

ORIGINAL ARTICLE

Relative IgE predominance within a core nsLTP panel may support clinical interpretation of multiplex sensitization profiles

Core-4 nsLTP predominance profiles

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Summary

Background. Non-specific lipid transfer proteins (nsLTPs) are major plant panallergens in Mediterranean plant-food allergy. In multiplex diagnostics, Pru p 3 reactivity often coexists with pollen-associated nsLTPs, making clinical interpretation difficult. **Methods.** We analyzed consecutive patients tested with ALEX² between 2021 and 2025. Among 5044 patients, 1271 were reactive to at least one molecule of a predefined core-4 nsLTP panel: Pru p 3, Art v 3, Pla a 3 and Ole e 7. High-confidence predominant profiles were defined by target IgE ≥ 0.30 kUA/L, target contribution $\geq 50\%$ of the total core-4 signal and molecule-specific log-ratio enrichment; the top 50 patients per molecule were selected. Food allergy and respiratory symptoms were analysed using adjusted logistic regression. Serum-pool inhibition experiments explored homologous and heterologous IgE inhibition. **Results.** The final sample included 200 patients equally distributed across the four predominant profiles. Pru p 3 predominance was associated with the highest frequency of food allergy, whereas Art v 3-, Pla a 3- and Ole e 7-predominant profiles showed greater respiratory involvement. In adjusted models, Art v 3- and Pla a 3-predominant profiles had lower odds of food allergy than Pru p 3 predominance, while all pollen-associated predominant profiles showed higher odds of respiratory

symptoms. Inhibition experiments showed strong homologous inhibition and variable heterologous inhibition, supporting partially overlapping but non-identical IgE recognition patterns. **Conclusions.** Relative IgE predominance within a core-4 nsLTP panel may help contextualize complex multiplex profiles beyond the canonical Pru p 3–food allergy association, without assigning sensitization chronology.

Key words

Non-specific lipid transfer proteins; Pru p 3; pollen-associated nsLTPs; molecular allergy diagnostics; IgE predominance.

IMPACT STATEMENT

This study shows that relative IgE predominance within a focused core-4 nsLTP panel can improve the clinical interpretation of complex multiplex allergy profiles. By distinguishing Pru p 3-centred food-associated patterns from pollen-associated Art v 3, Pla a 3 and Ole e 7 profiles, this approach may support more precise risk orientation in patients with LTP sensitisation, while avoiding unsupported assumptions about primary sensitisation pathways.

Introduction

Non-specific lipid transfer proteins (nsLTPs) are stable plant panallergens involved in plant-food allergy (1) and, in selected contexts, pollen-associated sensitisation (2). Their resistance to heat and digestion, together with their broad distribution across botanically unrelated plant sources, underlies their clinical relevance, particularly in Mediterranean populations, where nsLTP sensitisation is a major cause of plant-food allergy and systemic reactions (3–5). However, LTP allergy is clinically heterogeneous, ranging from asymptomatic sensitisation or mild oral symptoms to urticaria, angioedema, food-dependent cofactor-associated reactions and anaphylaxis (6–8). This heterogeneity is influenced not only by the molecular IgE profile, but also by cofactors, co-sensitisation to other plant panallergens and geographical exposure patterns (8–14).

Pru p 3, the peach nsLTP, is widely regarded as the prototypic marker of food-associated LTP allergy in Southern Europe (15–18). Nevertheless, nsLTPs are not restricted to plant foods. Pollen-associated nsLTPs, including Art v 3 from mugwort (19), Pla a 3 from plane tree (20) and Ole e 7 from olive pollen (21–24), are increasingly detected by molecular diagnostics and may coexist with food nsLTP reactivity within complex IgE profiles (9,11,21,25–27). Previous work from our centre showed that Pru p 3 was the most frequently recognised nsLTP (9), whereas Art v 3 and Pla a 3 have been associated with respiratory symptoms in independent studies (19,20,28). Previous analyses also highlighted the relevance of PR-10, profilin and Par j 2 co-sensitisation in modulating clinical expression (8,9).

Despite these observations, the relationship between food-associated and pollen-associated nsLTP reactivity remains unresolved. It is still unclear whether Pru p 3-centred profiles and pollen-associated nsLTP profiles reflect clinically distinguishable patterns, or whether they represent different quantitative configurations within a shared cross-reactive molecular network. The identification of the primary sensitiser in LTP syndrome remains one of the major open questions in the field, and cross-sectional IgE data alone cannot establish the temporal sequence of sensitisation (2,12).

The increasing use of multiplex molecular diagnostics has made it possible to assess these profiles in greater detail. However, quantitative IgE values obtained from different platforms are not directly interchangeable. In a previous comparison between ISAC and ALEX², dichotomised results showed substantial agreement, whereas raw quantitative values showed poor agreement, particularly for cross-reactive plant panallergens (29). These findings support the analysis of quantitative IgE hierarchies within a single diagnostic platform (30,31).

In the present study, we analysed consecutive patients tested with ALEX² at a single tertiary referral centre and focused on a predefined core-4 nsLTP panel including one reference food-associated molecule (Pru p 3) and three pollen-associated nsLTPs (Art v 3, Pla a 3 and Ole e 7). The panel was designed to address a specific clinical question: whether relative IgE predominance within food- and pollen-associated nsLTPs identifies clinical patterns that extend beyond binary positivity and beyond the canonical Pru p 3–food allergy association. Using proportional contribution and log-ratio enrichment, we identified high-confidence molecular profiles and compared their clinical correlates across food allergy and respiratory symptoms. These profiles were interpreted as cross-sectional representations of the current IgE architecture rather than as discrete endotypes or evidence of primary sensitisation pathways.

Methods

2.1 | Study design, setting and source population

This real-world cross-sectional study analysed prospectively recorded clinical phenotypes and molecular IgE profiles from patients referred for suspected allergic disease to the Clinical and Laboratory Molecular Allergy Unit of IDI-IRCCS, Rome, Italy. The centre is a tertiary referral unit for allergic and dermatological diseases serving Central and Southern Italy.

Between January 2021 and July 2025, 5044 consecutive patients underwent ALEX²-based molecular allergy testing and were considered for inclusion. Clinical information was recorded at the first allergological evaluation and before interpretation of the molecular IgE profile. Food allergy and respiratory symptoms were analysed as the two main clinical domains; atopic dermatitis was retained as an atopic comorbidity.

All participants provided written informed consent. The study protocol was approved by the local Ethics Committee of IDI-IRCCS and was conducted in accordance with the Declaration of Helsinki.

2.2 | Molecular IgE assessment

Allergen-specific IgE was assessed using the ALEX²® multiplex immunoassay (MacroArray Diagnostics, Vienna, Austria), according to the manufacturer's instructions. Specific IgE values were expressed in kUA/L, and sensitisation was defined as specific IgE ≥ 0.30 kUA/L.

The analysis focused on four a priori selected nsLTP molecules relevant to food- and pollen-associated recognition: Pru p 3 from peach, Art v 3 from mugwort, Pla a 3 from plane tree and Ole e 7 from olive pollen. These four molecules were defined as the core-4 nsLTP panel, a predefined analytical set including one reference food-associated nsLTP (Pru p 3) and three pollen-associated nsLTPs (Art v 3, Pla a 3 and Ole e 7). The core-4 panel was therefore intentionally restricted to molecules with a clear a priori biological contrast between food-associated and pollen-associated recognition. Broader food nsLTPs available on ALEX², including Ara h 9, Cor a 8, Mal d 3, Tri a 14 and others, were not used to define the enrichment denominator because they address a different question: expansion of the food-nsLTP repertoire rather than orientation between food- and pollen-associated nsLTP signals.

Other ALEX² nsLTP molecules were used for descriptive analyses only. Par j 2, an 11-kDa Parietaria LTP, was not included in the core-4 denominator because the present analysis was not designed to map all pollen LTPs, but to contrast one reference food-associated nsLTP with three clinically relevant pollen-associated nsLTPs already implicated in mugwort-, plane tree- and olive-related molecular profiles. Par j 2 was therefore retained as a descriptive marker of Parietaria sensitisation (32).

2.3 | Participant flow and analytical sample

The participant flow is shown in Figure 1. Among the 5044 ALEX²-tested patients, 1271 showed IgE reactivity ≥ 0.30 kUA/L to at least one core-4 nsLTP molecule and constituted the core-4-reactive source population.

High-confidence predominant profiles were then identified using the log-ratio enrichment procedure described below. For each target molecule, patients were eligible if the target IgE was ≥ 0.30 kUA/L and represented at least 50% of the total core-4 IgE signal. The 50 patients with the highest molecule-specific enrichment score were selected for each target molecule, yielding four balanced groups: Pru p 3-

predominant, Art v 3-predominant, Pla a 3-predominant and Ole e 7-predominant. The final analytical sample consisted of 200 patients.

These predominant groups were used for subsequent clinical comparisons and were not interpreted as discrete biological endotypes or as evidence of primary sensitisation routes.

2.4 | Core-4 log-ratio enrichment analysis

High-confidence core-4 molecular profiles were identified using a compositional log-ratio enrichment approach. For each patient and each core-4 molecule, the proportional contribution of the target molecule to the total core-4 IgE signal was calculated as:

$$p_j = \frac{IgE_j}{IgE_{Prup3} + IgE_{Artv3} + IgE_{Pla a 3} + IgE_{Ole e 7}}$$

where j represents the target molecule. This value ranges from 0 to 1 and quantifies the relative contribution of the target molecule to the total core-4 nsLTP IgE signal.

A molecule-specific enrichment score was calculated as:

$$E_j = \log \left[\frac{IgE_j + 0.01}{\sqrt[3]{(IgE_a + 0.01)(IgE_b + 0.01)(IgE_c + 0.01)}} \right]$$

where IgE_j is the target molecule and IgE_a , IgE_b and IgE_c are the remaining three core-4 molecules. A pseudo-value of 0.01 was added to allow calculation in the presence of values close to zero. Higher enrichment scores indicate a stronger relative excess of the target molecule compared with the remaining three core-4 nsLTPs.

For each target molecule, predominant cases were selected according to three criteria: target IgE ≥ 0.30 kUA/L, target proportional contribution $\geq 50\%$ of the total core-4 IgE signal, and ranking by molecule-specific enrichment score. The top 50 patients for each target molecule were retained.

Principal component analysis was performed in the full core-4-reactive population using log10-transformed and z-standardised IgE values to Pru p 3, Art v 3, Pla a 3 and Ole e 7. The 200 selected predominant profiles were overlaid on the PCA plot.

2.5 | Serum-pool inhibition experiments

To functionally explore IgE cross-reactivity within the core-4 nsLTP network, serum-pool inhibition experiments were performed as an exploratory substudy. Four serum pools were generated, each including five patients representative of one predominant molecular profile: Ole e 7-predominant, Art v 3-predominant, Pla a 3-predominant and Pru p 3-predominant. A fifth mixed pool was obtained by combining aliquots from the four profile-specific pools.

Each pool was incubated overnight with selected homologous and heterologous inhibitors before repeat IgE testing. The Ole e 7-predominant pool was inhibited with Pru p 3 and Ole e 7; the Art v 3-predominant pool with Pru p 3 and Art v 3; the Pla a 3-predominant pool with Pru p 3 and Platanus extract, used as a surrogate inhibitor because purified Pla a 3 was not commercially available; and the Pru p 3-predominant pool with Pru p 3, Art v 3, Platanus extract and Ole e 7. The mixed pool was inhibited with Pru p 3 and Par j 2.

Percentage inhibition was calculated for each tested allergen as the reduction in IgE signal after inhibitor incubation relative to the non-inhibited baseline. These experiments were considered exploratory and descriptive, without formal statistical testing.

2.6 | Clinical variables and presentation subsets

Food allergy and respiratory symptoms were classified from the clinical history recorded at the first allergological evaluation. For the main analyses, both domains were dichotomised as absent versus present. Food allergy severity was recorded when available but was not analysed as a primary endpoint because severity and anaphylaxis data were not systematically available for all patients.

Atopic dermatitis was not considered a primary clinical outcome of nsLTP sensitisation and was retained only as an atopic comorbidity.

For descriptive analyses, patients were assigned to four mutually exclusive clinical presentation subsets according to food allergy and respiratory symptom status: **neither**, **food-only**, **respiratory-only** and **mixed**. These subsets were used to describe presentation patterns and were not interpreted as mechanistic endotypes.

2.7 | Statistical analysis

Continuous variables are reported as mean $\pm$ standard deviation or median with interquartile range, as appropriate. Categorical variables are reported as counts and percentages.

Clinical and molecular characteristics were compared across the four predominant profiles. Kruskal–Wallis tests were used for continuous variables. Categorical variables were compared using χ^2 tests, or Fisher's exact tests when expected cell counts were small. Given the exploratory nature of between-profile comparisons, p values were not adjusted for multiple testing and should be interpreted descriptively.

Multivariable logistic regression models were used to evaluate the association between predominant molecular profile and the two main clinical domains: food allergy and respiratory symptoms. The Pru p 3-predominant group was used as the reference category. Models were kept parsimonious because of the selected sample size and included only prespecified covariates: age, sex, PR-10 co-sensitisation and profilin co-sensitisation.

Adjusted odds ratios with 95% confidence intervals were reported. Atopic dermatitis was not modelled as a primary outcome. Given the cross-sectional design, no causal or temporal inference regarding primary sensitisation routes was made.

Results

3.1 | Study population

Of 5044 consecutive patients tested with ALEX² between January 2021 and July 2025, 1271 were reactive to at least one molecule of the predefined core-4 nsLTP panel (Pru p 3, Art v 3, Pla a 3 or Ole e 7; ≥ 0.30 kUA/L). Using the predefined proportional contribution and log-ratio enrichment procedure, 50 patients were selected for each predominant profile. The final analytical sample included 200 patients equally distributed across Pru p 3-, Art v 3-, Pla a 3- and Ole e 7-predominant groups (Figure 1).

3.2 | Clinical and demographic characteristics

The selected sample had a mean age of 34.1 ± 17.8 years and included 113 female patients (56.5%), with no significant between-profile differences in age or sex distribution.

Food allergy was most frequent in the Pru p 3-predominant group, but it was also present across pollen-associated nsLTP profiles, indicating that non-Pru p 3 reactivity does not exclude food-related disease. Conversely, respiratory symptoms were least frequent in Pru p 3-predominant patients and more prevalent in profiles dominated by pollen-associated nsLTPs, particularly Art v 3, Pla a 3 and Ole e 7.

Atopic dermatitis was recorded in 36 patients (18.0%) and did not differ significantly across profiles. Co-sensitisation to PR-10, but not profilin alone, differed across predominant profiles. Combined PR-10 and/or profilin co-sensitisation was also unevenly distributed, as was Par j 2 positivity, which was most frequent in the Pla a 3-predominant group (Table 1).

3.3 | Molecular characteristics of predominant profiles

Core-4 molecular characteristics are reported in Table 2 and shown in Figure 3 and Figure S2. Each predominant group showed the highest IgE signal for its target molecule, with significant between-profile differences for all four core components.

Target core-4 proportion and log-ratio enrichment scores confirmed the molecular separation of the four selected profiles. Target contribution was highest in the Pru p 3- and Ole e 7-predominant groups, intermediate in the Pla a 3-predominant group and lowest in the Art v 3-predominant group. Par j 2 marker IgE was highest in the Pla a 3-predominant group.

In exploratory analyses, Can s 3 sensitisation was detected in 271/1271 core-4-reactive patients (21.3%). Only 10 patients fulfilled a $\geq 50\%$ core-5 contribution criterion for Can s 3, precluding definition of a balanced Can s 3-predominant group.

In exploratory screening of ALEX² molecular components, food allergy showed stronger positive associations with several food-associated nsLTPs, including Mal d 3, Cor a 8, Zea m 14, Tri a 14 and Ara h 9, whereas respiratory symptoms were more strongly associated with established inhalant markers, including Fel d 1, Der p 23, Phl p 1, Cup a 1, Der p 2 and Ole e 1. These analyses were considered exploratory and were not used to redefine the core-4 panel.

3.4 | Serum-pool inhibition patterns

Serum-pool inhibition experiments are shown in Figure 4. Homologous inhibition was generally strong within the corresponding predominant profiles. In the Ole e 7-predominant pool, Ole e 7 inhibited Ole e 7 reactivity by 96%, while Pru p 3 produced complete inhibition of Pru p 3 reactivity but only partial inhibition of Ole e 7 reactivity (24%). In the Art v 3-predominant pool, Art v 3 inhibited Art v 3 reactivity by 49%, whereas Pru p 3 strongly inhibited Pru p 3 reactivity (98%) but weakly inhibited Art v 3 reactivity (9%).

In the Pla a 3-predominant pool, Platanus extract inhibited the Ole e 7_RUO signal by 70% and produced partial inhibition of the tested Platanus-related profile, whereas Pru p 3 strongly inhibited Pru p 3 reactivity (97%) with minimal inhibition of the Platanus signal (3%). In the Pru p 3-predominant pool, Pru p 3 almost completely inhibited Pru p 3 reactivity (99%), whereas Art v 3, Ole e 7 and Platanus extract produced limited or absent inhibition of most heterologous signals.

In the mixed pool, Pru p 3 inhibited Pru p 3 reactivity by 98% but showed minimal inhibition of Par j 2 (5%), while Par j 2 selectively inhibited Par j 2 reactivity (93%) with no inhibition of Pru p 3. Overall, the inhibition patterns supported partially overlapping but non-identical IgE recognition within the core-4 nsLTP network and suggested that Par j 2 behaves as a distinct LTP marker outside the Pru p 3-centred cross-reactive axis.

3.5 | Clinical presentation subsets and PCA

Clinical presentation subsets are reported in Table 3 and Figure 2. Overall, half of the selected patients had food allergy without respiratory symptoms, whereas 17.0% had respiratory symptoms without food allergy and 22.0% had both. The distribution of clinical presentation subsets differed across predominant profiles. Food-only presentation predominated in the Pru p 3-predominant group, whereas respiratory-only and mixed presentations were more represented among Art v 3-, Pla a 3- and Ole e 7-predominant patients.

Principal component analysis performed in the full core-4-reactive population showed a continuous distribution of IgE profiles. The 200 selected predominant profiles were distributed toward the peripheral regions of the PCA space (Figure S1).

3.6 | Adjusted associations with clinical outcomes

Adjusted logistic regression models are shown in Table 4 and Figure 5. The Pru p 3-predominant group was used as reference. Models were adjusted for age, sex, PR-10 co-sensitisation and profilin co-sensitisation.

In adjusted models, Art v 3- and Pla a 3-predominant profiles showed lower odds of food allergy than the Pru p 3-predominant reference group; the estimate for Ole e 7 was in the same direction but did not reach statistical significance. Conversely, Art v 3-, Pla a 3- and Ole e 7-predominant profiles all showed higher odds of respiratory symptoms than the Pru p 3-predominant group.

Discussion

In this real-life ALEX²-based study, relative IgE enrichment within a predefined core-4 nsLTP panel identified clinically meaningful patterns among patients otherwise classified simply as LTP-sensitised. The restricted core-4 design was not intended to represent the full nsLTP repertoire, but to test whether the relative contribution of one prototypic food-associated nsLTP, Pru p 3, and three pollen-associated nsLTPs, Art v 3, Pla a 3 and Ole e 7, could improve interpretation of complex multiplex profiles.

Overall, the relative weight of individual nsLTPs was not clinically neutral. Pru p 3 predominance retained the strongest food-allergy signal, identifying the group with the highest frequency of food allergy (1,32), in keeping with its role as the reference food-associated nsLTP marker in Mediterranean populations (7,16). Conversely, profiles dominated by Art v 3, Pla a 3 or Ole e 7 were associated with greater respiratory involvement, supporting the view that nsLTP sensitisation includes clinically relevant pollen-associated patterns rather than a single Pru p 3-centred model. These associations remained evident after adjustment for age, sex, PR-10 co-sensitisation and profilin co-sensitisation.

The exploratory inhibition substudy added functional support. Strong homologous inhibition within predominant profiles and incomplete heterologous inhibition between food- and pollen-associated nsLTPs

suggest partially overlapping, but non-identical, IgE recognition patterns. Pru p 3 did not uniformly abolish reactivity to pollen-associated nsLTP signals, indicating that Art v 3, Pla a 3 and Ole e 7 reactivity should not be interpreted simply as passive extension of Pru p 3 sensitisation. However, these pooled experiments cannot establish primary sensitisation routes.

Food allergy was also observed among patients with Art v 3-, Pla a 3- or Ole e 7-predominant profiles. Therefore, a pollen-associated predominant profile does not exclude food allergy and should never replace careful assessment of culprit foods, cofactors and reaction patterns. Exploratory analyses further indicated that food allergy was not captured by Pru p 3 alone: other food-associated nsLTPs, particularly Mal d 3 and Cor a 8, showed relevant associations, supporting the concept that food-related LTP allergy reflects a broader food-nsLTP repertoire.

The pollen-associated profiles provide a complementary message. Art v 3, Pla a 3 and Ole e 7 predominance oriented the clinical picture towards respiratory involvement, giving practical meaning to pollen-associated nsLTP signals detected by multiplex testing, especially in polysensitised patients with several low-level nsLTP positivities. However, these molecules should be considered orientation markers rather than standalone respiratory biomarkers. Ole e 7 appeared less segregated than expected for a purely pollen-associated marker, as Ole e 7-predominant patients showed both food and respiratory presentations (22,23). Moreover, respiratory symptoms were more strongly associated with genuine inhalant markers than with pollen-associated nsLTPs alone.

PR-10 and profilin were included as covariates because they may modify plant-food allergy expression and confound the relationship between nsLTP enrichment and clinical manifestations (9,17). Previous data indicate that PR-10/profilin co-sensitisation and other modifiers influence the severity and clinical expression of LTP syndrome (3,9). Atopic dermatitis was retained only as an atopic comorbidity. Par j 2 was kept outside the core-4 denominator because it is an 11-kDa Parietaria LTP not considered part of the Pru p 3-related cross-reactive nsLTP network and was reported descriptively (33). This choice was supported by selective Par j 2 inhibition in the mixed serum pool, with negligible inhibition of Pru p 3. Similarly, Can s 3 did not define a clinically usable enrichment axis, although it was detectable in approximately one fifth of core-4-reactive patients (34,35).

The practical implication is to move nsLTP interpretation beyond binary positivity. In routine multiplex diagnostics, the relevant question is not only whether a patient is nsLTP-positive, but which molecule carries most of the IgE signal. A Pru p 3-predominant profile should prompt detailed assessment of plant-food reactions and cofactors, whereas Art v 3-, Pla a 3- or Ole e 7-predominant profiles should raise attention to respiratory disease and relevant pollen sensitisation. Predominant profiles should be integrated with food

history, respiratory history, cofactor exposure and local pollen context (36). They provide clinical orientation, not proof of sensitisation origin.

Several limitations should be acknowledged. The cross-sectional design precludes inference on sensitisation chronology; the study describes clinical correlates of current IgE architecture. The balanced selection of 50 patients per predominant profile was intentional and aimed to compare high-confidence molecular extremes, not to estimate profile prevalence. The enrichment method remains an analytical construct: the $\geq 50\%$ threshold and top-50 ranking define comparison groups, not natural clusters. Therefore, prevalence estimates from the selected sample cannot be extrapolated to the full nsLTP-reactive population. Inhibition experiments were descriptive, performed on pooled sera and included a limited number of patients per profile. Platanus extract was used as a surrogate inhibitor for Pla a 3 because purified Pla a 3 was not commercially available. Clinical severity, including anaphylaxis, could not be robustly compared across clinical presentation subsets because severity data were available only for a subset of patients. This aspect should be addressed in future studies with systematic severity adjudication. Clinical phenotypes were based on real-life recorded history; oral food challenges, culprit-food stratification, systematic cofactor assessment and residence-level pollen exposure data were not available for all patients.

In conclusion, relative IgE predominance within a predefined core-4 nsLTP panel helped identify clinical patterns extending beyond the canonical Pru p 3–food allergy association. Pru p 3 predominance aligned with a more food-oriented presentation, whereas pollen-associated nsLTP predominance was linked to greater respiratory involvement. This approach does not resolve the primary sensitiser question, but offers a pragmatic framework for contextualising complex multiplex profiles. Future longitudinal studies integrating culprit foods, cofactors, pollen exposure, systematic severity adjudication and functional assays are needed to determine whether these profiles are stable markers of clinical expression.

Funding | This study was supported by the “Progetto Ricerca Corrente” of the Italian Ministry of Health, Rome, Italy.

Potential conflict of interest | ES has received consultant arrangements and participated in speakers' bureaus for Dienes, Dasit, and Thermo Fisher Scientific. The remaining authors declare that they have no relevant conflicts of interest.

Acknowledgements | We extend our gratitude to Mr. Mauro Helmer Citterich for conducting the in vitro tests, and to the nursing and medical staff. Their daily dedication and selflessness made it possible for us to provide care to every patient who visited our Centre.

Institutional Review Board Statement | The research was conducted in accordance with ethical standards outlined in the World Medical Association Declaration of Helsinki. The study protocol underwent review and approval by the IDI–IRCCS's committee, with approval number IDI-IRCCS CE | 493/1.

Data Availability Statement | The data supporting the findings of this study are available upon request from the corresponding author. However, please note that the data are not publicly available due to privacy or ethical restrictions.

Author Contributions | Conceptualization: ES; Data curation: ES, EC; Formal analysis: ES; Funding acquisition: not specified; Investigation: ES, EC, MG, ECG, ML, GM, LP, DQ, AZ; Methodology: ES; Project administration: ES; Resources: ES, MG, ECG, ML, GM, LP, DQ, AZ; Software: ES; Supervision: ES, RA, LG, DV, LC, VP; Validation: ES, RA, LG, DV, LC, VP; Visualization: ES; Writing – original draft: ES; Writing – review & editing: ES, EC, MG, ECG, ML, GM, LP, DQ, AZ, RA, LG, DV, LC, VP. All authors reviewed, edited and approved the final version of the manuscript.

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Table 1 | Clinical and demographic characteristics of the high-confidence core-4 predominant profiles.

Variable	Overall	Prun p 3	Art v 3	Pla a 3	Ole e 7	P value
N	200	50	50	50	50	
Age, years (mean $\pm$ SD)	34.1 $\pm$ 17.8	28.5 $\pm$ 16.5	37.4 $\pm$ 18.4	35.3 $\pm$ 18.5	35.1 $\pm$ 17.1	0.058
Female sex, n (%)	113 (56.5%)	30 (60.0%)	28 (56.0%)	31 (62.0%)	24 (48.0%)	0.505
Food allergy present, n (%)	144 (72.0%)	44 (88.0%)	31 (62.0%)	33 (66.0%)	36 (72.0%)	0.021
Respiratory symptoms present, n (%)	78 (39.0%)	9 (18.0%)	28 (56.0%)	20 (40.0%)	21 (42.0%)	0.001
Atopic dermatitis comorbidity present, n (%)	36 (18.0%)	5 (10.0%)	6 (12.0%)	12 (24.0%)	13 (26.0%)	0.079
PR-10 co-sensitisation, n (%)	97 (48.5%)	22 (44.0%)	35 (70.0%)	27 (54.0%)	13 (26.0%)	<0.001
Profilin co-sensitisation, n (%)	19 (9.5%)	1 (2.0%)	5 (10.0%)	5 (10.0%)	8 (16.0%)	0.124
PR-10 and/or profilin co-sensitisation, n (%)	104 (52.0%)	23 (46.0%)	36 (72.0%)	29 (58.0%)	16 (32.0%)	<0.001
Par j 2 positive, n (%)	99 (49.5%)	19 (38.0%)	21 (42.0%)	36 (72.0%)	23 (46.0%)	0.003
Clinical subset: Neither, n (%)	22 (11.0%)	4 (8.0%)	6 (12.0%)	8 (16.0%)	4 (8.0%)	0.009
Clinical subset: Food-only, n (%)	100 (50.0%)	37 (74.0%)	16 (32.0%)	22 (44.0%)	25 (50.0%)	
Clinical subset: Respiratory-only, n (%)	34 (17.0%)	2 (4.0%)	13 (26.0%)	9 (18.0%)	10 (20.0%)	
Clinical subset: Mixed, n (%)	44 (22.0%)	7 (14.0%)	15 (30.0%)	11 (22.0%)	11 (22.0%)	

Note. Data are shown as n (%) unless otherwise stated. P values refer to across-group comparisons among the four predominant profiles.

Table 2 | Core-4 molecular characteristics across predominant profiles.

Variable	Overall	Pru p 3	Art v 3	Pla a 3	Ole e 7	P value
Pru p 3 IgE, median (IQR)	0.49 (0.01–6.14)	10.18 (6.82–20.76)	1.24 (0.01–3.20)	0.01 (0.01–1.02)	0.01 (0.01–0.11)	<0.001
Art v 3 IgE, median (IQR)	0.01 (0.01–1.03)	0.01 (0.01–0.01)	5.31 (1.88–8.81)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	<0.001
Pla a 3 IgE, median (IQR)	0.01 (0.01–2.12)	0.01 (0.01–0.19)	0.01 (0.01–1.04)	3.33 (2.15–6.14)	0.01 (0.01–0.01)	<0.001
Ole e 7 IgE, median (IQR)	0.01 (0.01–1.96)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	0.01 (0.01–0.01)	6.25 (4.07–11.00)	<0.001
Target molecule IgE, median (IQR)	6.01 (3.02–11.40)	10.18 (6.82–20.76)	5.31 (1.88–8.81)	3.33 (2.15–6.14)	6.25 (4.07–11.00)	<0.001
Target core-4 proportion, median (IQR)	0.98 (0.74–0.99)	0.99 (0.97–1.00)	0.72 (0.57–0.95)	0.94 (0.68–0.99)	0.99 (0.98–1.00)	<0.001
Target log-ratio enrichment, median (IQR)	5.07 (3.77–5.83)	5.81 (5.46–6.13)	3.38 (2.77–4.03)	4.06 (3.59–4.95)	5.38 (4.82–5.99)	<0.001
Second-highest other core-4 IgE, median (IQR)	0.01 (0.01–1.33)	0.01 (0.01–0.50)	1.35 (0.01–3.35)	0.16 (0.01–1.80)	0.01 (0.01–0.16)	<0.001
Margin vs second-highest, median (IQR)	5.33 (2.12–10.12)	10.05 (6.81–19.95)	2.66 (0.89–7.46)	2.27 (1.32–5.32)	5.96 (4.06–10.25)	<0.001
Ratio vs second-highest, median (IQR)	51.36 (3.57–280.88)	261.25 (37.95–419.25)	3.13 (1.80–29.72)	18.41 (2.39–102.37)	169.00 (49.82–345.69)	<0.001
Par j 2 marker IgE, median (IQR)	0.17 (0.01–22.55)	0.01 (0.01–9.47)	0.01 (0.01–6.90)	22.86 (0.01–38.04)	0.01 (0.01–10.63)	<0.001

Note. IgE values are expressed in kUA/L. Target proportion is the target molecule contribution to the total core-4 IgE signal. Enrichment was calculated as a log-ratio against the geometric mean of the other three core-4 molecules.

Table 3 | Distribution of clinical presentation subsets across core-4 predominant profiles.

Predominant_profile	Neither	Food-only	Respiratory-only	Mixed
Pru p 3	4 (8.0%)	37 (74.0%)	2 (4.0%)	7 (14.0%)
Art v 3	6 (12.0%)	16 (32.0%)	13 (26.0%)	15 (30.0%)
Pla a 3	8 (16.0%)	22 (44.0%)	9 (18.0%)	11 (22.0%)
Ole e 7	4 (8.0%)	25 (50.0%)	10 (20.0%)	11 (22.0%)

Note. Clinical presentation subsets were defined by food allergy and respiratory symptoms: neither, food-only, respiratory-only and mixed.

Table 4 | Adjusted logistic regression models for food allergy and respiratory symptoms.

Outcome	Contrast	OR (95% CI)	P value
Food allergy	Art v 3 vs Pru p 3	0.24 (0.08–0.70)	0.009
Food allergy	Pla a 3 vs Pru p 3	0.27 (0.09–0.77)	0.014
Food allergy	Ole e 7 vs Pru p 3	0.38 (0.13–1.14)	0.083
Respiratory symptoms	Art v 3 vs Pru p 3	5.38 (2.11–13.74)	<0.001
Respiratory symptoms	Pla a 3 vs Pru p 3	2.94 (1.16–7.46)	0.023
Respiratory symptoms	Ole e 7 vs Pru p 3	3.38 (1.31–8.71)	0.012

Note. Models were adjusted for age, sex, PR-10 co-sensitisation and profilin co-sensitisation. The Pru p 3-predominant group was used as the reference category.

Figure Captions

Figure 1 | Participant flow diagram.

Consecutive patients undergoing ALEX² molecular allergy diagnostics at IDI-IRCCS between January 2021 and July 2025 were screened for IgE reactivity to the predefined core-4 nsLTP panel, including Pru p 3, Art v 3, Pla a 3 and Ole e 7. Among 5044 ALEX²-tested patients, 1271 showed IgE reactivity ≥ 0.30 kUA/L to at least one core-4 nsLTP molecule. High-confidence predominant profiles were identified using a molecule-specific log-ratio enrichment approach requiring target IgE ≥ 0.30 kUA/L and target contribution $\geq 50\%$ of the total core-4 IgE signal. The 50 patients with the highest enrichment score were selected for each molecule, yielding four balanced predominant groups and a final analytical sample of 200 patients.

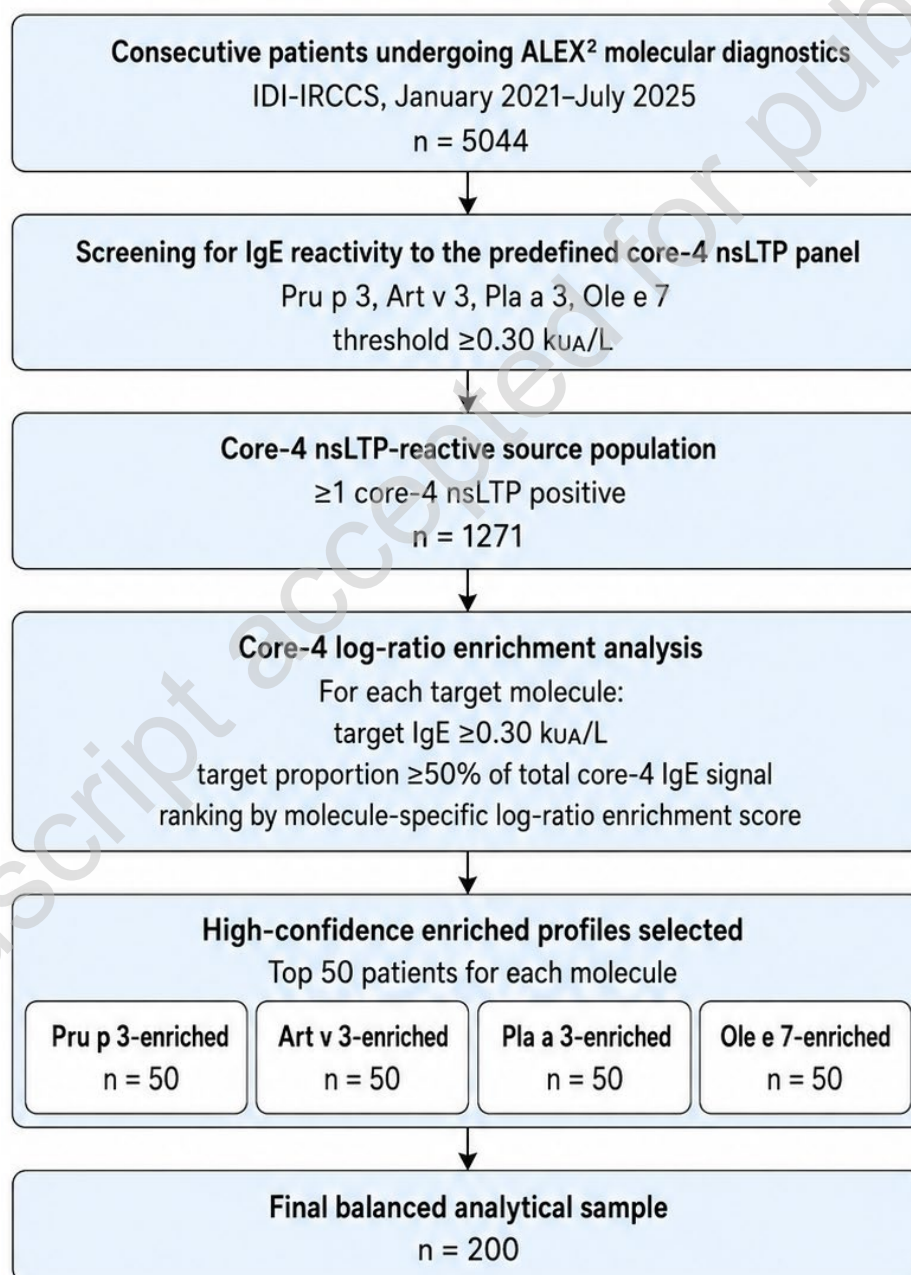


Figure 2 | Clinical presentation subsets across high-confidence predominant molecular profiles.

Stacked bar plot showing the distribution of neither, food-only, respiratory-only and mixed clinical presentations within each predominant core-4 molecular profile.

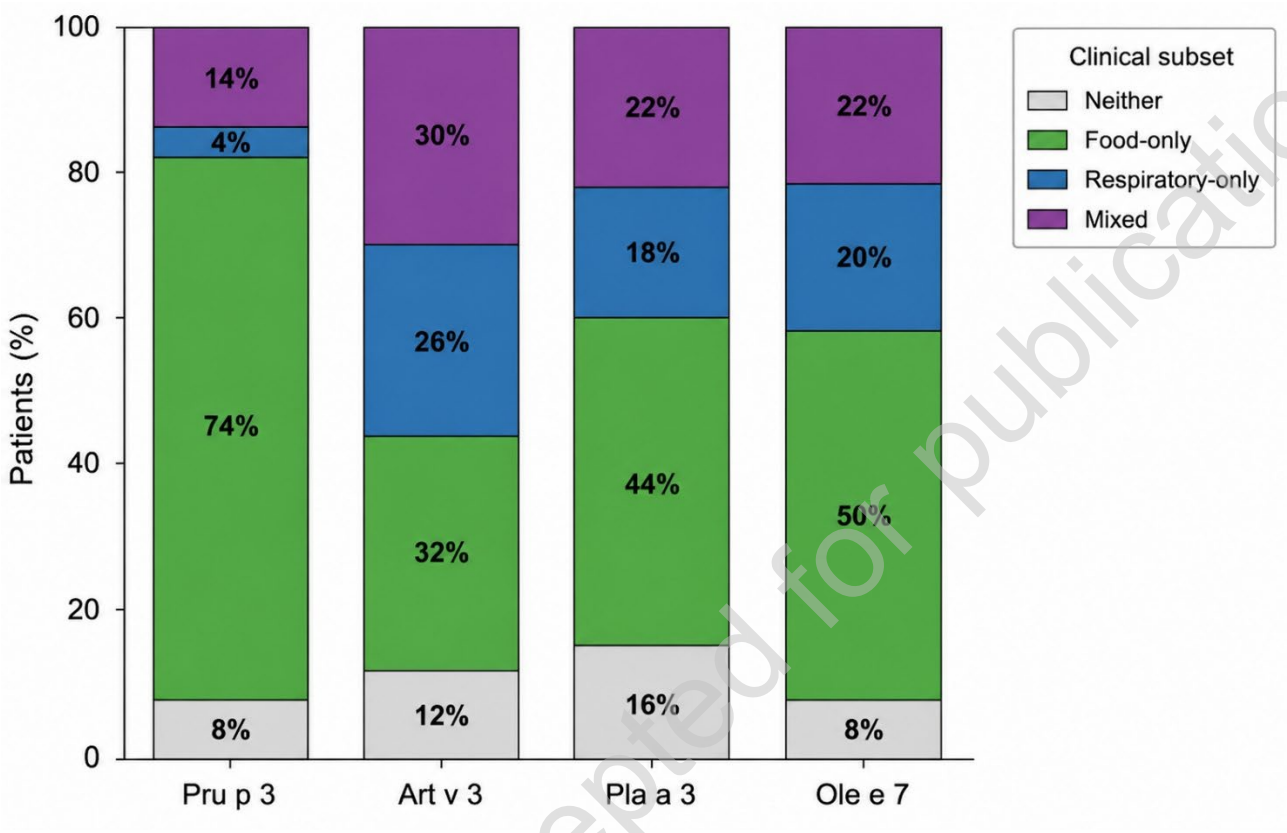


Figure 3 | Core-4 nsLTP IgE distributions across predominant molecular profiles.

Box-and-dot plots showing IgE levels to Pru p 3, Art v 3, Pla a 3 and Ole e 7 across the four high-confidence predominant profiles. IgE values are displayed on a logarithmic scale.

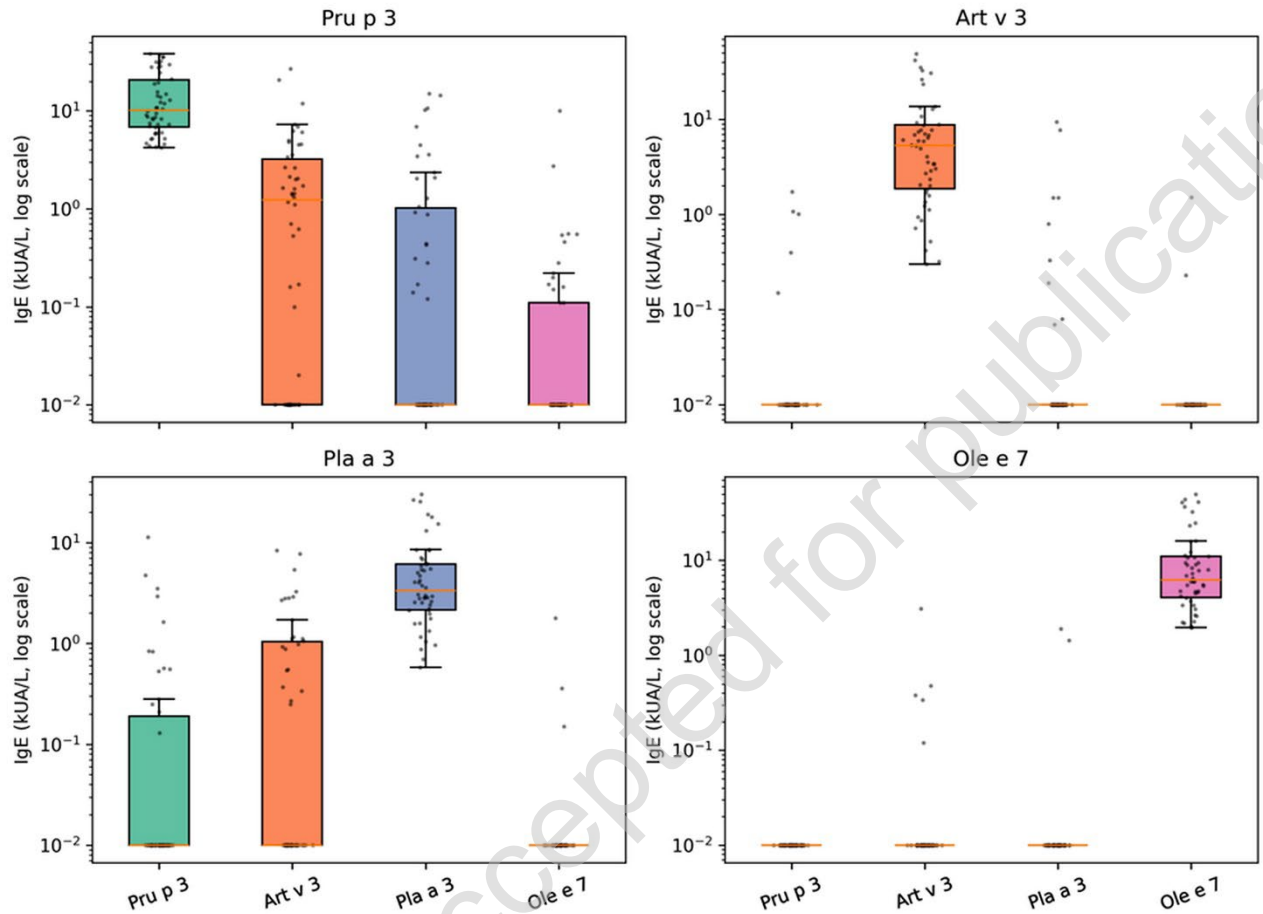


Figure 4 | Serum-pool inhibition patterns across core-4 nsLTP predominant profiles and mixed pool

Heatmap showing percentage inhibition of IgE reactivity after overnight incubation of predominant serum pools with the indicated inhibitors. Columns indicate inhibitors and rows the re-tested molecular signals. Four predominant pools (Ole e 7, Art v 3, Pla a 3 and Pru p 3; n = 5 each) and one mixed pool (n = 20) were analysed. Values represent percentage inhibition relative to the non-inhibited baseline. *Platanus extract was used as surrogate inhibitor for Pla a 3; Par j 2 was included as an external LTP control.

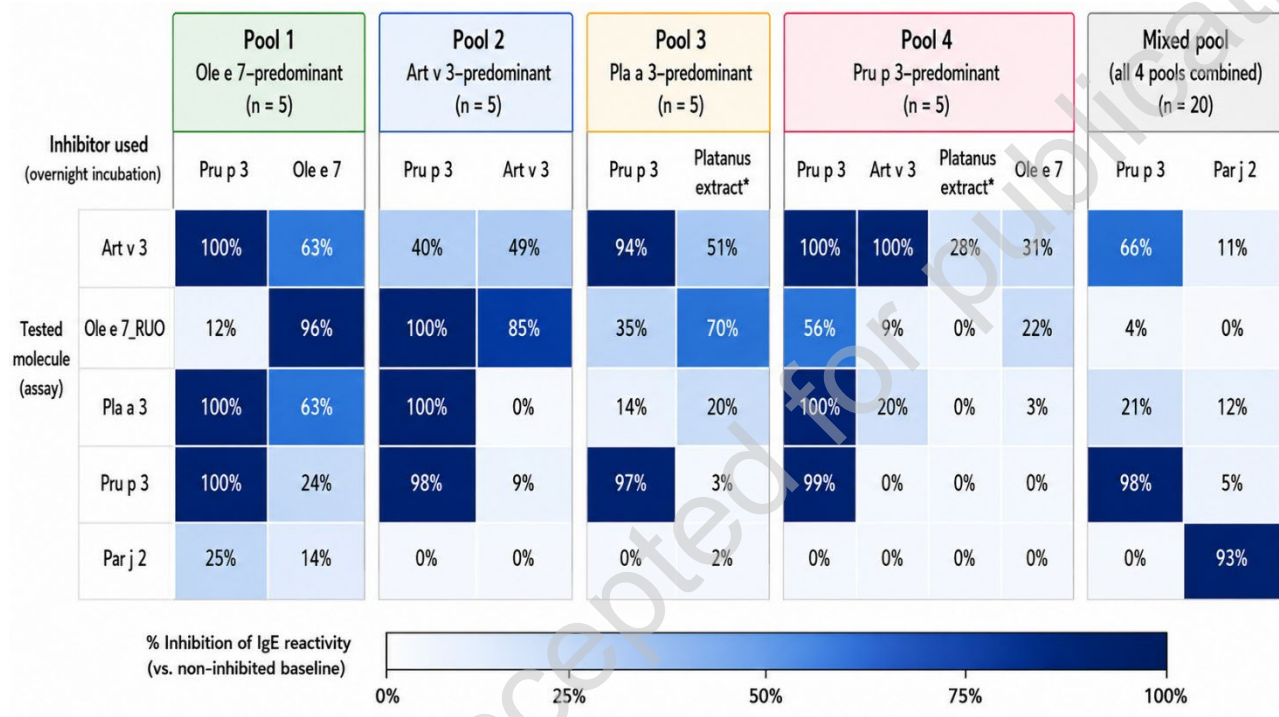


Figure 5 | Adjusted odds ratios

Adjusted associations between predominant molecular profiles and clinical outcomes.

Forest plot showing adjusted odds ratios for food allergy and respiratory symptoms. Models were adjusted for age, sex, PR-10 co-sensitisation and profilin co-sensitisation. The Pru p 3-predominant group was used as the reference category.

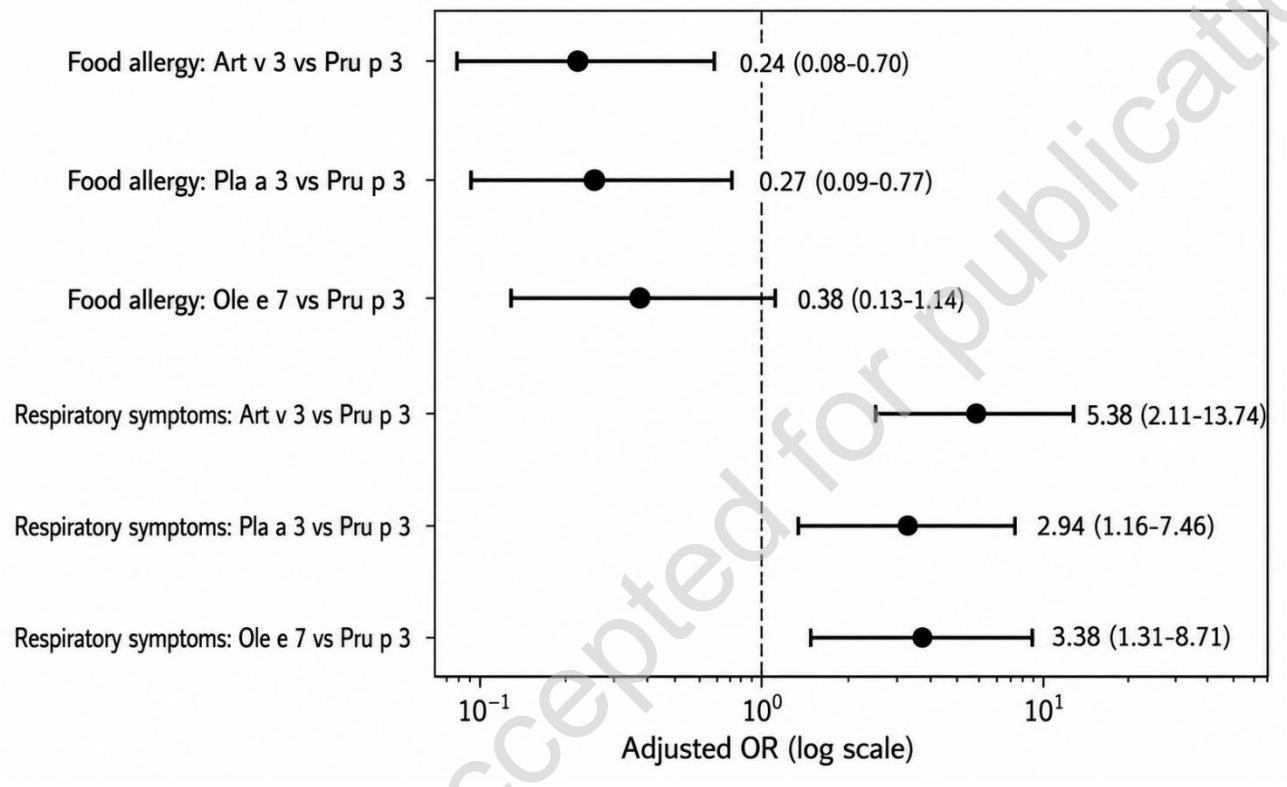


Figure S1 | PCA of the core-4 nsLTP IgE continuum

PCA was performed on log10-transformed and standardised IgE values to Pru p 3, Art v 3, Pla a 3 and Ole e 7 in the full core-4-reactive population. Points indicate the overall core-4-reactive population; overlaid points indicate the 200 high-confidence predominant profiles selected by proportional contribution and log-ratio enrichment. The first two principal components explained 78.7% of the total variance.

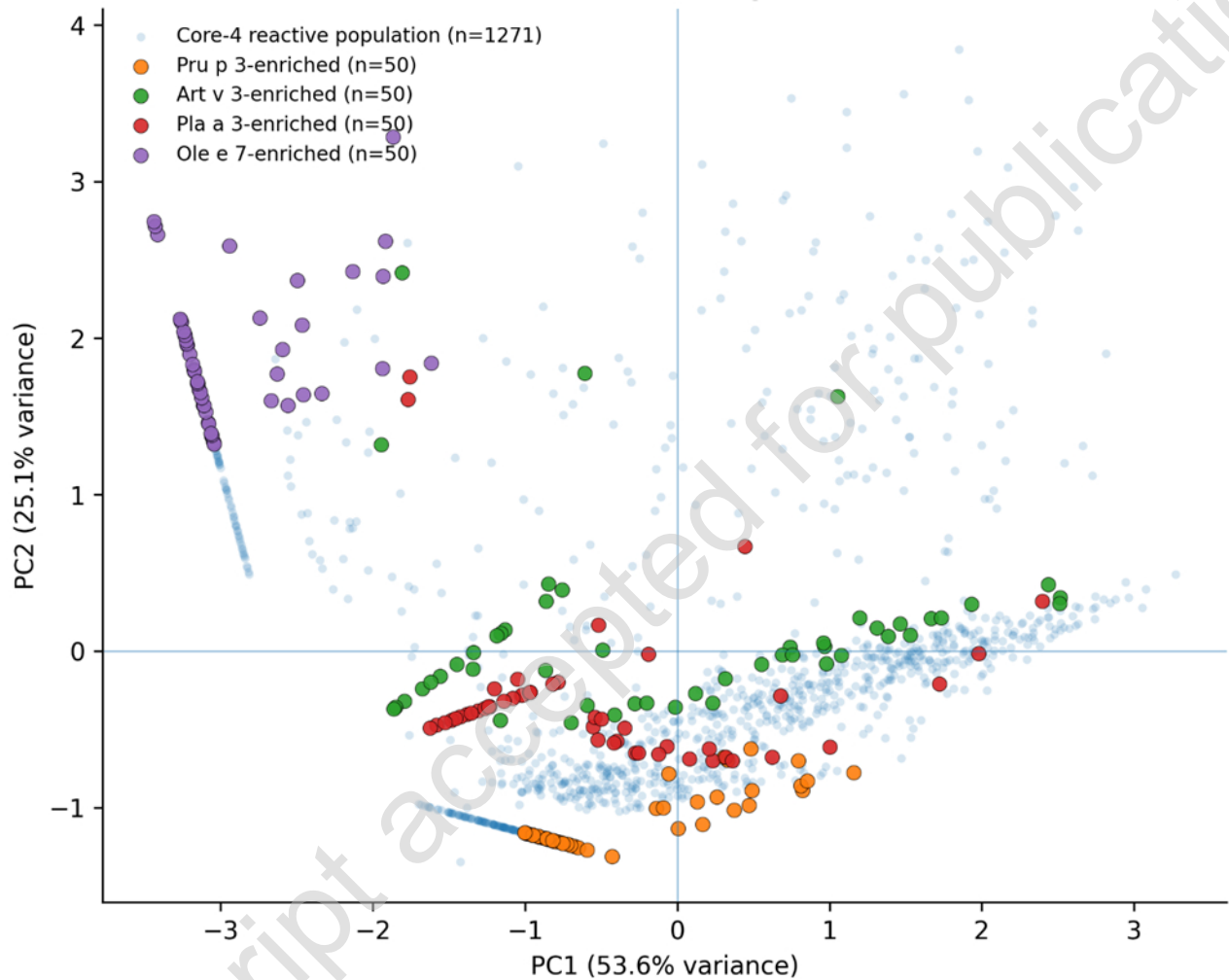


Figure S2 | Median relative contribution of each core-4 nsLTP molecule.

Heatmap showing the median proportional contribution of Pru p 3, Art v 3, Pla a 3 and Ole e 7 to the total core-4 IgE signal within each predominant molecular profile.

