

Letter to the Editor

IgE-reactivity profiles to non-specific lipid transfer proteins in a northwestern European country

To the Editor:

Nonspecific lipid transfer proteins (ns-LTPs) have increasingly been recognized to constitute a significant cause of plant food allergy, especially in the Mediterranean basin.^{1,2} Sensitization to ns-LTPs is generally believed to be a risk factor for generalized allergic reactions, although symptoms restricted to the oropharynx (oral allergy syndrome [OAS]), skin, and respiratory or gastrointestinal tract are also described.^{1,2} Moreover, IgE reactivity to ns-LTPs might remain asymptomatic or require cofactors to become clinically overt.^{1,3} Because of the extensive cross-reactive nature of this panallergen, ns-LTP-sensitized patients frequently display multiple plant food allergies designated as the “ns-LTP syndrome.”¹ Hitherto, the ns-LTP of peach (Pru p 3, *Prunus persica*) is oftentimes described as the primary sensitizer.⁴ However, like for other pollen and plant food-related allergies, the primary sensitizer for a ns-LTP syndrome and the clinical relevance of such a sensitization might differ according to age and geographic region.⁵⁻⁷ Epidemiological surveys on sensitization to ns-LTPs have mainly been performed in southern European and are generally gathered in adolescents and adults.^{2,3,5,8} Therefore, this study aimed at evaluating the prevalence of sIgE reactivity to 6 ns-LTPs in children and adults suffering from pollen and/or plant food allergies in a northwestern European country. Furthermore, we sought to investigate the clinical significance of sIgE reactivity to these ns-LTPs. For this purpose, we focused on Pru p 3 and evaluated whether the basophil activation test (BAT) could discriminate between the different clinical phenotypes of sIgE reactivity to rPru p 3.

Details of the experimental methods used are provided in the [Methods](#) section in the Online Repository at www.jacionline.org. Briefly, consecutive patients with a compelling history of inhalant and/or plant food allergies were included by qualified physicians via the outpatient clinics of Allergology and Pediatrics of the Antwerp University Hospital between September 2013 and May 2015. Measurement of total IgE and sIgE to 6 ns-LTP components (rAra h 9, nArt v 3, rCor a 8, rMal d 3, rPar j 2, and rPru p 3) and the major birch pollen component (Bet v 1) was performed in all patients. BATs with rPru p 3 were performed to evaluate the clinical significance of sIgE reactivity to rPru p 3.

A total of 718 patients allergic to pollen and/or plant food were included. As shown in [Fig E1](#) in this article's Online Repository at www.jacionline.org, statistical analysis revealed no significant differences in sIgE reactivity to ns-LTP(s) between the 3 different clinical phenotypes (pollen allergic, plant food allergic, pollen and plant food allergic), nor between sexes or different age groups. For further evaluation of the ns-LTP reactivity profiles, patients with sIgE reactivity to ns-LTPs (ns-LTP⁺) out of the 3 clinical groups were pooled together, resulting in a total of 177 ns-LTP⁺ patients (25%; 95% CI, 22-28) out of 718 patients allergic to pollen and/or food. The total IgE level was higher in the ns-LTP⁺ patients (median, 777 kU/L; 276-1848) as compared to patients without IgE reactivity to ns-LTPs (median, 156 kU/L; 62-341) (Mann-Whitney *U* test; *P* < .05). However, as indicated by [Fig E2](#) in this article's Online Repository at www.jacionline.org,

various sera with high total IgE levels were negative for ns-LTP, ruling out a major effect of false-positives due to nonspecific binding. [Fig 1](#) and [Fig E3](#) in this article's Online Repository at www.jacionline.org display the individual sIgE results of the evaluated ns-LTPs for patients out of the different clinical groups. sIgE reactivity to rPru p 3 was most frequently observed (152 of 177 [86%]; 95% CI, 80-90), followed by rMal d 3 (129 of 177 [73%]; 95% CI, 66-79). sIgE reactivity to Bet v 1 was demonstrated in 140 of 177 (79%; 95% CI, 72-84) ns-LTP⁺ patients. Furthermore, as demonstrated in [Fig 1](#), there is a clustering between all studied ns-LTP sIgE results, indicating high cross-reactivity between all ns-LTPs. In [Fig E4, A-E](#), in this article's Online Repository at www.jacionline.org, correlations are shown between sIgE rPru p 3 and the other ns-LTP sIgEs. Among the 177 ns-LTP⁺ patients, the majority did not display an overt food allergy upon eating the respective plant foods, as shown in [Fig 2, A](#). Actually, in the patients with sIgE reactivity to rPru p 3, rMal d 3, rAra h 9, and rCor a 8, tolerance to peach, apple, peanut, and hazelnut, respectively, is seen in 35% to 59% of patients. A possible explanation for this absence of overt allergy could be the elevated prevalence of Bet v 1 sensitization.²

To investigate whether the BAT could help to disentangle the clinical relevance of a positive rPru p 3 sIgE result, we decided to perform a BAT with rPru p 3. As displayed in [Fig 2, B and C](#), all patients allergic to peach exhibiting generalized reactions and 2 of 7 Pru p 3⁺ patients with OAS showed basophil activation and, most importantly, rPru p 3 failed to activate basophils of Pru p 3⁺ patients tolerating peach. Note that all 7 patients demonstrating an OAS to peach were sensitized to Bet v 1, which could explain their clinical manifestations.

To our knowledge, this is the first study evaluating the prevalence of sensitization to different ns-LTPs in a northwestern European country. In summary, it emerges that sIgE reactivity to ns-LTP(s), using a panel of 4 food ns-LTPs and 2 weed pollen ns-LTPs, is demonstrable in about one-quarter of our patients presenting with symptoms of a pollen and/or plant food allergy, irrespective of sex or age. The exact reason(s) for this relatively high prevalence of ns-LTP sIgE reactivity remains elusive. Although it could reflect a genuine high sensitization rate to ns-LTP in our patients, it cannot entirely be excluded that our findings (to some extent) relate to the applied methodology. First, our study involved quantification of sIgE to a set of 6 different ns-LTPs. Second, we applied a single-plexed solid-phase assay with a threshold value of 0.10 kUa/L, which might be more sensitive than a multiplexed microarray method.⁹ Another particularity of our study relates to the observation that most patients with sIgE reactivity to ns-LTPs do not clinically react to the respective plant food(s). Moreover, it appears that basophil activation experiments might be helpful in discriminating between patients with a clinically significant sensitization to ns-LTPs and patients who are merely sensitized.

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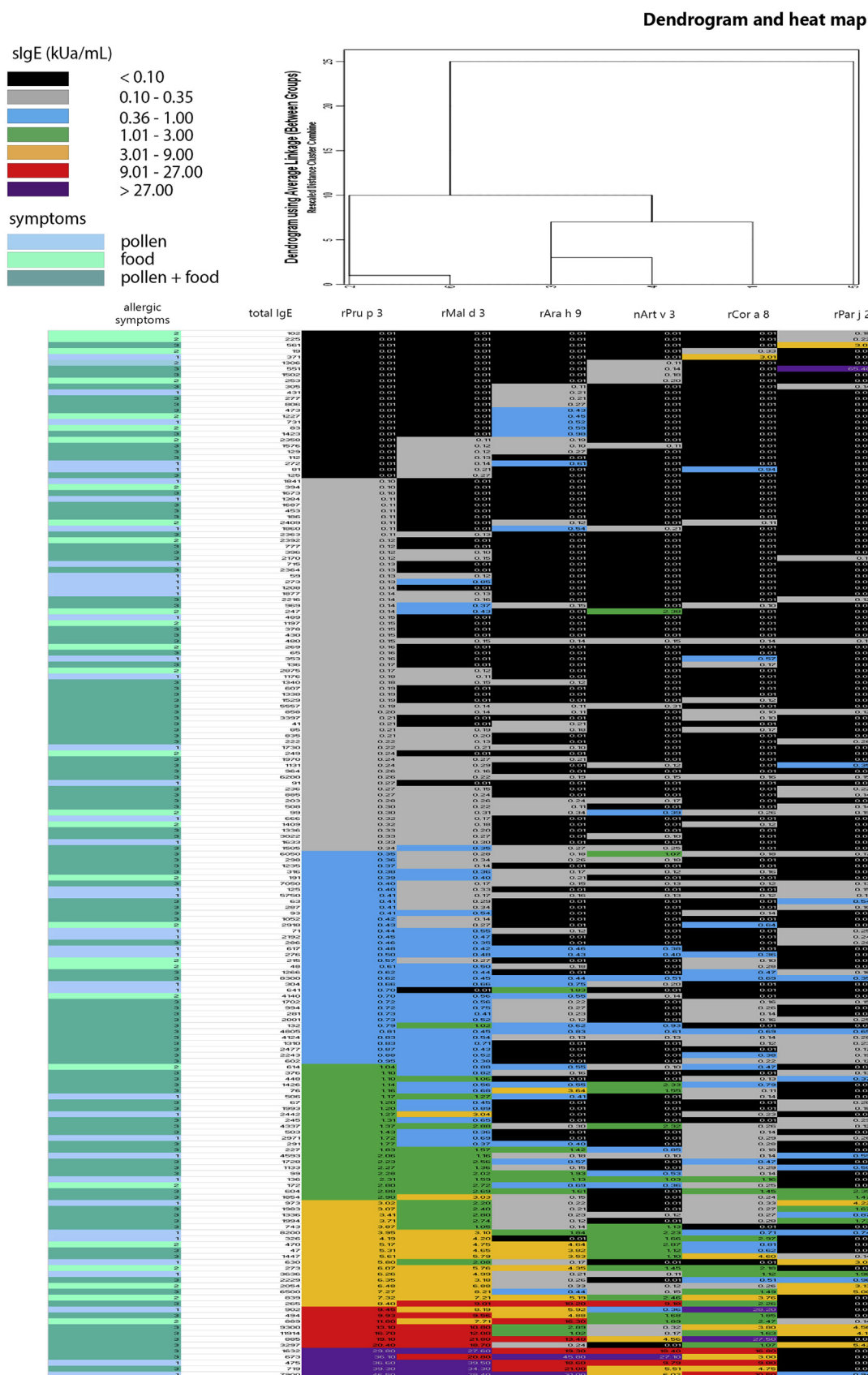


FIG 1. Dendrogram and heat map of total IgE and specific IgE results to rPru p 3, rMal d 3, rAra h 9, rCor a 8, nArt v 3, and rPar j 2. In the first column, clinical symptoms of the patients (pollen allergic, plant food allergic, pollen and plant food allergic) are displayed.

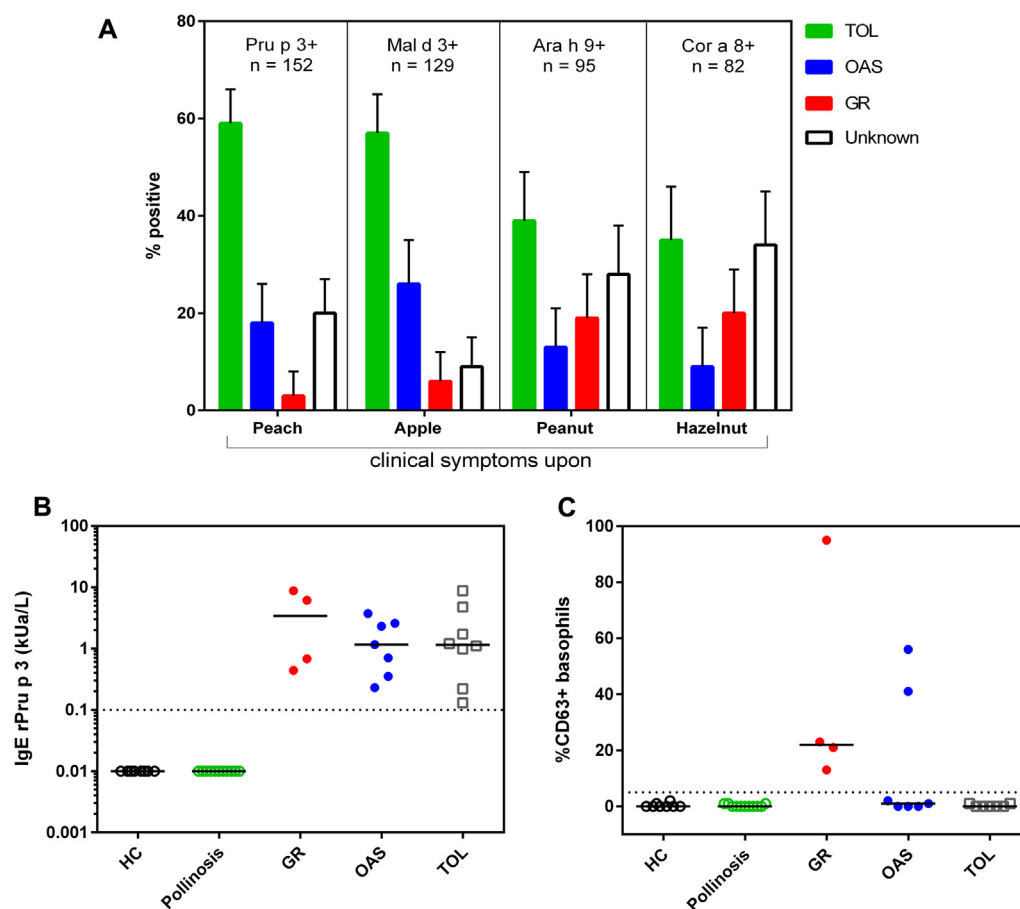


FIG 2. **A**, Food-specific clinical manifestations for peach in Pru p 3⁺ patients, apple in Mal d 3⁺ patients, peanut in Ara h 9⁺ patients, and hazelnut in Cor a 8⁺ patients. Frequencies were displayed with 95% CI. **B**, Specific IgE to rPru p 3 in 38 individuals in whom a BAT with rPru p 3 was performed; healthy control individuals (HC, n = 8), patients with pollinosis (n = 11), patients allergic to peach experiencing generalized reactions (GR, n = 4) or OAS (n = 7), and patients tolerating peach (TOL, n = 8) but showing specific IgE reactivity to Pru p 3. **C**, Results of BAT with 1 μ g/mL rPru p 3 expressed as net percentage of CD63 upregulation in the same groups as described above. GR, Generalized reaction and unknown; TOL, tolerating the indicated food.

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REFERENCES

- Pascal M, Munoz-Cano R, Reina Z, Palacin A, Vilella R, Picado C, et al. Lipid transfer protein syndrome: clinical pattern, cofactor effect and profile of molecular sensitization to plant-foods and pollens. *Clin Exp Allergy* 2012;42:1529-39.
- Scala E, Till SJ, Asero R, Abeni D, Guerra EC, Pirrotta L, et al. Lipid transfer protein sensitization: reactivity profiles and clinical risk assessment in an Italian cohort. *Allergy* 2015;70:933-43.
- Gonzalez-Mancebo E, Gonzalez-de-Olano D, Trujillo MJ, Santos S, Gandolfo-Cano M, Melendez A, et al. Prevalence of sensitization to lipid transfer proteins and profilins in a population of 430 patients in the south of Madrid. *J Invest Allergol Clin Immunol* 2011;21:278-82.
- Schulten V, Nagl B, Scala E, Bernardi ML, Mari A, Ciardiello MA, et al. Pru p 3, the nonspecific lipid transfer protein from peach, dominates the immune response to its homolog in hazelnut. *Allergy* 2011;66:1005-13.
- Andersen MB, Hall S, Dragsted LO. Identification of European allergy patterns to the allergen families PR-10, LTP, and profilin from Rosaceae fruits. *Clin Rev Allergy Immunol* 2011;41:4-19.
- Asero R, Antonicecchi L, Arena A, Bommarito L, Caruso B, Crivellaro M, et al. Epidemiology of food allergy in Italian adults attending allergy clinics: a multi-centre study. *Clin Exp Allergy* 2009;39:547-55.

7. Ma S, Yin J, Jiang N. Component-resolved diagnosis of peach allergy and its relationship with prevalent allergenic pollens in China. *J Allergy Clin Immunol* 2013; 132:764-7.
8. Barber D, de la Torre F, Feo F, Florido F, Guardia P, Moreno C, et al. Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. *Allergy* 2008;63:1550-8.
9. Cabrera-Freitag P, Goikoetxea MJ, Gamboa PM, Martinez-Aranguren R, Beorlegui C, Fernandez J, et al. A study of the variability of the in vitro component-based microarray ISAC CDR 103 technique. *J Investig Allergol Clin Immunol* 2011; 21:414-5.

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METHODS

Patients

Consecutive patients with a compelling history of inhalant and/or plant food allergies were included by qualified physicians via the outpatient clinics of Allergology and Pediatrics of the Antwerp University Hospital between September 2013 and May 2015. These patients were stratified into 3 age groups (preschool [<7 years], schoolchildren [7-18 years], and adults [>18 years]). Demographic details and signs of clinical reactions were obtained by a standardized questionnaire. With respect to inhalant allergens, this questionnaire focused on the presence of seasonal rhinoconjunctivitis. With respect to plant food allergy, the patients were thoroughly questioned about the presence of allergic symptoms restricted to the oral cavity (eg, itchy mouth and/or scratchy throat and/or swelling of the lips, mouth, tongue, throat, and/or itchy ears) or more generalized reactions with urticaria, angioedema, gastrointestinal symptoms (cramps, nausea, vomiting), bronchospasm, or generalized anaphylaxis immediately related to the consumption of different fruits, vegetables, and/or nuts.^{E1} Tolerance was defined as no clinical allergic symptoms to age-appropriate portions of the plant food in patients' diet. The local ethics committee approved this study (B300201524055), and patients or their representatives approved an informed consent in accordance with the Declaration of Helsinki.

Total and specific IgE

Total IgE and specific IgE to the Crossreactive Carbohydrate Determinants (CCD)-free recombinant (r) and native (n) ns-LTP components of 4 foods (rAra h 9 from peanut [*Arachis hypogaea*], rCor a 8 from hazelnut [*Corylus avellana*], rMal d 3 from apple [*Malus domestica*], and rPru p 3 from peach [*Prunus persica*]) and 2 pollens (nArt v 3 from mugwort [*Artemisia vulgaris*] and rPar j 2 from wall pellitory [*Parietaria judaica*]) were quantified. Birch pollen sensitization was evaluated by specific IgE to rBet v 1, the major allergen from birch (*Betula verrucosa*).

Total and specific IgE quantifications were performed using the FEIA ImmunoCAP technique (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions. Results were considered positive for levels of 0.10 kUa/L or more.

BATs

BATs were performed as detailed elsewhere.^{E2} Briefly, prewarmed heparinized blood samples were stimulated with 4 concentrations (0.001, 0.01, 0.1, and 1 $\mu\text{g/mL}$) of recombinant Pru p 3. This recombinant protein was obtained, cloned, and characterized as described elsewhere.^{E3} Antihuman IgE served as a positive control (10 $\mu\text{g/mL}$, BD Biosciences, Erembodegem, Belgium), and stimulation buffer alone was used to measure spontaneous CD63 expression. Basophils were characterized by side scatter, anti-IgE+, and CD203c+. Subsequently, within this gate, the percentage of activated basophils, that is, those expressing CD63, was measured. Results were expressed as net percentages of CD63+ basophils, namely, by subtraction of the spontaneous expression from the allergen-induced CD63 expression. Dose-finding experiments revealed that the best discriminative stimulation concentration was 1 $\mu\text{g/mL}$ rPru p 3 (data not shown).

Statistical analysis

Data are expressed as medians with interquartile ranges or percentages with 95% CIs. IBM SPSS version 23.0 (IBM Corp, Armonk, NY) software was used for data analysis. Hierarchical cluster analysis was performed according to Wald's method. Nonparametric tests and chi-square analysis were used where appropriate. A *P* value of less than .05 was regarded as statistically significant.

REFERENCES

- E1. Kim H, Fischer D. Anaphylaxis. *Allergy Asthma Clin Immunol* 2011;7:S6.
- E2. Bridts CH, Sabato V, Mertens C, Hagendorens MM, De Clerck LS, Ebo DG. Flow cytometric allergy diagnosis: basophil activation techniques. *Methods Mol Biol* 2014;1192:147-59.
- E3. Diaz-Perales A, Garcia-Casado G, Sanchez-Monge R, Garcia-Selles FJ, Barber D, Salcedo G. cDNA cloning and heterologous expression of the major allergens from peach and apple belonging to the lipid-transfer protein family. *Clin Exp Allergy* 2002;32:87-92.

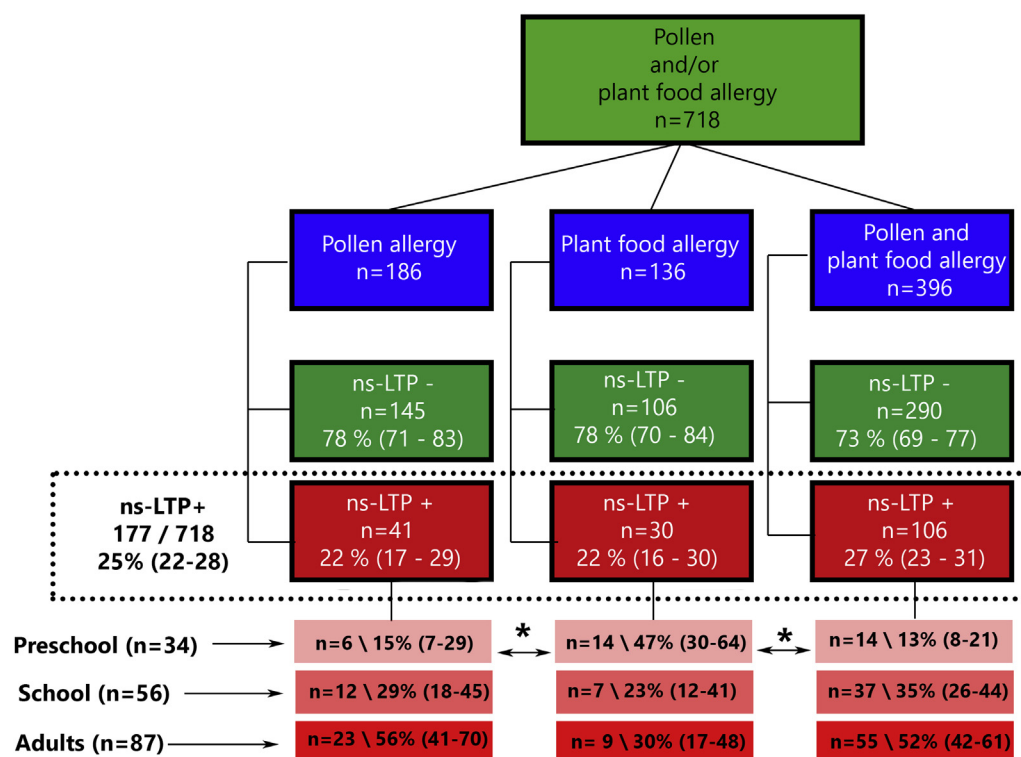


FIG E1. Overview of the study group stratified according to clinical symptoms on exposure to pollen and/or plant food with a subdivision based on specific IgE reactivity to at least 1 of the 6 evaluated ns-LTPs (nArt v 3, rAra h 9, rCor a 8, rMal d 3, rPar j 2, and rPru p 3). Patients were further stratified according to age (preschool children, 0-6 years; school children, 7-18 years; and adults, >18 years). Frequencies (%) were displayed including 95% CI. *Specific IgE reactivity to ns-LTPs was more frequent in children at preschool with only plant food allergy as compared with the other 2 clinical phenotypes (χ^2 ; $P < .05$).

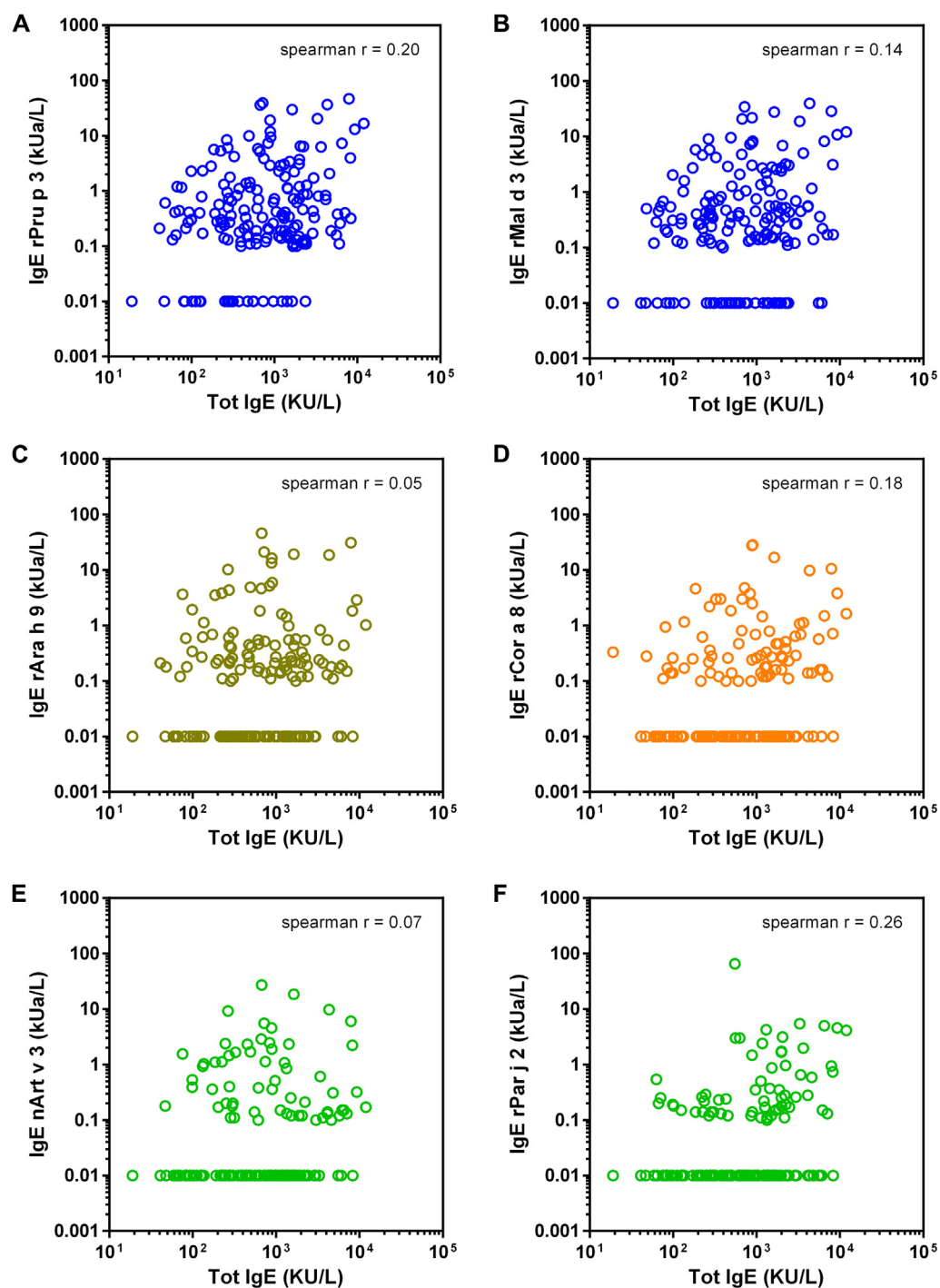


FIG E2. Graphical representation of the correlation of total IgE (tIgE) with specific IgE to rPru p 3 (A), rMal d 3 (B), rAra h 9 (C), rCor a 8 (D), nArt v 3 (E), and rPar j 2 (F). Spearman ranking coefficients (r) are displayed in the right upper corner of each graph.

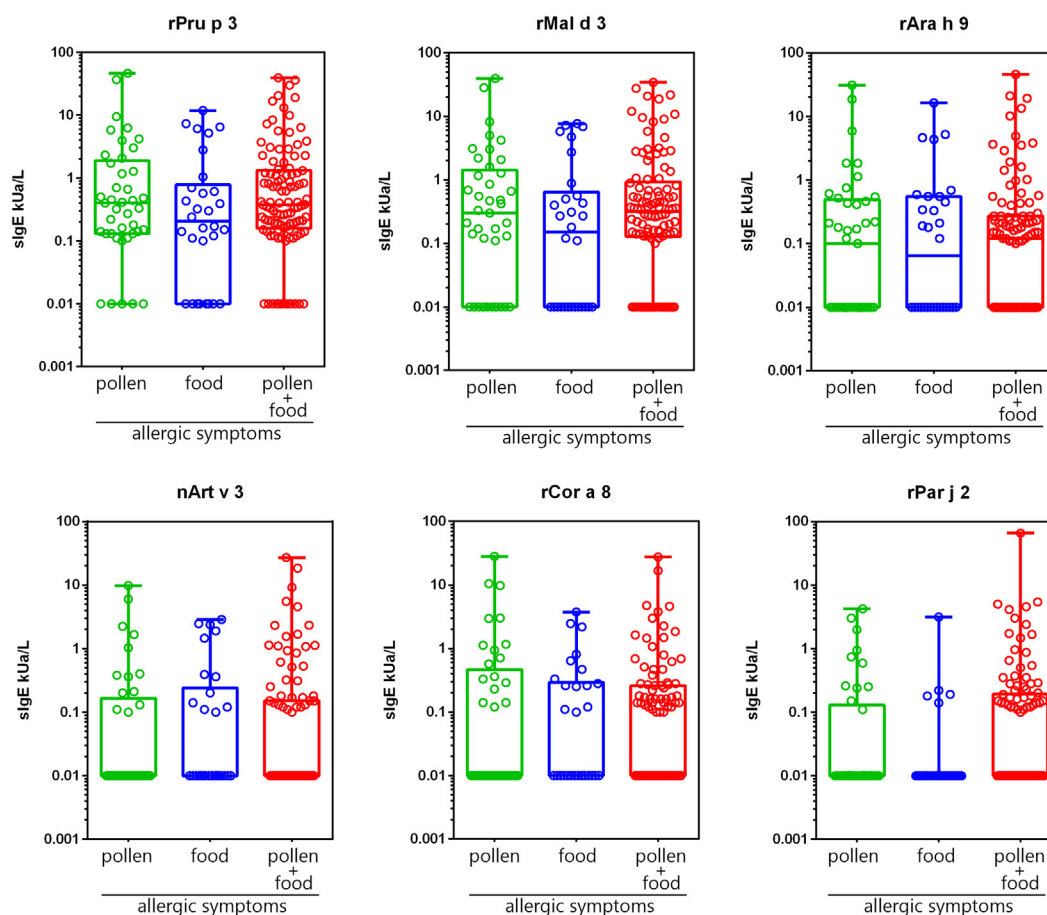


FIG E3. Specific IgE to rPru p 3, rMal d 3, rAra h 9, nArt v 3, rCor a 8, and rPar j 2 in patients with pollen allergic symptoms (*green*), plant food allergic symptoms (*blue*), and pollen and plant food allergic symptoms (*red*). Levels less than 0.10 kUa/L (or negative) were set to 0.01 kUa/L.

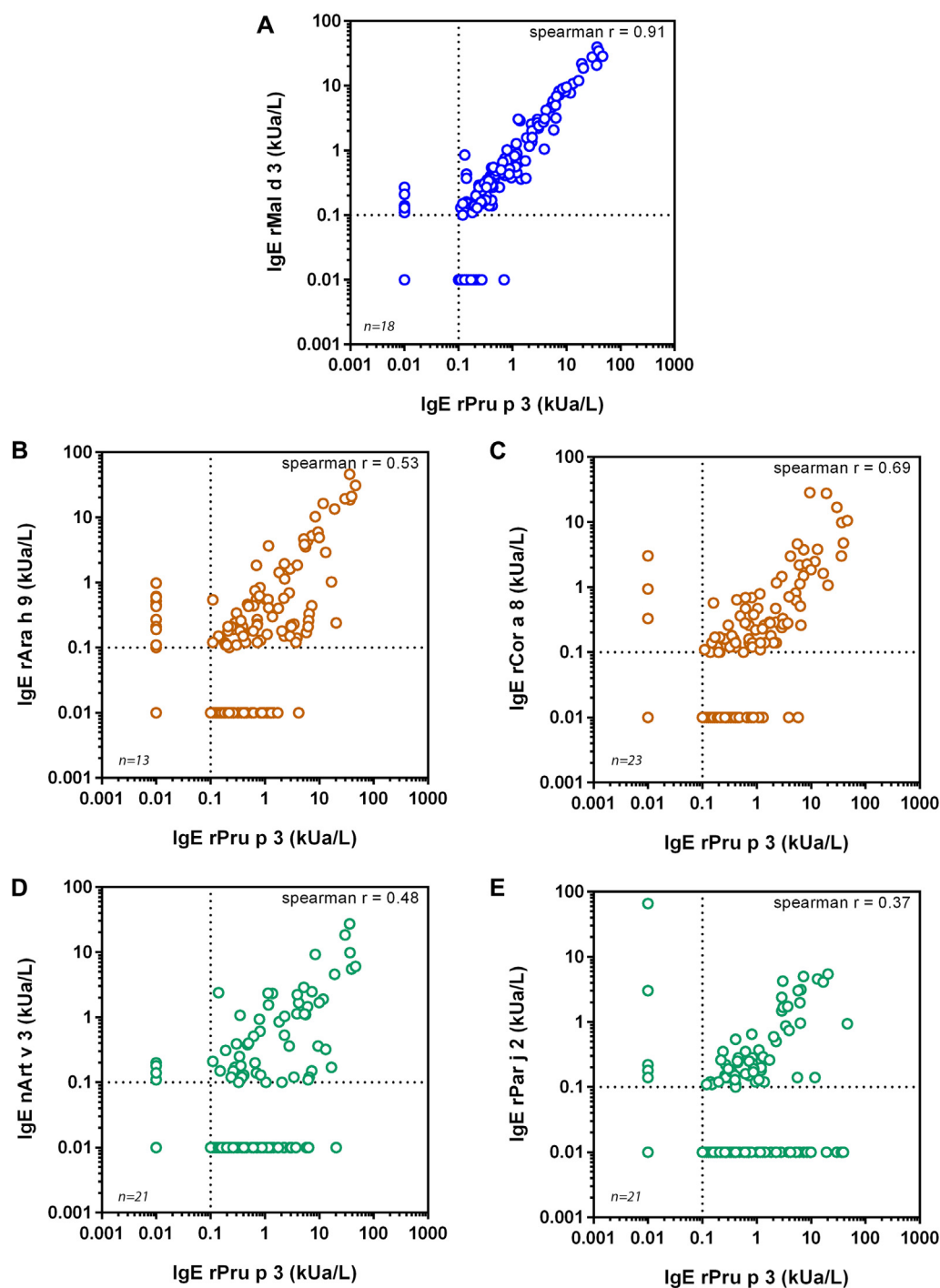


FIG E4. Graphical representation of the correlation of specific IgE to rMal d 3 (A), rAra h 9 (B), rCor a 8 (C), nArt v 3 (D), and rPar j 2 (E) with rPru p 3. Spearman correlation coefficients are displayed in the right corner of the different figures. The number of patients with double-negative specific IgE results is displayed in the bottom left corner of each figure.