

ORIGINAL ARTICLE

Multiplex IgE reactivity without a documented corresponding phenotype: cross-sectional patterns and routine-care follow-up

Multiplex IgE without documented phenotype

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Summary

Background. Multiplex diagnostics may identify IgE reactivity without a corresponding phenotype documented at testing. This record-level discordance can reflect incomplete history, limited exposure, cross-recognition, or broader atopy and is not equivalent to asymptomatic sensitisation. We described concordance across six allergen groups and routine-care reassessment of selected Pru p 3/nsLTP and Hymenoptera findings. **Methods.** We retrospectively analyzed ALEX2 assessments, retaining one index-positive test per patient and allergen group. Pairs were classified according to whether a compatible phenotype was documented at index assessment. Follow-up of selected Pru p 3/nsLTP and Hymenoptera findings were non-standardized, descriptive, and restricted to patients retained in routine care by July 2026. **Results.** Among 3,636 patients, 1,241 were positive to at least one group, yielding 1,698 pairs: 667 (39.3%) had a documented corresponding phenotype and 1,031 (60.7%) did not. Atopic dermatitis did not increase record-level discordance compared with respiratory atopy without atopic dermatitis. Among 316 Pru p 3-positive patients without a documented phenotype, 290 were retained; 219 reported a compatible post-index reaction after plant-food exposure and 71 did not, including 55 with documented tolerance. Among 204 Hymenoptera-reactive patients without a documented sting phenotype, 112 were retained; 31 reported a post-index sting-associated event and 81 reported no post-index sting. **Conclusions.** In this tertiary-

care cohort, absence of a documented corresponding phenotype was frequent. Routine-care reassessment yielded heterogeneous exposure-based outcomes but cannot estimate asymptomatic sensitisation, clinical penetrance, or future risk. The findings support clinical verification rather than automatic dismissal or allergy labelling.

IMPACT STATEMENT

Multiplex IgE testing frequently identifies reactivities that are not matched by a corresponding phenotype in routine clinical records. This study shows that such findings should be interpreted as record-level phenotype discordance rather than as confirmed asymptomatic sensitisation or established allergy. Structured reassessment of exposure, tolerance, cofactors and subsequent clinical events may help distinguish clinically relevant sensitisation from incidental molecular recognition while avoiding unnecessary allergy labelling.

KEY WORDS

Atopic dermatitis; food allergy; longitudinal follow-up; molecular allergology; multiplex testing; sensitization.

1 | INTRODUCTION

Multiplex molecular diagnostics map broad IgE repertoires, distinguish genuine sensitisation from cross-reactivity, and identify uncommon recognition profiles (1-7). Their breadth also reveals molecular positivity for which no corresponding reaction phenotype is documented at the time of testing, across settings that include food, cross-reactive, nsLTP, alpha-gal, and Hymenoptera sensitisation (8-16). This record-level discordance may reflect incomplete clinical documentation, limited or absent exposure, avoidance, cross-recognition, broad atopy, or a compatible phenotype that was not captured in the available record.

A molecular result extending beyond the presenting phenotype may be dismissed as incidental or translated prematurely into an allergy diagnosis. Neither interpretation is justified by IgE positivity alone. Clinical relevance requires reassessment of history, exposure, avoidance, tolerated ingestion, and cofactors (6,7,12-16). This is particularly important for nsLTPs, whose real-life exposure varies with cultivar, ripening, food portion, matrix, processing, exercise, NSAIDs, and alcohol, and for Hymenoptera, for which an informative re-sting may not occur for years (14-16,29-34,36-38,40).

Sensitisation and clinical allergy are not interchangeable: specific IgE indicates immunological recognition but does not establish symptoms on exposure, while absence of a documented phenotype does not establish tolerance or asymptomatic sensitisation (6,7,12-16,34,36-38). Atopic dermatitis (AD) adds further complexity because barrier dysfunction and type 2 inflammation may broaden IgE recognition while the recorded phenotype remains predominantly cutaneous (17-28,39).

We evaluated six prespecified allergen groups - food-associated nsLTPs, seed-storage proteins, arginine kinases, tropomyosins, parvalbumins, and Hymenoptera components - in a tertiary-care cohort. We described phenotype documentation at index assessment, related atopic and molecular contexts, and routine-care follow-up of selected Pru p 3/nsLTP and Hymenoptera findings. The study was not designed to estimate asymptomatic sensitisation or prospective risk.

2 | METHODS

2.1 | Study design and data sources

This single-centre retrospective observational study comprised a cross-sectional classification module and a routine-care follow-up module at the Allergy Unit of Fondazione Luigi Maria Monti, IDI-IRCCS, Rome. All routine-care ALEX2 assessments from 8 July 2020 to 27 June 2026 were screened and linked to structured clinical fields and records. The cohort represents patients selected for multiplex testing in a tertiary referral setting; analyses were not stratified by testing indication and were not intended to represent an unselected population. The study flow is shown in Supplementary Figure S1.

Each positive patient-group pair was classified at the index assessment as having a documented corresponding phenotype or lacking a documented corresponding phenotype. For brevity, the latter operational category is referred to as record-level non-concordance. Food pairs in this category were then stratified by AD status; Hymenoptera classification remained AD-independent. The longitudinal module focused on two selected signals considered relevant to routine counselling: Pru p 3-positive nsLTP reactivity and Hymenoptera venom-component reactivity. Post-index analyses included patients retained in routine care by July 2026 and were descriptive.

Age was unavailable for 6/3,636 patients and 2/1,698 positive patient-group observations; sex and structured phenotype codes were complete. Classification was based on the information available in structured phenotype fields and linked clinical records at the index assessment. A pair was classified as having a documented corresponding phenotype only when a compatible phenotype was recorded. When the relevant phenotype information was present but no compatible phenotype was documented, the pair was classified as coded absence; missing phenotype information was handled separately. Thus, coded absence was an operational record-level category and did not establish systematic baseline questioning, relevant exposure, explicit symptom denial, or tolerance.

2.2 | Allergen-group definitions and molecular variables

The primary food-associated nsLTP model included assignable food nsLTPs and excluded Par j 2, Art v 3, Pla a 3, and Ole e 7. This operational restriction defined the analytical scope and should not be interpreted as evidence that pollen-associated nsLTPs lack clinical relevance; it may underestimate broader nsLTP recognition. Seed-storage proteins comprised 2S albumins, 7S vicilins, and 11S legumins. Arginine kinase, tropomyosin, and

parvalbumin models included all assignable components; the Hymenoptera model comprised Api m 1, Api m 10, Pol d 5, Ves v 1, and Ves v 5 (Supplementary Table S3).

Specific IgE >0.3 kUA/L defined positivity. For each patient and group, the earliest positive assessment was retained; same-day duplicates were collapsed using the maximum component value and the union of positive components. Analyses used patients for overall cohort proportions and patient-group pairs for group-specific outcomes.

Human-homologous exogenous molecular allergens (HEMAs) were defined as exogenous allergen molecules sharing at least 25% amino-acid sequence similarity with a human homolog; HEMA reactivity required positivity to at least one of the 48 molecules included in this predefined panel (27). Restricted food-associated nsLTP recognition was defined a priori as fewer than five positive components within the primary nsLTP model, and PR-10/profilin co-recognition as positivity to either family. Edible-insect extract reactivity was assessed exploratorily among record-level non-concordant Hymenoptera-reactive patients. The HEMA panel is listed in Supplementary Table S2.

2.3 | Phenotype concordance and secondary AD context

Food-group concordance required a compatible immediate reaction history documented at the index assessment: plant-food allergy for food-associated nsLTPs and seed-storage proteins, shellfish or mollusc allergy for arginine kinases and tropomyosins, and fish allergy for parvalbumins. AD was coded as absent (0), previous/mild (1), moderate (2), or severe (3); codes 1-3 defined AD present. This was a history/status variable rather than an active-disease-only definition: the available coding did not include a separate measure of AD activity at the index assessment and did not permit childhood-only AD to be isolated as a distinct subgroup.

Food results without a documented corresponding immediate reaction history were classified as record-level non-concordant and secondarily stratified by AD. This classification did not imply food-triggered dermatitis, delayed food allergy, asymptomatic sensitisation, or a causal explanation for the absence of a documented phenotype. Hymenoptera concordance depended only on a documented compatible sting reaction, in keeping with venom-allergy diagnostic principles (36-38).

Record-level non-concordance therefore denoted absence of a corresponding phenotype in the available routine-care record. It did not denote verified exposure, systematic baseline questioning, explicit symptom denial, challenge-confirmed tolerance, or biological irrelevance.

2.4 | Routine-care longitudinal follow-up

Patients with record-level non-concordant Pru p 3 or Hymenoptera reactivity were reassessed during routine specialist care, generally at six- or 12-month intervals, with direct access to the referral specialist for interim events. Follow-up duration varied with the index date and was not protocol-defined. Records captured post-index exposure or sting, compatible events, trigger, timing, severity, cofactors, treatment, documented tolerance,

avoidance or no exposure, and final clinical adjudication. Follow-up was not a research-mandated exposure protocol and included no standardised food or sting challenge. Only events documented as occurring after the index test were counted as post-index reactions.

For nsLTPs, routine adjudication considered temporal association with ingestion, symptom pattern, the implicated food, cofactors, and alternative explanations. Exposure without reaction was distinguished from explicit documented tolerance, which did not constitute a standardised challenge. Outcomes were classified as absent, mild, moderate, or severe. Adjudication was routine, unblinded, and not independently duplicated. For Hymenoptera, follow-up distinguished reported absence of a post-index sting from a reported sting-associated large local or systemic reaction. Absence of a reported re-sting was not interpreted as tolerance, and unreported asymptomatic stings cannot be excluded.

2.5 | Statistical analysis

Analyses used Python 3.13.5 and statsmodels 0.14.6. Categorical data are counts and percentages with exact two-sided Clopper-Pearson 95% CIs; continuous data are medians, IQRs, and ranges. Group-specific analyses used patient-group pairs. AD proportions across food groups were descriptive because patients could contribute to multiple groups.

All models fitted using generalised estimating equations (GEE) used a binomial distribution, logit link, independence working correlation, robust sandwich standard errors, and patient clustering. Age was scaled per 10 years, sex was male versus female, and allergen group was categorical. Pair-level models examined record-level non-concordance in relation to AD, adjusted for age, sex, and allergen group, across all six groups and within food groups. A food-group sensitivity GEE compared AD-positive patients with AD-negative patients with respiratory atopy, defined as rhinitis and/or asthma, using record-level non-concordance as the binary outcome.

Among food-group-positive patients without a documented corresponding phenotype, patient-level logistic regression examined HEMA reactivity in relation to AD, adjusted for age and sex, and additionally for the number of positive food groups. A pair-level sensitivity GEE adjusted for age, sex, and allergen group. Restricted food-associated nsLTP recognition was compared across nsLTP clinical strata using chi-square tests.

Family-maximum specific IgE was the highest component value within each group and represented family-level recognition intensity. When total IgE was available, the specific-to-total ratio was $100 \times \text{family-maximum specific IgE} / \text{total IgE}$. Pru p 3-specific IgE and its ratio were also evaluated. Ratios were exploratory analytical descriptors, not biological IgE fractions or validated diagnostic indices. One ratio exceeded 100%; exclusion of this value did not alter conclusions.

Skewed quantitative variables were compared using Mann-Whitney or Kruskal-Wallis tests. Benjamini-Hochberg FDR correction was applied across six groups separately for family-maximum specific IgE, total IgE, and their ratio; three-stratum food analyses used Kruskal-Wallis tests followed by prespecified AD-present versus AD-absent Mann-Whitney contrasts, with global and pairwise FDR correction across five food groups. Five residual-

nsLTP quantitative comparisons were likewise FDR-adjusted. Total-IgE analyses used available cases without imputation.

Adjusted analyses used complete cases; two pairs with missing age were excluded. No a priori sample-size calculation was performed. Unless an FDR-adjusted q value is reported, P values are two-sided and nominal. GEE and HEMA models were not multiplicity-adjusted. Follow-up analyses were descriptive, restricted to patients retained in care, and did not estimate incidence, clinical penetrance, diagnostic accuracy, or prospective risk.

3 | RESULTS

3.1 | Analytical cohort and allergen-group frequency

Of 3,720 assessments, 18 were excluded for missing, implausible, or out-of-period dates, leaving 3,702 assessments from 3,636 patients. Median age was 33 years (IQR 21-50; range 0-95; n=3,630), 2,319 (63.8%) were female, and 58 (1.6%) had repeated assessments (Supplementary Table S1).

At least one prespecified group was positive in 1,241 patients (34.1%; 95% CI 32.6-35.7), yielding 1,698 patient-group pairs. Within this selected multiplex-tested cohort, positivity was most frequent for food-associated nsLTPs (730; 20.1%), followed by seed-storage proteins (290; 8.0%), Hymenoptera (279; 7.7%), tropomyosins (155; 4.3%), arginine kinases (144; 4.0%), and parvalbumins (100; 2.8%) (Table 1). Within each allergen group, phenotype-classification categories were mutually exclusive; allergen-group positivity was not mutually exclusive across patients, who could contribute to more than one group.

3.2 | Documented phenotype concordance and record-level discordance

Among 1,698 positive patient-group pairs, 667 (39.3%) had a documented corresponding phenotype and 1,031 (60.7%) did not: 458 food pairs occurred in patients with AD, 369 food pairs in patients without AD, and 204 Hymenoptera pairs were classified independently of AD (Table 1; Figure 1A). The 60.7% proportion describes the available index record and does not represent verified asymptomatic sensitisation or tolerance.

Among food-group pairs without a documented phenotype, the AD-absent proportion ranged from 21.0% for seed-storage proteins to 36.1% for tropomyosins. Among Hymenoptera-reactive patients, 204/279 (73.1%; 95% CI 67.5-78.2) lacked a documented corresponding sting history; 92/204 (45.1%) had AD. Edible-insect extract reactivity occurred in 38/204 (18.6%; 95% CI 13.5-24.7), including 28 reactive to at least two extracts and 18 to all three.

3.3 | AD and molecular context without a documented food phenotype

Among 637 food-sensitised patients without a documented corresponding immediate reaction history, 334 (52.4%) had AD and 303 (47.6%) did not (Table 2; Figure 2A). At pair level, AD was present in 50.5%-65.2% across food groups (Table 2; Figure 2B).

Of 382 record-level non-concordant food-associated nsLTP cases, 193 had AD and 189 did not. Among AD-absent cases, 88 (46.6%) had restricted recognition, 73 (38.6%) had PR-10/profilin co-recognition, and 27 had both; 55 (29.1%) had neither and formed an operational residual subgroup (Table 1; Figure 1B). Restricted recognition did not differ significantly across clinical strata ($P=0.202$). Because a non-significant result does not establish equivalence, this exploratory finding was not used to define groups; restricted recognition was retained only as an individual interpretative context. The residual subgroup was exploratory and was not considered a naturally occurring endotype.

In exploratory analyses, HEMA reactivity was more frequent with than without AD (228/334, 68.3% vs 177/303, 58.4%; Figure 2C). The association persisted after age- and sex-adjustment (OR 1.46, 95% CI 1.05-2.04; $P=0.025$) and pair-level adjustment (OR 1.48, 95% CI 1.02-2.15; $P=0.039$), but attenuated after accounting for the number of positive food groups (OR 1.34, 95% CI 0.95-1.88; $P=0.093$; Supplementary Table S4). These cross-sectional HEMA analyses were exploratory and were not interpreted as evidence of causality.

AD did not increase record-level non-concordance: adjusted ORs were 0.78 (95% CI 0.62-0.99; $P=0.039$) across all groups and 0.79 (95% CI 0.60-1.02; $P=0.070$) within food groups. Compared specifically with respiratory atopy without AD, the adjusted OR was 0.82 (95% CI 0.60-1.14; $P=0.236$). Thus, AD was an interpretative context rather than a determinant of absent phenotype documentation.

3.4 | Exploratory quantitative IgE context

Total IgE was available for 1,562/1,698 pairs (92.0%). Family-maximum specific IgE largely overlapped between pairs with and without a documented corresponding phenotype; after FDR correction, higher values in phenotype-concordant pairs remained only for tropomyosins and Hymenoptera. Exploratory specific-to-total IgE ratios were higher in phenotype-concordant pairs for nsLTPs (3.57% vs 1.67%), seed-storage proteins (0.85% vs 0.31%), and tropomyosins (1.18% vs 0.66%; Supplementary Table S5). No diagnostic threshold or incremental clinical value was evaluated.

In exploratory comparisons among food pairs without a documented corresponding phenotype, AD was associated mainly with higher total IgE. For nsLTPs, family-maximum specific IgE was similar by AD status, whereas total IgE was higher and the specific-to-total ratio lower with AD (0.99% vs 2.33%; FDR $q<0.001$). Total IgE was also higher with AD for seed-storage proteins, arginine kinases, and tropomyosins, but only the nsLTP ratio differed between AD strata after FDR correction.

The exploratory residual 55-patient nsLTP subgroup had higher family-maximum specific IgE (8.95 vs 4.56 kUA/L; $q=0.002$), Pru p 3-specific IgE (7.14 vs 3.31 kUA/L; $q=0.001$), and Pru p 3-specific-to-total ratio (6.71% vs 2.54%; $q=0.001$) than phenotype-concordant cases, while total IgE was similar (108.5 vs 133 kU/L; $P=0.140$). Pru p 3 was positive in all 55. Because recognition of at least five food-associated nsLTP components partly defined this subgroup, these comparisons are structurally dependent on the selection criteria and should be interpreted descriptively, not as independent predictors.

3.5 | Descriptive routine-care reassessment of Pru p 3 and Hymenoptera findings

Among 316 Pru p 3-positive patients without a documented corresponding phenotype, 290 (91.8%; 95% CI 88.2-94.6) were retained in routine care. All had documented post-index plant-food exposure. A compatible reaction was reported by 219/290 (75.5%; 95% CI 70.1-80.4), whereas 71 had exposure without a compatible reaction. Of these, 55 had explicit documented tolerance and 16 had insufficient information to classify sustained tolerance. Routine adjudication was mild in 96 cases, moderate in 99, and severe in 24; adrenaline was used in 21 severe reactions. Cofactors were mainly post-prandial exercise (n=31) or NSAID-associated reactions (n=18) (Supplementary Table S6).

Within the exploratory residual 55-patient AD-absent nsLTP subgroup, 52 (94.5%; 95% CI 84.9-98.9) were retained and three were lost. All 52 had post-index plant-food exposure; 45 (86.5%; 95% CI 74.2-94.4) reported a compatible reaction and seven tolerated exposure. Adjudication was absent in seven, mild in 14, moderate in 25, and severe in six; all six severe cases required adrenaline. These findings are descriptive within a molecularly selected subgroup (Table 3).

Among 204 Hymenoptera-reactive patients without a documented sting phenotype, 112 (54.9%; 95% CI 47.8-61.9) were retained. Thirty-one reported a post-index sting-associated event and 81 reported no post-index sting. Events comprised 18 large local and 13 systemic reactions; adrenaline was used in three cases. Because retention was incomplete, exposure was not standardised, and asymptomatic stings may be under-reported, these data do not estimate reaction probability after re-sting (Table 3).

4 | DISCUSSION

4.1 | Main findings

In this selected tertiary-care cohort, 60.7% of positive patient-group pairs lacked a documented corresponding phenotype at index assessment. This is a measure of record-level discordance, not the prevalence of asymptomatic sensitisation, tolerance, or clinically irrelevant IgE recognition. AD was common in this category but did not increase discordance relative to respiratory atopy without AD; it was associated mainly with broader sensitisation and higher total IgE.

Quantitative IgE findings differed across groups. Family-maximum specific IgE differed by documented phenotype only for tropomyosins and Hymenoptera after FDR correction, whereas exploratory specific-to-total ratios differed for nsLTPs, seed-storage proteins, and tropomyosins. No thresholds or incremental clinical value were assessed. The residual nsLTP subgroup showed that absent phenotype documentation was not confined to low-level IgE. However, because this subgroup was partly defined by broad nsLTP recognition (at least five positive food-associated nsLTP components), comparisons of nsLTP IgE measures with phenotype-concordant cases were structurally conditioned by the selection criterion and cannot be interpreted as independent evidence of stronger sensitisation.

Routine-care reassessment documented both compatible post-index events and exposure without compatible symptoms among Pru p 3-positive patients. Most retained Hymenoptera-reactive patients reported no subsequent sting, while 31 reported sting-associated events. These observations illustrate heterogeneous outcomes but cannot establish clinical penetrance or risk because follow-up was non-standardised, depended on retention and patient reporting, and may preferentially capture symptomatic exposures. The 31/31 proportion among reported stings must not be interpreted as a universal reaction rate.

4.2 | AD as a clinical context

AD may broaden IgE recognition through barrier dysfunction and type 2 inflammation without establishing immediate food allergy or food-triggered eczema (17-28,39). The higher prevalence of HEMA reactivity among patients with AD was an exploratory cross-sectional finding. HEMA enrichment weakened after adjustment for the number of positive food groups, consistent with a broader sensitisation burden, although these data cannot distinguish confounding from mediation or establish causality. AD should therefore modify interpretation, not trigger elimination based on IgE positivity alone (18,19,23).

4.3 | Allergen-group-specific interpretation and post-test reassessment

Restricted food-associated nsLTP recognition and PR-10/profilin co-recognition overlapped among AD-absent patients without a documented phenotype. The residual subgroup had higher IgE measures than phenotype-concordant cases, partly because it was defined by broader food-associated nsLTP recognition. Exposure, avoidance, cofactors, and challenge outcomes remain central to assessment. Even oral food challenge may not reproduce real-life nsLTP exposure, which varies with cultivar, ripening, food portion, matrix, processing, and cofactors (29-34,40).

The nsLTP model was deliberately restricted to food-associated components. Art v 3, Pla a 3, and Ole e 7 may participate in food-pollen cross-recognition, while Par j 2 represents another pollen-associated LTP context. Their exclusion defines the scope of this analysis and may underestimate nsLTP recognition; expanded definitions should be examined in future sensitivity analyses.

Among retained Pru p 3-positive patients, 219/290 reported compatible post-index events, whereas 71 had exposure without compatible symptoms and 55 had explicit tolerance. The exploratory residual subgroup showed the same heterogeneity. These findings support exposure-based reassessment but cannot demonstrate that initially asymptomatic sensitisation later became clinically expressed, because baseline exposure and questioning were not standardised.

For Hymenoptera, absent phenotype documentation often reflects an uninformative sting history (14-16,36-38). Among retained patients, 81/112 reported no post-index sting and 31 reported a sting-associated event, including 18 large local and 13 systemic reactions. Re-sting is episodic, and tolerated or minimally symptomatic stings may

be under-reported. These data therefore describe reported events and cannot estimate reaction probability. Edible-insect co-reactivity provided molecular context but did not establish the source or relevance of venom reactivity.

The proportion of positive pairs without a documented corresponding phenotype was also substantial for seed-storage proteins, arginine kinases, tropomyosins, and parvalbumins. This should not be interpreted as low or high clinical penetrance because systematic exposure, tolerance, and challenge outcomes were not available. Only tropomyosin family-maximum IgE differed quantitatively after FDR correction.

4.4 | Clinical implications

IgE reactivity without a documented phenotype should prompt verification of the baseline history, clarification of exposure, avoidance, tolerance, and cofactors, and consideration of AD and co-recognition. Figure 1B provides the nsLTP-specific interpretative branch, while Figure 3 summarises the overall routine-care reassessment framework without equating sensitisation with allergy. Specific-to-total ratios showed exploratory group-level differences, but no validated diagnostic threshold or incremental value was derived (6,7,18,19,23,34,36-38).

A proportionate sequence is appropriate: verify baseline documentation; establish whether relevant exposure was tolerated, avoided, or symptomatic; consider AD, broad IgE responsiveness, co-recognition, nsLTP spreading, and cofactors; and maintain follow-up when the result is potentially actionable. Tolerated exposure argues against unnecessary restriction, whereas an uninformative exposure history warrants verification rather than reassurance or diagnosis based on IgE alone.

4.5 | Strengths and limitations

Methodological strengths include reconstruction of the earliest positive test, explicit patient- and pair-level denominators, a prespecified record-level definition of phenotype concordance, secondary AD contextualisation, and a routine-care reassessment module for two selected molecular signals. The study also distinguishes reported absence of re-exposure from documented tolerated exposure and avoids interpreting no Hymenoptera re-sting as tolerance.

The principal limitations are the retrospective, single-centre design and selected tertiary-care population. Testing indication was not incorporated into the analyses, creating referral and spectrum bias. Index classification relied on routine records rather than standardised baseline questioning and cannot establish adequate exposure, explicit symptom denial, asymptomatic sensitisation, or tolerance. Exposures and reactions were self-reported and heterogeneous; adjudication was unblinded and not independently duplicated. Oral food challenges were not systematically available, and live-insect sting challenge was not performed. Although only post-index events were counted, recall and temporal misclassification remain possible.

Follow-up duration was variable and analyses included only patients retained in care by July 2026. Attrition was limited for Pru p 3 but substantial for Hymenoptera, and symptom-dependent reporting may have under-recorded tolerated exposures. The food-associated nsLTP model excluded Art v 3, Pla a 3, Ole e 7, and Par j 2. AD and

residual nsLTP strata were operational, and the residual subgroup was partly defined by variables subsequently compared. GEE and HEMA analyses were not multiplicity-adjusted, whereas quantitative IgE comparisons were FDR-adjusted. Family-maximum IgE varied by component, and the specific-to-total ratio is unvalidated. Prospective multicentre studies require standardised history, exposure documentation, predefined adjudication, broader nsLTP models, and systematic follow-up of symptomatic and tolerated exposures.

In this selected tertiary-care cohort, multiplex IgE reactivity frequently lacked a corresponding phenotype in the index record. This should establish neither clinical allergy nor asymptomatic sensitisation. Routine-care reassessment of retained Pru p 3-positive and Hymenoptera-reactive patients documented compatible post-index events, exposure without symptoms, tolerance, and absence of re-exposure. These descriptive findings support targeted verification and proportionate follow-up but do not estimate clinical penetrance or future risk. Prospective multicentre studies should use standardised baseline questioning, explicit exposure assessment, predefined adjudication, broader nsLTP models, and systematic follow-up.

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Potential conflict of interest

ES has received consultancy fees or participated in speakers' bureaus for Diesse, Dasit, MADx, and Thermo Fisher Scientific. The remaining authors declare no relevant conflicts of interest.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, was approved by the IDI-IRCCS Ethics Committee (approval IDI-IRCCS CE | 493/1), and written informed consent was obtained from all participants.

Data Availability Statement

Data supporting the findings are available from the corresponding author on reasonable request, subject to privacy and ethical restrictions.

Author Contributions

Conceptualization: ES; data curation: ES, MG, ECG, ML, GM, LP, AZ, DQ, EC, and GC; formal analysis: ES; investigation: ES, MG, ECG, ML, GM, LP, EC, and GC; methodology: ES; project administration: ES; supervision: ES, LC, DV, and RA; validation: ES, LC, DV, and RA; visualization: ES; writing—original draft: ES; writing—review and editing: all authors. All authors approved the final manuscript.

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Table 1. Documented phenotype concordance and record-level absence of a corresponding phenotype across six allergen-group models.

Allergen group	Positive	Documented phenotype, n (%)	No documented phenotype, AD present, n (%)	No documented phenotype, AD absent, n (%; 95% CI)	No documented sting phenotype, AD-independent, n (%; 95% CI)
nsLTP	730	348 (47.7%)	193 (26.4%)	189 (25.9%; 22.7-29.2)	-
Seed-storage proteins	290	138 (47.6%)	91 (31.4%)	61 (21.0%; 16.5-26.2)	-
Arginine kinase	144	39 (27.1%)	66 (45.8%)	39 (27.1%; 20.0-35.1)	-
Tropomyosin	155	36 (23.2%)	63 (40.6%)	56 (36.1%; 28.6-44.2)	-
Parvalbumins	100	31 (31.0%)	45 (45.0%)	24 (24.0%; 16.0-33.6)	-
Hymenoptera	279	75 (26.9%)	-	-	204 (73.1%; 67.5-78.2)

Categories were mutually exclusive within each allergen group. Food-group pairs without a documented corresponding phenotype were secondarily subdivided by AD status. Hymenoptera classification was based only on the documented sting history and remained independent of AD. Absence of a documented phenotype did not establish systematic exposure, explicit symptom denial, asymptomatic sensitisation, or tolerance. Allergen-group models were not mutually exclusive between patients.

Table 2. AD status among food-group pairs without a documented corresponding immediate reaction phenotype.

Food allergen group	Pairs without documented phenotype	AD present, n (%; 95% CI)	AD absent, n (%; 95% CI)
nsLTP	382	193 (50.5%; 45.4-55.6)	189 (49.5%; 44.4-54.6)
Seed-storage proteins	152	91 (59.9%; 51.6-67.7)	61 (40.1%; 32.3-48.4)
Arginine kinase	105	66 (62.9%; 52.9-72.1)	39 (37.1%; 27.9-47.1)
Tropomyosin	119	63 (52.9%; 43.6-62.2)	56 (47.1%; 37.8-56.4)
Parvalbumins	69	45 (65.2%; 52.8-76.3)	24 (34.8%; 23.7-47.2)

Percentages and exact 95% confidence intervals are descriptive. Formal contingency-table comparisons were not performed because patients could contribute to more than one food allergen group. Absence of a documented phenotype refers to the index routine-care record and does not imply verified tolerance.

Table 3. Descriptive routine-care reassessment of Pru p 3-positive nsLTP and Hymenoptera reactivity without a documented corresponding phenotype.

Variable	Pru p 3-positive without documented phenotype	Hymenoptera-reactive without documented phenotype
Index denominator	316 Pru p 3-positive patients without a documented corresponding phenotype	204 Hymenoptera-reactive patients without a documented corresponding sting phenotype
Not lost to routine follow-up by July 2026	290/316 (91.8%; 95% CI 88.2-94.6)	112/204 (54.9%; 95% CI 47.8-61.9)
Post-test exposure or sting among followed	290/290 (100.0%; 95% CI 98.7-100.0)	31/112 (27.7%; 95% CI 19.6-36.9)
Compatible post-index reaction/event among followed	219/290 (75.5%; 95% CI 70.1-80.4)	31/112 (27.7%; 95% CI 19.6-36.9)
Compatible post-index reaction/event among exposed/stung	219/290 (75.5%; 95% CI 70.1-80.4)	31/31 (100.0%; 95% CI 88.8-100.0)
Final severity/adjudication	Absent 71; mild 96; moderate 99; severe 24	No post-test sting 81; large local reaction 18; systemic reaction 13
Residual nsLTP subgroup	55 patients: 52 followed, 3 lost; among followed, 45 reactions, 7 tolerated exposure; severity 7 absent, 14 mild, 25 moderate, 6 severe	Not applicable
Treatment reported for reactions	H1 antihistamine 93; corticosteroid 101; adrenaline 21; residual subgroup: adrenaline 6/6 severe cases	Corticosteroid 28; adrenaline 3
Cofactor context	FDEIA 31; NSAID-associated 18; alcohol/unspecified 4; residual subgroup: FDEIA 7, NSAID-associated 6, unspecified 1	None recorded

Follow-up analyses included patients retained in routine care by July 2026 and are based on reported post-index exposures and events. Percentages should not be interpreted as incidence, clinical penetrance, diagnostic accuracy, or prospective risk in the full index population. For Hymenoptera, the 31/31 proportion among reported stings is vulnerable to symptom-dependent reporting and does not establish that every re-sting caused a reaction.

Figure 1. Clinical classification of allergen-group reactivity and food-associated nsLTP-specific interpretation. Panel A shows pairs with and without a documented corresponding phenotype as proportions of the full analytical cohort (N=3,636); patients could contribute to more than one allergen group. To avoid redundant labelling, subcategory numbers and percentages are omitted from the bars and are provided in Table 1; only total group n (%) is shown. Food pairs without a documented phenotype are stratified by AD, whereas Hymenoptera classification is AD-independent. Panel B shows the nsLTP interpretative pathway. Among 189 AD-absent cases without a documented corresponding phenotype, 88 had restricted recognition (<5 positive food-associated nsLTP components), 73 had PR-10/profilin co-recognition, 27 had both, and 55 had neither. The residual subgroup is operational and exploratory and does not establish absence or presence of clinically relevant allergy.

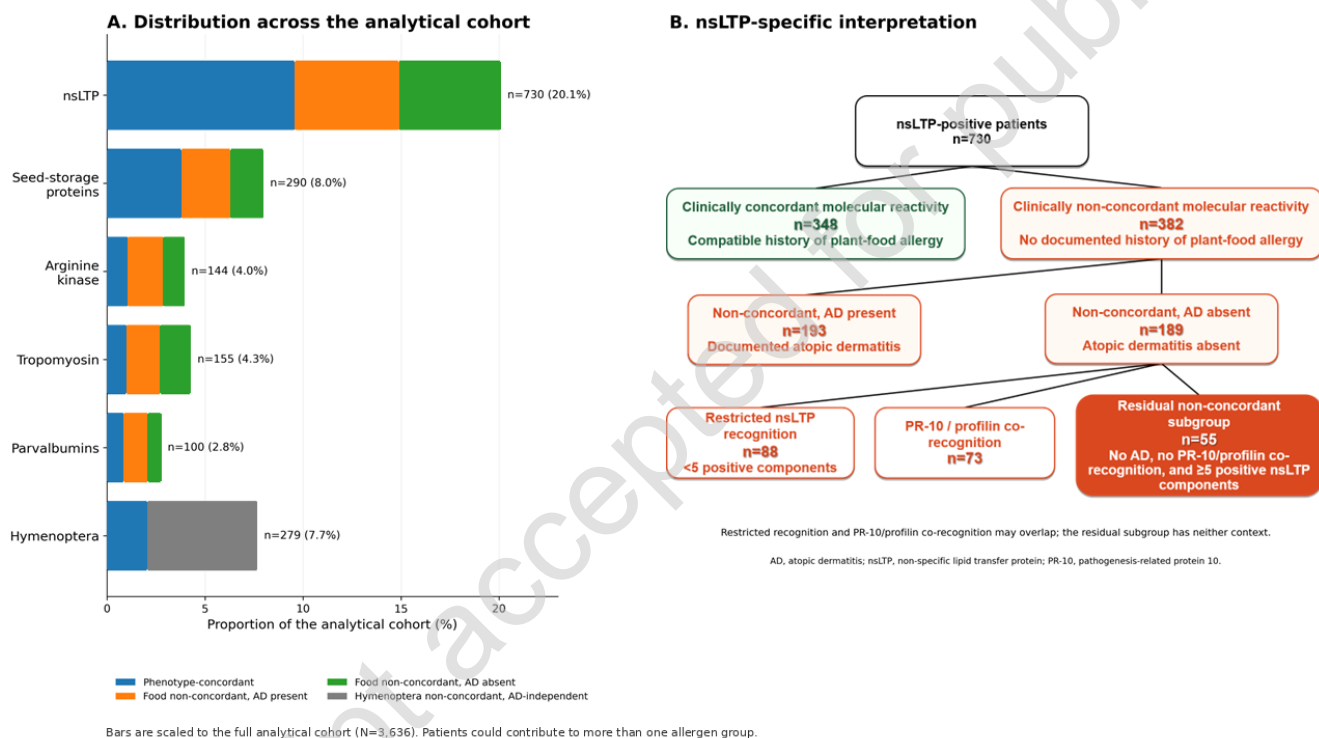


Figure 2. AD as a clinical context among food-sensitised patients without a documented corresponding immediate reaction phenotype. Panel A shows the 637 patients by AD status; Panel B, the corresponding patient-group pairs; and Panel C, HEMA reactivity by AD status. The odds ratio is adjusted for age and sex. The figure does not imply that AD caused absent phenotype documentation or that food sensitisation caused dermatitis.

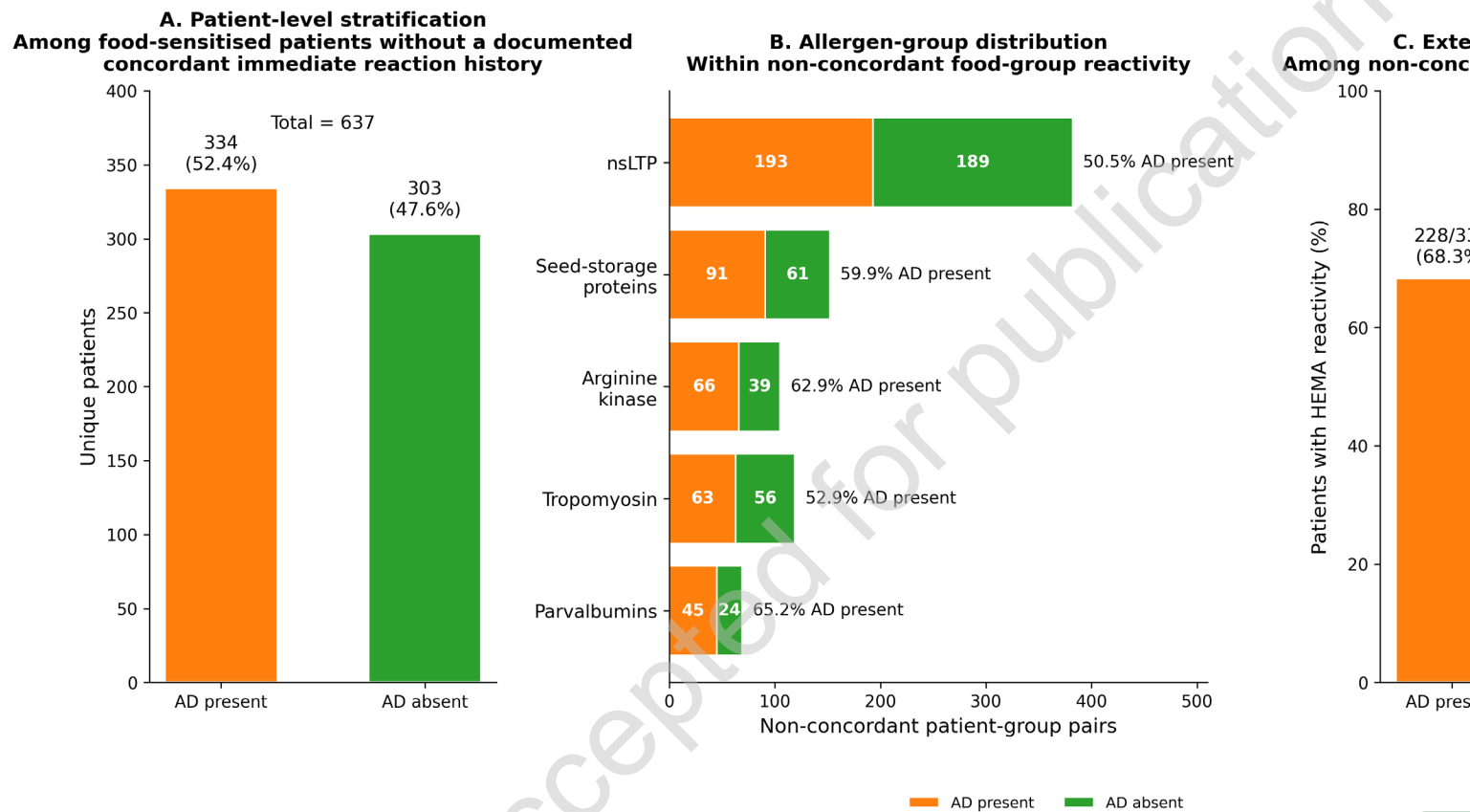
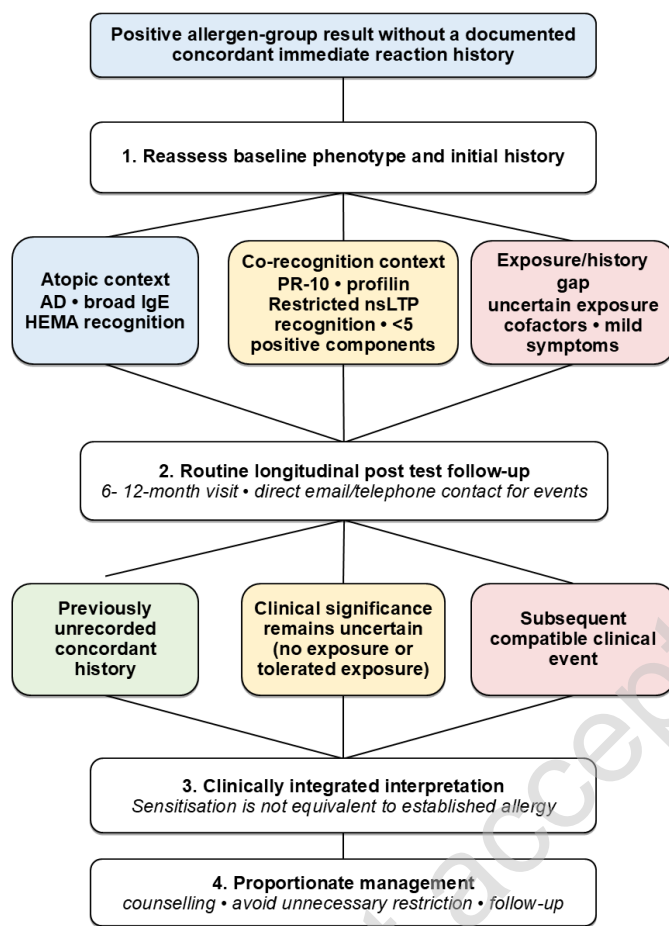
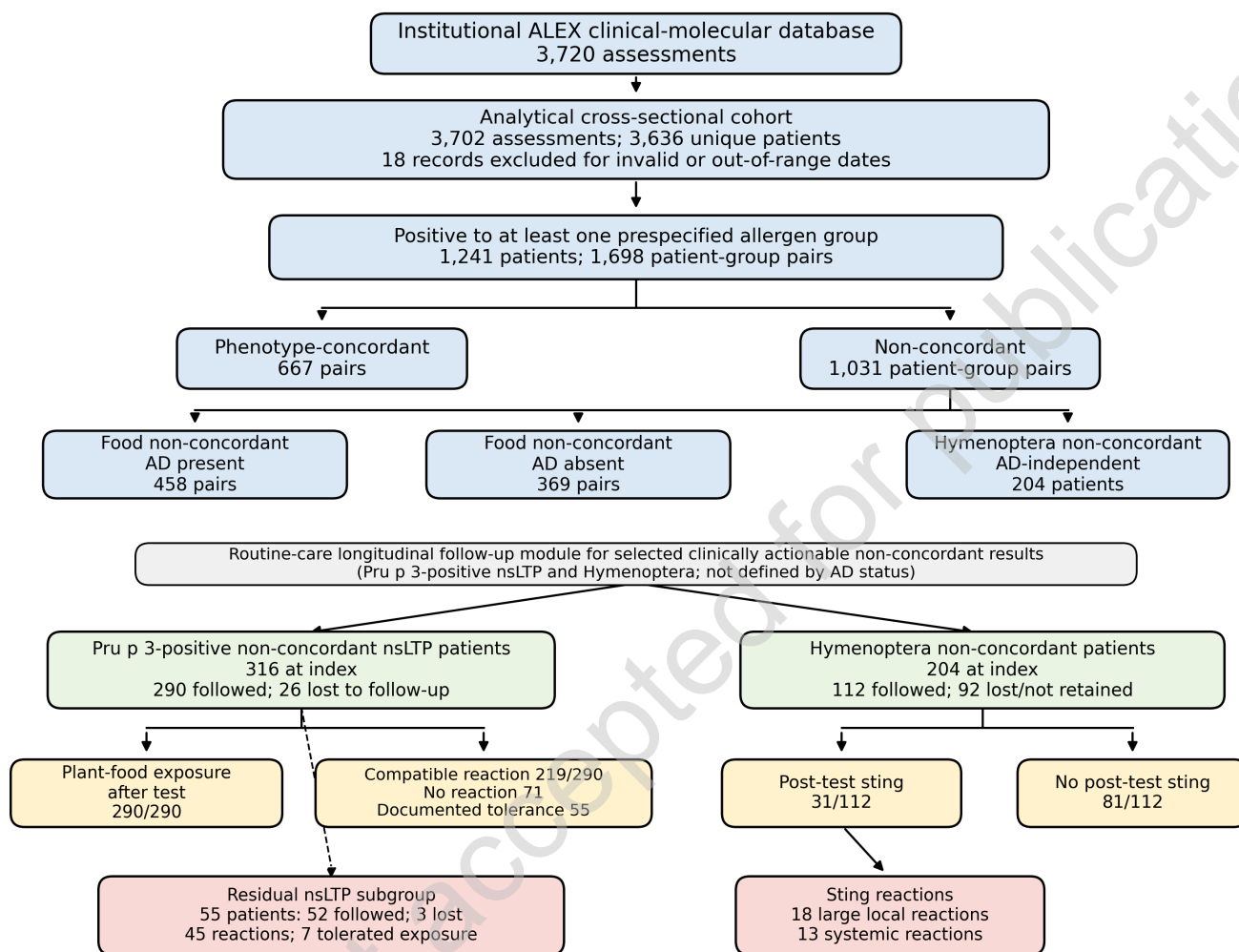


Figure 3. Routine-care reassessment framework for multiplex IgE reactivity without a documented corresponding phenotype. The framework separates sensitisation from established allergy and directs reassessment towards baseline documentation, atopic context, HEMA reactivity, co-recognition, restricted nsLTP recognition, exposure, cofactors, reported post-index events, tolerated exposure, absence of re-exposure, and proportionate management. It reflects routine clinical reassessment and does not imply diagnostic validation or prospective risk estimation



Supplementary Figure S1. The diagram distinguishes cross-sectional patient-group classification from descriptive routine-care reassessment of selected molecular findings without a documented corresponding phenotype. The Pru p 3-positive nsLTP follow-up group was drawn from patients without a documented corresponding nsLTP phenotype irrespective of AD status. Follow-up denominators include patients retained in routine care by July 2026; absence of a reported post-index sting was not interpreted as tolerance.



Supplementary Table S1. Cohort characteristics, assessment frequency, and data completeness.

Characteristic	Value
Assessments screened	3,720
Assessments excluded because of missing, implausible, or out-of-period test date	18 (0.5%)
Eligible assessments	3,702
Unique patients	3,636
Female sex	2,319 (63.8%)
Male sex	1,317 (36.2%)
Age, median (IQR); range, years	33 (21-50); 0-95 [n=3,630]
Missing age	6 (0.2%)
One eligible assessment per patient	3,578 (98.4%)
More than one eligible assessment per patient	58 (1.6%): 52 with two, 4 with three, and 2 with four assessments
Positive to at least one prespecified allergen group	1,241 (34.1%)

Supplementary Table S2. Forty-eight human-homologous exogenous molecular allergens included in the HEMA variable.

HEMA protein family (n)	ALEX2 allergen molecules	Human homolog
Alpha-lactalbumin (1)	Bos d 4	LALBA, alpha-lactalbumin
Prostatic kallikrein (1)	Can f 5	KLK1, kallikrein-1
Arginine kinase (3)	Bla g 9; Der p 20; Pen m 2	KCRB, creatine kinase B-type
Beta-lactoglobulin (1)	Bos d 5	PAEP, glycodelin
Beta-parvalbumin (7)	Clu h 1; Cyp c 1; Gad m 1; Sal s 1; Sco s 1; Thu a 1; Xip g 1	PVALB, parvalbumin
Alpha-S1-casein (1)	Bos d 8	CSN1S1, alpha-S1-casein
Cyclophilin (1)	Mala s 6	PPIA, cyclophilin
Cysteine protease (3)	Act d 1; Der f 1; Der p 1	CATK, cathepsin K
Enolase (1)	Alt a 6	ENO1, alpha-enolase
Latherin (1)	Equ c 4	BPIFA4P
Lipocalin (2)	Can f 1; Fel d 7	LCN1, lipocalin-1
Lipophilin CL2 (1)	Ory c 3	SCGB2A1, mammaglobin-B
Manganese superoxide dismutase (2)	Asp f 6; Mala s 11	SOD2, manganese superoxide dismutase
Mite group 2/NPC2 (4)	Der p 2; Gly d 2; Lep d 2; Tyr p 2	NPC2, intracellular cholesterol transporter 2
Polcalcine (2)	Aln g 4; Phl p 7	CALM1, calmodulin-1
Serum albumin (5)	Bos d 6; Can f 3; Equ c 3; Fel d 2; Sus s 1	ALBU, albumin
Tropomyosin (5)	Ani s 3; Blo t 10; Der p 10; Pen m 1; Per a 7	TPM1, tropomyosin alpha-1 chain
Profilin (6)	Hev b 8; Pho d 2; Phl p 12; Cuc m 2; Mer a 1; Bet v 2	PROF2, profilin-2
Uteroglobulin (1)	Fel d 1	SCGB1A1, uteroglobulin

Supplementary Table S3. Molecular components included in each prespecified allergen-group model.

Allergen-group model	Components used
Food-associated nsLTP	Act d 10; Api g 6; Ara h 9; Can s 3; Cor a 8; Jug r 3; Mal d 3; Pru p 3; Sola l 6; Tri a 14; Vit v 1; Zea m 14
Seed-storage proteins	Ana o 2; Ana o 3; Ara h 1; Ara h 2; Ara h 3; Ara h 6; Ber e 1; Cor a 9; Cor a 11; Cor a 14; Fag e 2; Gly m 5; Gly m 6; Gly m 8; Jug r 1; Jug r 2; Jug r 4; Jug r 6; Mac i 2S Albumin; Pap s 2S Albumin; Pis v 1; Pis v 2; Pis v 3; Ses i 1; Sin a 1
Arginine kinase	Bla g 9; Der p 20; Pen m 2
Tropomyosin	Ani s 3; Blo t 10; Der p 10; Pen m 1; Per a 7
Parvalbumins	Clu h 1; Cyp c 1; Gad m 1; Raj c alpha-Parvalbumin; Sal s 1; Sco s 1; Thu a 1; Xip g 1
Hymenoptera	Api m 1; Api m 10; Pol d 5; Ves v 1; Ves v 5

Supplementary Table S4. Sensitivity analyses of AD as a covariate for record-level absence of a corresponding phenotype and as a clinical context for HEMA reactivity. The five food allergen groups were food-associated nsLTPs, seed-storage proteins, arginine kinases, tropomyosins, and parvalbumins.

Analysis	Adjusted OR	95% CI	P value
Binary GEE, all six allergen groups: AD and absence of documented phenotype	0.78	0.62-0.99	.039
Binary GEE, five food allergen groups: AD and absence of documented phenotype	0.79	0.60-1.02	.070
Patient-level logistic model: HEMA reactivity and AD; adjusted for age and sex	1.46	1.05-2.04	.025
Pair-level GEE: HEMA reactivity and AD; adjusted for age, sex, and allergen group	1.48	1.02-2.15	.039
Patient-level logistic model: HEMA reactivity and AD; additionally adjusted for number of positive food groups	1.34	0.95-1.88	.093
Pair-level GEE, five food allergen groups: AD versus respiratory atopy without AD and absence of documented phenotype	0.82	0.60-1.14	.236

Supplementary Table S5. Exploratory quantitative IgE measures according to documented phenotype status, atopic dermatitis status, and the operational residual nsLTP subgroup. Values are reported to document the analyses underlying Section 3.4 and should not be interpreted as diagnostic thresholds or independent predictors. Panel A. Pairs with versus without a documented corresponding phenotype across the six allergen groups.

Allergen group	Measure	Documented phenotype	No documented phenotype	P	FDR q
nsLTP	Family-maximum sIgE, kUA/L	n=348; 4.56 [1.77-9.99]	n=382; 3.65 [1.27-9.43]	.036	.072
nsLTP	Total IgE, kU/L	n=328; 133 [57.8-355.8]	n=348; 189 [77.8-662.5]	<.001	.002
nsLTP	Family-maximum sIgE/total IgE, %	n=328; 3.57 [1.06-9.53]	n=348; 1.67 [0.47-5.94]	<.001	<.001
Seed-storage proteins	Family-maximum sIgE, kUA/L	n=138; 2.31 [0.60-9.97]	n=152; 1.68 [0.56-4.99]	.065	.098

Seed-storage proteins	Total IgE, kU/L	n=130; 331.5 [150.8-1055.8]	n=140; 694 [229.1-2117.2]	.001	.004
Seed-storage proteins	Family-maximum sIgE/total IgE, %	n=130; 0.85 [0.19-2.63]	n=140; 0.31 [0.08-1.16]	<.001	<.001
Arginine kinase	Family-maximum sIgE, kUA/L	n=39; 4.01 [1.03-10.25]	n=105; 3.65 [0.67-10.91]	.868	.868
Arginine kinase	Total IgE, kU/L	n=36; 258.5 [112.8-523.8]	n=99; 667 [165.5-1643.5]	.005	.010
Arginine kinase	Family-maximum sIgE/total IgE, %	n=36; 1.48 [0.37-5.53]	n=99; 0.88 [0.16-2.31]	.062	.074
Tropomyosin	Family-maximum sIgE, kUA/L	n=36; 5.76 [2.94-11.12]	n=119; 2.12 [0.67-8.50]	.006	.019
Tropomyosin	Total IgE, kU/L	n=33; 312 [129-726]	n=106; 432 [134.2-1383]	.256	.384
Tropomyosin	Family-maximum sIgE/total IgE, %	n=33; 1.18 [0.38-7.90]	n=106; 0.66 [0.17-2.50]	.015	.030
Parvalbumins	Family-maximum sIgE, kUA/L	n=31; 1.89 [0.64-27.19]	n=69; 2.76 [0.89-7.98]	.809	.868
Parvalbumins	Total IgE, kU/L	n=27; 491 [206-1080]	n=63; 347 [171-1267]	.940	.940
Parvalbumins	Family-maximum sIgE/total IgE, %	n=27; 1.47 [0.32-4.97]	n=63; 0.63 [0.15-2.55]	.238	.238
Hymenoptera	Family-maximum sIgE, kUA/L	n=75; 2.30 [0.73-4.52]	n=204; 0.99 [0.51-2.20]	<.001	.002
Hymenoptera	Total IgE, kU/L	n=65; 222 [114-784]	n=187; 243 [102-825]	.611	.733
Hymenoptera	Family-maximum sIgE/total IgE, %	n=65; 0.68 [0.29-2.12]	n=187; 0.56 [0.13-1.48]	.036	.055

Panel B1. Family-maximum sIgE (kUA/L) across food-group clinical strata.

Food allergen group	Documented phenotype	No documented phenotype, AD present	No documented phenotype, AD absent	Global P	Global FDR q	AD+ vs AD- FDR q
nsLTP	n=348; 4.56 [1.77-9.99]	n=193; 3.34 [1.27-9.04]	n=189; 4.05 [1.32-9.46]	.073	.122	.414
Seed-storage proteins	n=138; 2.31 [0.60-9.97]	n=91; 1.87 [0.86-6.96]	n=61; 1.00 [0.51-3.37]	.019	.046	.121
Arginine kinase	n=39; 4.01 [1.03-10.25]	n=66; 4.67 [0.81-21.19]	n=39; 1.49 [0.62-7.90]	.256	.320	.273
Tropomyosin	n=36; 5.76 [2.94-11.12]	n=63; 2.14 [0.75-9.85]	n=56; 2.08 [0.58-7.06]	.015	.046	.414
Parvalbumins	n=31; 1.89 [0.64-27.19]	n=45; 4.28 [0.94-11.45]	n=24; 2.34 [0.51-5.59]	.554	.554	.394

Panel B2. Total IgE (kU/L) across food-group clinical strata.

Food allergen group	Documented phenotype	No documented phenotype, AD present	No documented phenotype, AD absent	Global P	Global FDR q	AD+ vs AD- FDR q
nsLTP	n=328; 133 [57.8-355.8]	n=172; 281 [95-1153.2]	n=176; 143.5 [71.2-391.2]	<.001	<.001	<.001
Seed-storage proteins	n=130; 331.5 [150.8-1055.8]	n=82; 1113 [303.5-2500]	n=58; 398.5 [172.5-1120]	<.001	<.001	.002
Arginine kinase	n=36; 258.5 [112.8-523.8]	n=60; 1068.5 [187.8-2427.5]	n=39; 261 [127.5-858]	<.001	<.001	.003
Tropomyosin	n=33; 312 [129-726]	n=53; 842 [202.5-2042]	n=53; 231 [92-907]	.002	.002	.002
Parvalbumins	n=27; 491 [206-1080]	n=41; 355 [186-1579]	n=22; 325 [164-959]	.615	.615	.338

Panel B3. Family-maximum sIgE/total IgE ratio (%) across food-group clinical strata.

Food allergen group	Documented phenotype	No documented phenotype, AD present	No documented phenotype, AD absent	Global P	Global FDR q	AD+ vs AD- FDR q
nsLTP	n=328; 3.57 [1.06-9.53]	n=172; 0.99 [0.32-4.31]	n=176; 2.33 [0.75-7.82]	<.001	<.001	<.001
Seed-storage proteins	n=130; 0.85 [0.19-2.63]	n=82; 0.24 [0.07-1.02]	n=58; 0.41 [0.11-1.17]	<.001	<.001	.607
Arginine kinase	n=36; 1.48 [0.37-5.53]	n=60; 0.77 [0.17-2.12]	n=39; 0.94 [0.16-3.24]	.133	.167	.612
Tropomyosin	n=33; 1.18 [0.38-7.90]	n=53; 0.32 [0.12-2.30]	n=53; 0.98 [0.34-2.53]	.005	.008	.073
Parvalbumins	n=27; 1.47 [0.32-4.97]	n=41; 1.11 [0.14-2.96]	n=22; 0.58 [0.27-1.48]	.493	.493	.891

Panel C. Exploratory quantitative comparison of the residual AD-absent nsLTP subgroup without a documented phenotype with phenotype-concordant nsLTP cases.

Measure	Documented phenotype	Exploratory residual subgroup	P	FDR q
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Family-maximum sIgE, kUA/L	n=348; 4.56 [1.77-9.99]	n=55; 8.95 [3.54-17.76]	.001	.002
Pru p 3 sIgE, kUA/L	n=348; 3.31 [0.71-8.79]	n=55; 7.14 [2.92-12.29]	<.001	.001
Total IgE, kU/L	n=328; 133 [57.8-355.8]	n=50; 108.5 [66-169.2]	.140	.140
Family-maximum sIgE/total IgE, %	n=328; 3.57 [1.06-9.53]	n=50; 8.04 [2.67-20.29]	<.001	.001
Pru p 3 sIgE/total IgE, %	n=328; 2.54 [0.37-7.55]	n=50; 6.71 [1.94-17.80]	<.001	.001

Supplementary Table S6. Coding and aggregate results of descriptive routine-care reassessment.

Variable	Coding/definition	Aggregate result
AD	0 absent; 1 mild/previous; 2 moderate; 3 severe	Pru p 3 follow-up: 213 AD-absent, 77 AD-present; Hymenoptera follow-up: 91 AD-absent, 21 AD-present
Routine-care reassessment inclusion	Not lost to routine follow-up by July 2026	Pru p 3: 290/316 (91.8%; 95% CI 88.2-94.6); residual subgroup: 52/55 (94.5%; 95% CI 84.9-98.9); Hymenoptera: 112/204 (54.9%; 95% CI 47.8-61.9)
Successful contact	1 = positive contact among those retained	Pru p 3: 290/290; residual subgroup: 52/52; Hymenoptera: 112/112
Reported exposure/sting after index test	0 no; 1 yes	Pru p 3: 290/290; residual subgroup: 52/52; Hymenoptera: 31/112
Reported compatible reaction/event after index test	0 no; 1 yes	Pru p 3: 219/290; residual subgroup: 45/52; Hymenoptera: 31/112 and 31/31 among those stung
Pru p 3 final adjudication	0 absent; 1 mild; 2 moderate; 3 severe	All Pru p 3: 0=71, 1=96, 2=99, 3=24; residual subgroup: 0=7, 1=14, 2=25, 3=6
Hymenoptera symptom severity	No post-test sting/reaction; large local reaction; systemic reaction	No post-test sting: 81; large local reaction: 18; systemic reaction: 13
Cofactors	0 none; FDEIA; FDNIH/FDHIH; alcohol; unspecified	All Pru p 3: FDEIA 31; NSAID-associated 18; alcohol 1; unspecified 3. Residual subgroup: FDEIA 7; NSAID-associated 6; unspecified 1. Hymenoptera: none recorded
Exposure interpretation	Food exposure can recur; Hymenoptera re-sting is opportunistic and cannot be standardised in routine care	No post-test sting was recorded in 81/112 Hymenoptera patients; absence of re-sting was not interpreted as tolerance