy. Multiple logistic regression analysis, when incorporating all the cofactors together with age and sex into the model, confirmed the individual findings observed for the presence of more than 5 molecules simultaneously, FDEIA, FDNIH, and atopic dermatitis (see adjusted odds ratios, $OR_{adj}$, and respective significances in Table 3). It is worth noting that PR10 reactivity, which is strongly associated with atopic dermatitis, did not reach statistical significance in the final model.

## 5 | Discussion

### 5.1 | Key results

Although we studied a large number of LTP molecules, including both LTP1 and LTP2, Pru p 3 remains the molecule most commonly associated with LTP allergy, as it yielded a positive result in 78% of patients, making it the most suitable molecule of all those tested to detect LTP sensitization.

However, due to its broad reactivity, Pru p 3 seems to be less effective in detecting patients at higher risk for severe reactions to LTP than specific sensitizations such as Cor a 8, Mal d 3, or Ara h 9, at least considering the multiplex system used in this study.

At the same time, all these molecules as well as Pru p 3 showed significantly higher IgE levels in patients with severe adverse reactions (25)(26). This observation suggests that a comprehensive evaluation of the LTP reactivity profile is useful for distinguishing the different clinical phenotypes observed in LTP syndrome.

LTPs are homologous molecules with a structured identity that largely depends on the taxonomic relationships between the sources of origin. Therefore, botanically related molecules will have a higher identity and a higher likelihood of IgE cross-reactivity compared to taxonomically distant molecules. Interestingly, proportions greater than 60 per cent are rarely recorded, indicating that LTPs are homologous molecules with low structural identity, unlike other panallergens (i.e., profilins)(27). Upon comparing the prevalence of individual LTPs as determined by the macroarray with the structural identity information documented in the IUIS database, we encountered instances of complete concordance as well as complete discordance between the two measurements. The total disagreement may be due to the hook effect of the corecognition of other molecules, even if taxonomically unrelated, but widely detected in our LTP cohort. For instance, Pru p 3 or Mal d 3 exhibited positive reactivity in up to 90% of Can s 3 or Act d 10 reactors, despite exhibiting low sequence identity with these molecules. This leads us to hypothesize that some sensitizations to homologous molecules belonging to taxonomically distant biological sources may result from independent sensitizations (for example, Act d 10 of kiwi and Pru p 3 of peach). Therefore, the prevalence of molecular recognition within a specific group of homologous proteins is not always determined solely by sequence identity and may be influenced by the recognition of other molecules, even those not currently available for *in vitro* evaluation, which can result in unexpected recognition rates.

One of the main goals of this study was to comprehensively evaluate the mutual relationship of several known cofactors in the same population of patients with LTP sensitization. Among all the known cofactors, the 5-molecule cut-off proves to have the highest combination of sensitivity and specificity, both considering reactivity to Pru p 3 and the clinical outcome of patients. It proves to be a useful tool for the rapid classification of LTP reactors.

Once again, Pru p 3 hypersensitivity is widely expressed in patients with both more and fewer than 5 molecular recognitions, so it does not allow discrimination between the two groups. However, some molecules that are scarcely detected in subjects showing IgE reactivity to less than 5 LTPs are widely represented in >5 LTPs reactors and may represent a further useful tool for the immediate discrimination of patient's subsets (i.e., Ara h 9, Mal d 3, Zea m 14, or Vit v 1). It is noteworthy that most subjects with FDEIA and/or FDNIH fall into the group with more than 5

molecules, and it is possible to hypothesize that the two factors have an additive effect, as shown by the multiple logistic regression analysis.

Another crucial point is the importance of conducting a comprehensive evaluation of these patients, which should also involve testing for reactivity to other plant panallergens, such as profilins or PR10. This is because the presence of such reactivities allows the identification of patients with a more favorable prognosis (18)(21). Notably, this pattern of poly-reactivity is specific to patients observed in northern European territories (14)(3).

LTP sensitization is a characteristic of the more severe form of atopic dermatitis, as previously described (20). However, it is typically not associated with anaphylactic reactions in these patients. The discovery of this reactivity in patients with generalized and severe forms of atopic dermatitis suggests that LTP sensitization may contribute to a delayed re-exacerbation of T cell-driven dermatitis rather than immediate IgE-mediated symptoms, particularly in patients from the Mediterranean area where dietary habits often involve high consumption of plant foods. Probably, in these patients, sensitization occurs via a disrupted skin barrier, as demonstrated for peanut allergy in the USA(28), rather than via the gastrointestinal tract. It is well known that sensitization does not always correlate with clinical symptoms.

### 5.2 | Limitations

The clinical data of the patients were primarily based on self-reported history, and no food challenge was conducted. This selective criterion may not have affected the subset of patients who consistently tolerate LTP-containing foods. However, it is important to emphasize that the occurrence of severe food reactions (anaphylaxis) was always confirmed by emergency room records documenting the administration of adrenaline and steroids for symptom management.

The simultaneous presence of reactivity to PR10 and atopic dermatitis does not enable discrimination regarding which of the two factors plays a central role in mitigating the adverse effects of LTP reactivity in certain patients.

Finally, in this study, we did not establish causality but solely reported the association between cofactors (exercise, intake of NSAIDs, sensitization to other panallergens, presence of atopic dermatitis, etc.) and the consumption of LTP-containing foods.

### 5.3 | Generalisability

Simultaneous proteomic evaluation of multiple LTPs that are homologous but not identical molecules is useful to reveal the presence of sensitization profiles associated with different clinical

subgroups. The clinical severity of the LTP syndrome is associated with an expanded IgE repertoire characterized by increased numbers of recognized LTP components and elevated levels of specific IgE. Ara h 9, Cor a 8, and Mal d 3 showed the strongest association with clinical severity, and they were rarely detected in 'tolerant' individuals.

In addition, we investigated the potentially attenuating or exacerbating role of different cofactors within the same patient groups. We found that higher LTP detection capacity (5 molecules), even in combination with exercise and/or NSAID use, strongly correlated with a higher frequency of severe episodes.

In contrast, the concomitant presence of atopic dermatitis and hypersensitivity to other panallergens had a mitigating effect on the incidence of systemic or anaphylactic reactions in our patient cohort.

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**Potential conflict of interest**

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**Author contribution statement**

ES, EC, VV and MG carried out the experiments and data collection.

ES, VV and MG recruited the patients.

ES and DA performed the statistical analysis.

ES, DV, LC, VP and RA conceived the study and assisted in data interpretation.

All authors reviewed, edited and approved the final manuscript.

All Authors consent for publication, and confirm that this manuscript is original, has not been published before, is not currently being considered for publication elsewhere, and has not been posted to a preprint server.

**Data Avaiability**

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

**ETHICAL APPROVAL STATEMENT**

The research was conducted ethically following the World Medical Association Declaration of Helsinki. All subjects have given their written informed consent and that the study protocol was approved by the institute's committee.

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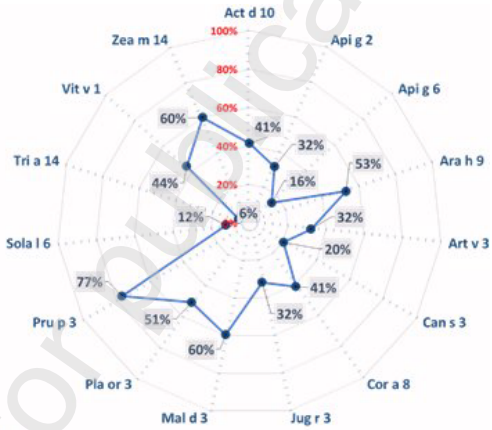
**Figure 1 | A** | The radar chart shows the prevalence of positive outcomes for the assessed LTPs in the study. **B** | Bivariate analysis of the reciprocal relationships among the 15 LTPs studied. Spearman coefficient values and the absolute number of IgE-positive subjects are given for the paired allergens. A correlation coefficient between 0.7 and 1.0 indicates a strong positive relationship (dark yellow), whereas a coefficient between 0.3 and 0.7 indicates a moderate positive relationship (light yellow).




| | Act d 10 | Api g 2 | Api g 6 | Ara h 9 | Art v 3 | Can s 3 | Cor a 8 | Jug r 3 | Mal d 3 | Pla a 3 | Pru p 3 | Sola l 6 | Tri a 14 | Vit v 1 | Zea m 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Act d 10 | 176 | | | | | | | | | | | | | | |
| Api g 2 | **0,576** 102 | 136 | | | | | | | | | | | | | |
| Api g 6 | 0,439 52 | 0,447 47 | 66 | | | | | | | | | | | | |
| Ara h 9 | **0,595** 139 | **0,612** 117 | *0,37* 52 | 225 | | | | | | | | | | | |
| Art v 3 | *0,493* 99 | **0,569** 87 | *0,427* 46 | **0,605** 118 | 137 | | | | | | | | | | |
| Can s 3 | 0,413 64 | **0,602** 72 | 0,449 42 | **0,522** 76 | **0,591** 68 | 87 | | | | | | | | | |
| Cor a 8 | **0,518** 117 | **0,585** 103 | *0,365* 46 | **0,647** 143 | **0,603** 110 | **0,561** 76 | 175 | | | | | | | | |
| Jug r 3 | **0,526** 98 | *0,642* 93 | *0,427* 45 | *0,68* 121 | **0,607** 92 | *0,67* 75 | **0,688** 115 | 135 | | | | | | | |
| Mal d 3 | **0,566** 148 | **0,575** 123 | 0,349 55 | **0,758** 195 | **0,618** 127 | **0,509** 79 | **0,727** 162 | **0,713** 129 | 254 | | | | | | |
| Pla a 3 | **0,544** 136 | **0,615** 117 | 0,38 54 | 0,63 167 | **0,563** 114 | **0,55** 81 | **0,621** 146 | **0,692** 123 | **0,715** 187 | 218 | | | | | |
| Pru p 3 | 0,487 161 | **0,509** 129 | 0,3 58 | **0,659** 208 | **0,531** 126 | 0,449 79 | **0,613** 164 | 0,631 132 | **0,859** 243 | **0,638** 197 | 326 | | | | |
| Sola l 6 | 0,21 32 | 0,246 28 | 0,544 34 | 0,171 33 | 0,249 30 | 0,326 28 | 0,211 33 | 0,247 28 | 0,171 35 | 0,244 38 | 0,136 38 | 53 | | | |
| Tri a 14 | 0,148 16 | 0,271 17 | 0,212 10 | 0,176 16 | 0,278 17 | 0,41 16 | 0,187 16 | 0,288 18 | 0,142 17 | 0,221 17 | 0,086 17 | 0,303 10 | 25 | | |
| Vit v 1 | **0,621** 127 | **0,732** 120 | 0,49 57 | **0,703** 156 | **0,647** 113 | **0,607** 80 | 0,692 135 | **0,727** 115 | **0,678** 163 | **0,693** 156 | **0,564** 170 | 0,273 39 | 0,27 18 | 187 | |
| Zea m 14 | **0,611** 148 | **0,663** 125 | 0,475 61 | **0,668** 191 | **0,523** 117 | 0,533 80 | **0,562** 145 | **0,601** 118 | **0,623** 195 | **0,568** 168 | 0,496 212 | 0,285 41 | 0,224 19 | **0,674** 159 | 255 |

**Figure 2** | LTP-IgE detection profiles and the distribution of reactivity in patients with fewer or more than 5 LTP. Percentages of each molecule indicate the proportion of patients positive for that molecule within the reactivity category. Significant differences ($p<0.05$) were observed in all cases except Tri a 14.

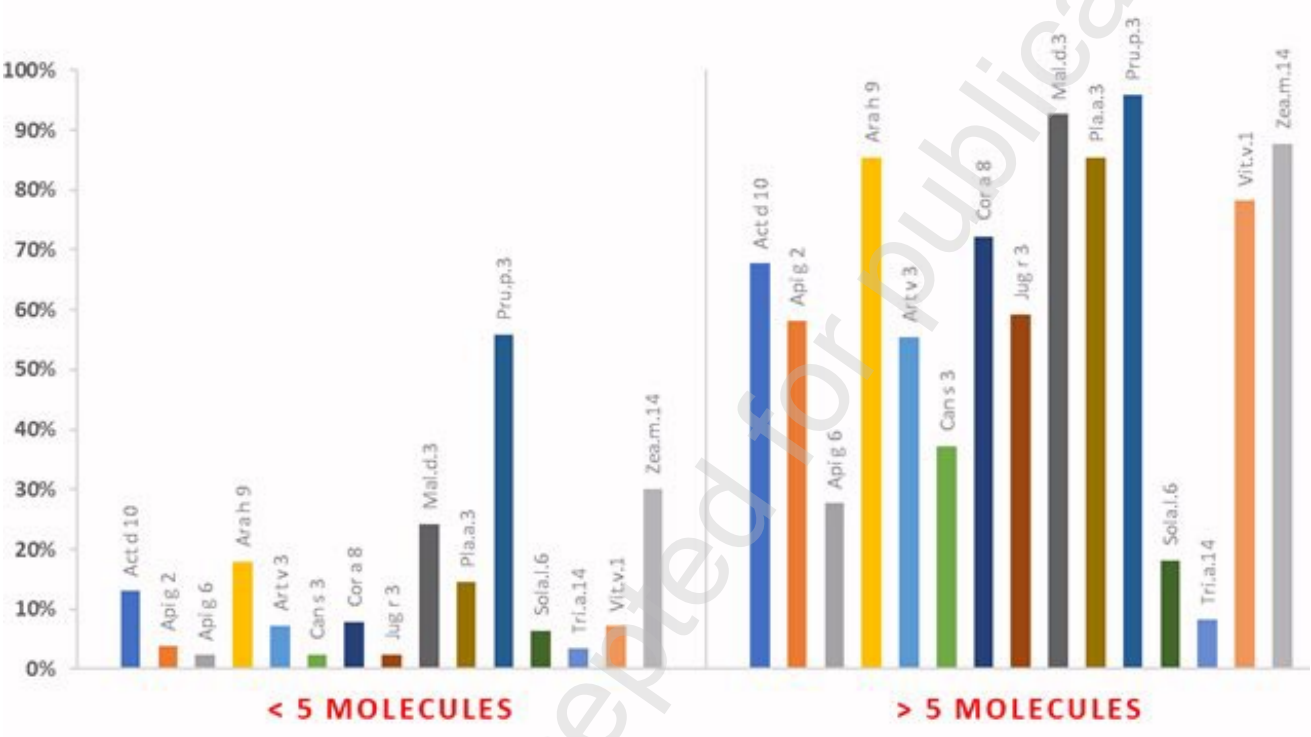


**Figure 3** | The Venn diagram illustrates the relationships between various cofactors that may influence the clinical outcome of patients with LTP allergy, indicating mitigating factors (co-sensitization to PR10 and/or Profilin and presence of atopic dermatitis) and aggravating factors (reactivity to more than 5 LTPs simultaneously, exercise, and/or intake of NSAIDs after consumption of LTP-containing sources). The figure provides an algorithm that can be used in daily clinical practice for the management of patients with LTP syndrome.

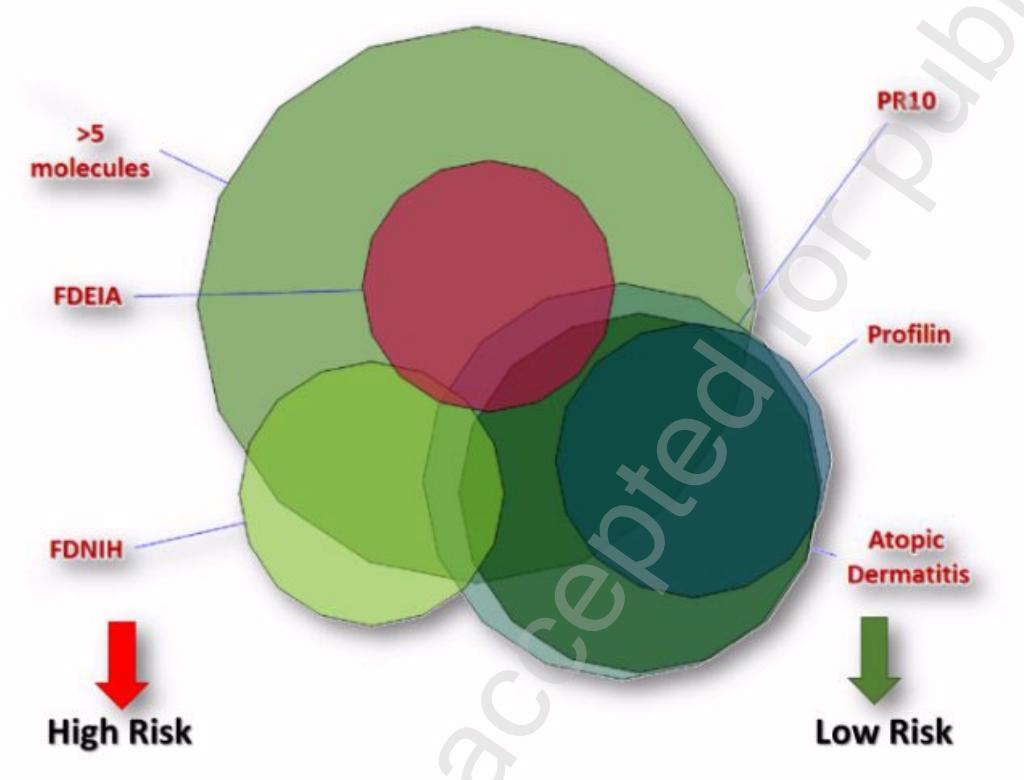


**Figure 1.repository** | Comparison of sequence identity between the 15 LTPs studied and those whose sequences are currently registered in the IUIS database. The red line represents 60% sequence identity.

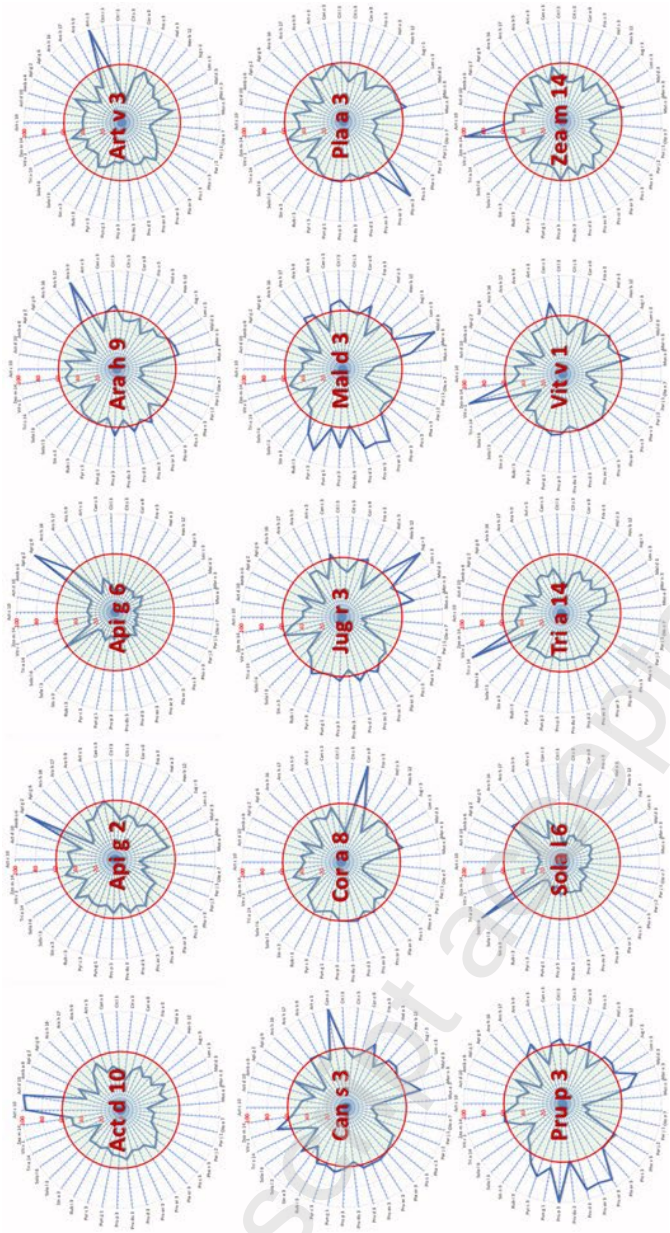


**Figure 2.repository** | The patients studied were divided into 15 different subgroups, each characterized by 100% reactivity to one of the 15 molecules studied. The figure consists of 15 radar plots comparing the prevalence of IgE detection (in brown) and the percentage of sequence identity reported by IUIS (in blue). These comparisons are made between the molecule characterizing the specific subgroup and the other LTPs tested in the macroarray. The molecule representing the subgroup of interest appears 100% on the respective radar plot, while the percentages of the other individual molecules indicate the proportion of patients positive for that molecule within the reactivity category.

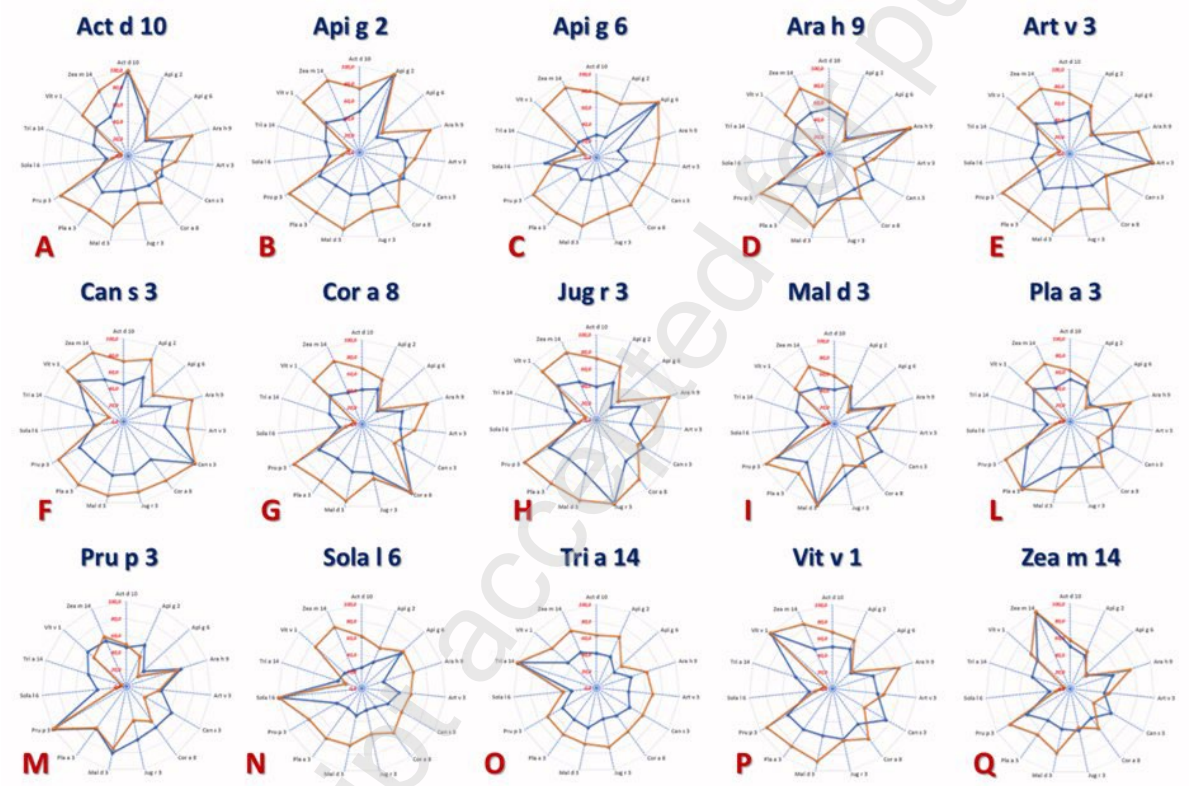


**Table 1** | The table provides a quantitative comparison of specific IgE levels (kU/L) directed against the different LTPs assessed in different subgroups. The subgroups include (A) men and women, (B) individuals who tolerate LTP-containing foods and those who do not, (C) patients with mild symptoms and those with severe symptoms, (D) individuals with atopic dermatitis, (E) patients with fewer or more than 5 nsLTP sensitizations, and (F) LTP monoreactors and LTP reactors to PR10 and/or Profilin. The yellow highlighted asterisk indicates a significant difference (p 0.05) between the two subgroups studied.

| | A | | | B | | | C | | | D | | | E | | | F | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | | Tolerant | Not Tolerant | | OAS | Moderate Severe | | AD Absent | AD Present | | LTP <5mol | LTP >5mol | | LTP Mono | LTP Poly | |
| Act d 10 | 1.8±3.9 | 1.7±3.9 | | 0.9±3.4 | 1.9±4.0 | * | 1.4±4.0 | 2.3±4.0 | * | 1.7±4.0 | 1.9±3.6 | | 0.2±0.4 | 3.2±5.0 | * | 1.7±4.2 | 1.8±3.5 | |
| Api g 2 | 1.3±3.3 | 1.0±3.1 | | 0.6±4.0 | 1.3±3.0 | | 0.9±3.1 | 1.5±2.9 | | 0.9±2.8 | 1.8±4.4 | * | 0.0±0.2 | 2.2±4.2 | * | 0.9±2.4 | 1.5±4.0 | * |
| Api g 6 | 0.8±3.5 | 0.4±2.0 | | 0.1±0.5 | 0.7±3.0 | | 0.6±2.5 | 0.8±3.3 | | 0.5±2.3 | 1.0±3.9 | | 0.0±0.2 | 1.2±3.8 | * | 0.6±3.3 | 0.6±1.9 | |
| Ara h 9 | 2.8±5.8 | 2.0±4.2 | | 0.8±3.7 | 2.7±5.2 | * | 1.7±4.8 | 3.5±5.3 | * | 2.3±4.7 | 2.6±5.9 | | 0.3±1.0 | 4.3±6.2 | * | 2.3±4.3 | 2.5±5.7 | |
| Art v 3 | 1.4±3.8 | 1.3±3.9 | | 0.7±3.2 | 1.5±3.9 | | 0.7±1.7 | 2.1±4.9 | * | 1.3±4.0 | 1.4±3.0 | | 0.2±0.9 | 2.4±5.0 | * | 1.4±4.3 | 1.2±3.2 | |
| Can s 3 | 0.9±3.3 | 0.5±2.5 | | 0.1±0,5 | 0.8±3.2 | * | 0,6±2,8 | 1.0±3.4 | | 0.5±2.1 | 1.4±4.7 | * | 0.1±0.1 | 1.2±3.8 | * | 1.1±3.7 | 0.5±2.3 | * |
| Cor a 8 | 1.8±4.7 | 1.5±3.5 | | 0.5±3.0 | 1.9±4.2 | * | 1.2±3.8 | 2.4±4.4 | * | 1.5±3.5 | 2.1±5.4 | | 0.1±0.4 | 3.1±5.2 | * | 1.5±2.8 | 1.9±5.1 | |
| Jug r 3 | 1.5±4.8 | 0.7±2.1 | * | 0.1±0.8 | 1.3±3.9 | * | 0.5±1.3 | 1.9±4.8 | * | 0.8±2.3 | 1.8±6.2 | * | 0.02±0.1 | 1.9±4.7 | * | 1.1±2.7 | 1.1±3.9 | |
| Mal d 3 | 3.6±7.0 | 2.7±4.6 | | 0.9±4.4 | 3.6±6.0 | * | 2.0±4.1 | 4.7±6.8 | * | 3.0±5.2 | 3.4±7.4 | | 0.4±1.5 | 5.6±7.1 | * | 3.2±6.0 | 2.9±5.5 | |
| Pla a 3 | 2.2±4.5 | 1.6±3.4 | | 0.8±3.5 | 2.1±4.0 | * | 1.4±2.6 | 2.6±4.6 | * | 1.7±3.5 | 2.4±4.9 | | 0.3±0.9 | 3.4±4.9 | * | 1.8±4.0 | 1.9±3.8 | |
| Pru p 3 | 5.6±9.3 | 4.9±7.0 | | 1.5±4.9 | 6.0±8.4 | * | 4.1±7.1 | 7.4±9.0 | * | 5.0±7.4 | 5.7±10.0 | | 1.6±3.9 | 8.5±9.5 | * | 5.4±8.2 | 4.9±7.9 | |
| Sola l 6 | 0.8±3.4 | 0.5±2.0 | | 0.1±0.4 | 0.7±3.0 | | 0.6±2.2 | 0.9±3.4 | | 0.6±2.8 | 0.8±2.3 | | 0.2±1.5 | 1.0±3.4 | * | 0.5±2.9 | 0.7±2.4 | |
| Tri a 14 | 0.2±0.7 | 0.1±0.9 | | 0.0±0.2 | 0.2±0.9 | | 0.1±0.6 | 0.2±1.1 | | 0.1±0.7 | 0.2±1.0 | | 0.1±0.7 | 0.2±0.9 | | 0.1±0.8 | 0.2±0.8 | |
| Vit v 1 | 2.6±6.5 | 1.5±3.7 | * | 0.9±4.9 | 2.2±5.2 | * | 1.4±3.7 | 2.8±5.9 | * | 1.8±4.8 | 2.6±6.0 | | 0.1±0.4 | 3.7±6.7 | * | 1.9±5.4 | 2.0±4.8 | |
| Zea m 14 | 4.1±8.1 | 3.3±7.1 | | 1.8±6.6 | 4.1±7.7 | * | 3.2±7.8 | 4.6±7.6 | | 3.2±6.4 | 5.2±10.4 | * | 0.5±1.4 | 6.6±9.5 | * | 3.4±7.4 | 4.0±7.7 | |

**Table 2** | The distribution of patients tolerating LTP-containing foods or showing adverse reactions are divided by gender and molecular reactivity. Within each clinical group (tolerant, OAS, urticaria/angioedema, or anaphylaxis), the percentage of patients reacting to each LTP studied is given. The linear relationship between all LTPs studied in alphabetical order and the clinical outcome is given molecule by molecule with chi-square values and significance.

| | Tolerant | Oral Allergy Syndrome | Urticaria Angioedema | Anaphylaxis | | |
|---|---|---|---|---|---|---|
| **No** | 80 (19%) | 144 (34%) | 133 (31%) | 69 (16%) | | |
| **Male** | 40 (9,40%) | 65 (15,30%) | 48 (11,30%) | 34 (8,00%) | | |
| **Female** | 40 (9,40%) | 79 (18,50%) | 85 (20,00%) | 35 (8,20%) | $\chi^2$ *linear-by-linear* | *p-value* |
| **Act d 10** | 18% | 32% | 54% | 64% | 45,91 | *< 0.0001* |
| **Api g 2** | 10% | 21% | 40% | 65% | 62,36 | *< 0.0001* |
| **Api g 6** | 5% | 15% | 20% | 20% | 8,66 | *<0.005* |
| **Ara h 9** | 15% | 40% | 72% | 87% | 105,54 | *< 0.0001* |
| **Art v 3** | 14% | 22% | 41% | 57% | 41,60 | *< 0.0001* |
| **Can s 3** | 5% | 15% | 26% | 38% | 29,45 | *< 0.0001* |
| **Cor a 8** | 9% | 26% | 58% | 78% | 101,24 | *< 0.0001* |
| **Jug r 3** | 6% | 22% | 43% | 59% | 61,68 | *< 0.0001* |
| **Mal d 3** | 20% | 46% | 83% | 88% | 109,25 | *< 0.0001* |
| **Pla a 3** | 19% | 44% | 62% | 81% | 66,98 | *< 0.0001* |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Pru p 3** | 46% | 72% | 91% | 93% | 59,68 | < *0.0001* |
| **Sola l 6** | 8% | 12% | 13% | 19% | 3,98 | *<0.05* |
| **Tri a 14** | 3% | 6% | 6% | 9% | ns | |
| **Vit v 1** | 13% | 33% | 58% | 77% | 79,83 | < *0.0001* |
| **Zea m 14** | 33% | 49% | 74% | 87% | 62,10 | < *0.0001* |

**Table 3** | The demographic and clinical characteristics of the study population are presented. Multiple logistic regression analysis including age, sex, and all cofactors shows a significant association between the presence of more than 5 nsLTPs, FDEIA, and FDNIH and a significantly higher risk of severe LTP-related food allergy. On the other hand, the presence of atopic dermatitis is associated with a less severe course.

| | | LTP symptomatic patients | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Severe | | Mild | | | | | | | |
| *Variable* | *Level* | *N* | *%* | *N* | *%* | *N* | *%* | *ORc* | *95% CI* | *P-value* | *ORadj* | *95% CI* | *P-value* |
| **Overall** | | 346 | 100 % | 202 | 58 % | 144 | 42 % | | | | | | |
| **>5 LTP molecules** | *Yes* | 213 | 62% | 160 | 79 % | 53 | 37 % | 1 | | | 1 | | |
| | *No* | 133 | 38% | 42 | 21 % | 91 | 63 % | 6,541 | 4,0-10,51 | <0.001 | 17,512 | 8,8-34,51 | <0.001 |
| **LTP Mono-reactivity** | *Yes* | 190 | 55% | 135 | 67 % | 55 | 38 % | 1 | | | | | |
| | *No* | 156 | 45% | 67 | 33 % | 89 | 62 % | 3,261 | 2,1-5,11 | <0.001 | | | |
| **PR10 Cosensitization** | *Yes* | 103 | 30% | 38 | 19 % | 65 | 45 % | 1 | | | | | |
| | *No* | 243 | 70% | 164 | 81 % | 79 | 55 % | 0,282 | 0,2-0,51 | <0.001 | | | |
| **Profilin Cosensitization** | *Yes* | 44 | 13% | 18 | 9% | 26 | 18 % | 1 | | | | | |
| | *No* | 302 | 87% | 184 | 91 % | 118 | 82 % | 0,444 | 0,2-0,81 | <0.001 | | | |
| **FDNIH** | *Yes* | 49 | 14% | 38 | 19 % | 11 | 8% | 1 | | | 1 | | |
| | *No* | 297 | 86% | 164 | 81 % | 133 | 92 % | 2,802 | 1,4-5,71 | <0,005 | 4,1118 | 1,6-10,65 | <0,005 |
| **FDEIA** | *Yes* | 46 | 13% | 44 | 22 % | 2 | 1% | 1 | | | 1 | | |
| | *No* | 300 | 87% | 158 | 78 % | 142 | 99 % | 19,77 | 4,7-83,01 | <0.001 | 71,398 | 13,9-1 | <0.001 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | 366,9 | |
| **Atopic Dermatitis** | *Yes* | 71 | 21% | 24 | 12% | 47 | 33% | 1 | | | | |
| | *No* | 275 | 79% | 178 | 88% | 97 | 67% | 0,278 | 0,2-0,5 | <0.001 | 0,3006 | 0,1-0,6 | <0,003 |
| **Bronchial Asthma** | *Yes* | 67 | 19% | 32 | 16% | 35 | 24% | 1 | | | | |
| | *No* | 279 | 81% | 170 | 84% | 109 | 76% | 0,586 | 0,3-1,0 | <0.05 | | |