Endotypes of pollen-food syndrome in children with seasonal allergic rhinoconjunctivitis: a molecular classification

ORIGINAL ARTICLE AIRWAY DISEASES

# Endotypes of pollen-food syndrome in children with seasonal allergic rhinoconjunctivitis: a molecular classification

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**To cite this article:** Mastrorilli C, Tripodi S, Caffarelli C, Perna S, Di Rienzo-Businco A, Sfika I, Asero R, Dondi A, Bianchi A, Dascola CP, Ricci G, Cipriani F, Maiello N, Miraglia-Del Giudice M, Frediani T, Frediani S, Macrì F, Pistoletti C, Dello Iacono D, Patria MF, Varin E, Peroni D, Comberiati P, Chini L, Moschese V, Lucarelli S, Bernardini R, Pingitore G, Pelosi U, Olcese R, Moretti M, Cirisano A, Faggian D, Travaglini A, Plebani M, Verga MC, Calvani M, Giordani P, Matricardi PM for the Italian Pediatric Allergy Network (I-PAN). Endotypes of pollen-food syndrome in children with seasonal allergic rhinoconjunctivitis: a molecular classification. *Allergy* 2016; DOI: 10.1111/all.12888

**Keywords**
children; classification; cluster analysis; endotypes; panallergens; pollen-food syndrome.

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Accepted for publication 14 March 2016

DOI:10.1111/all.12888

## Abstract

**Background:** Pollen-food syndrome (PFS) is heterogeneous with regard to triggers, severity, natural history, comorbidities, and response to treatment. Our study aimed to classify different endotypes of PFS based on IgE sensitization to panallergens.

**Methods:** We examined 1271 Italian children (age 4–18 years) with seasonal allergic rhinoconjunctivitis (SAR). Foods triggering PFS were acquired by questionnaire. Skin prick tests were performed with commercial pollen extracts. IgE to panallergens Phl p 12 (profilin), Bet v 1 (PR-10), and Pru p 3 (nsLTP) were tested by ImmunoCAP FEIA. An unsupervised hierarchical agglomerative clustering method was applied within PFS population.

**Results:** PFS was observed in 300/1271 children (24%). Cluster analysis identified five PFS endotypes linked to panallergen IgE sensitization: (i) cosensitization to ≥2 panallergens ('multi-panallergen PFS'); (ii–iv) sensitization to either profilin, or nsLTP, or PR-10 ('mono-panallergen PFS'); (v) no sensitization to panallergens ('no-panallergen PFS'). These endotypes showed peculiar characteristics: (i) 'multi-panallergen PFS': severe disease with frequent allergic comorbidities and multiple offending foods; (ii) 'profilin PFS': OAS triggered by *Cucurbitaceae*; (iii) 'LTP PFS': living in Southern Italy, OAS triggered by hazelnut and peanut;

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(iv) 'PR-10 PFS': OAS triggered by *Rosaceae*; and (v) 'no-panallergen PFS': mild disease and OAS triggered by kiwifruit.

**Conclusions:** In a Mediterranean country characterized by multiple pollen exposures, PFS is a complex and frequent complication of childhood SAR, with five distinct endotypes marked by peculiar profiles of IgE sensitization to panallergens. Prospective studies in cohorts of patients with PFS are now required to test whether this novel classification may be useful for diagnostic and therapeutic purposes in the clinical practice.

Pollen-food syndrome (PFS) is defined by allergic symptoms elicited promptly by the ingestion of fruits or vegetables in patients with seasonal allergic rhinoconjunctivitis (SAR) (1). Patients are sensitized to pollen allergenic molecules highly cross-reacting with their homologues in the offending foods (incomplete food allergens or nonsensitizing elicitors, class 2 food allergy) (2, 3). Symptoms of PFS are often restricted and isolated to the oral cavity and include labial and oropharyngeal pruritus, paresthesia and angioedema of the oral mucosa, tongue, lips, palate and oropharynx, or laryngeal tightness, which all together are labeled as oral allergy syndrome (OAS) (4). Gastrointestinal symptoms and, rarely, life-threatening wheezing and anaphylaxis occur in less than 10% of patients (5). Because of great heterogeneity in triggers, severity, natural history, comorbidities, and response to treatment, pollen-food syndrome is defined not as a simple disease but as a complex syndrome. Oral allergy syndrome can also be the clinical expression of primary sensitization to genuine and/or cross-reacting food allergens and OAS is frequently the first symptom of an allergic reaction in cases followed by systemic symptoms (class 1 food allergy). It can be induced by any type of food sources or any type of food allergens.

The highly cross-reacting molecules causing PFS are usually labile, degraded by heat and digestive enzymes and can induce allergic reactions only in already-sensitized patients (6). As these molecules are ubiquitous, they have also been classified as 'panallergens' (7, 8). The most important panallergens include three protein clusters: profilins (9), pathogenesis-related class 10 proteins (PR-10) (10), and nonspecific lipid transfer proteins (nsLTP) (11). PR-10s are the dominant trigger of PFS manifesting with symptoms restricted to the oral cavity in Northern and Central Europe, where pollen allergy is mainly linked to *birch* and *alder* pollens (9). In these countries, PFS is more similar to a single disease, where symptoms are mostly triggered by PR-10-containing *Rosaceae*, such as apples and peaches. By contrast, the spectrum of molecules mediating PFS in patients with SAR is much more complex in Mediterranean countries, where many more allergenic pollens are dispersed throughout the year (10). This higher complexity makes the classification of PFS in Southern Europe difficult, as most patients with SAR are highly pollen-polysensitized (8). Under these circumstances, many different molecules can be suspected as triggers of PFS in Southern European patients with SAR, and an etiologic diagnosis may be difficult.

The target of the present study is to investigate whether endotypes of PFS may indeed be described in Southern European patients affected by SAR. To test this hypothesis, we have examined with an unsupervised hierarchical agglomerative (bottom-up) clustering method a large cohort of 1271 Italian children affected by SAR.

**Abbreviations**

AR, allergic rhinitis; ARIA, Allergic Rhinitis and its Impact on Asthma; CI, confidence interval; GINA, Global Initiative for Asthma; I-PAN, Italian Pediatric Allergy Network; ISAAC, International Study of Allergy and Asthma in Childhood; OAS, oral allergy syndrome; OR, odds ratio; PAN-PED, Panallergens in Pediatrics; PFS, pollen-food syndrome; SAR, seasonal allergic rhinoconjunctivitis; SD, standard deviation; SPT, skin prick test.

## Materials and methods

### Study population

The study population was enrolled in the first Italian nationwide observational multicenter survey carried out by the Italian Pediatric Allergy Network (I-PAN) on the impact of sensitization to highly cross-reacting allergenic molecules on the management of respiratory allergies in childhood ('Panallergens in Pediatrics' [PAN-PED]) (12–14). Children were recruited between May 2009 and June 2011 by 16 pediatric outpatient clinics in 14 Italian cities distributed in three main geographic areas: Northern Italy (Milan, Verona, Genoa, Parma, and Bologna), Central Italy (Empoli, Ascoli Piceno, Ostia, three centers in Rome), and Southern Italy and Major Islands (Naples, Benevento, Iglesias, Palermo, and Crotone). Criteria for eligibility were as follows: (i) age 4–18 years; (ii) a history of pollen-induced allergic rhinitis and/or asthma in one of the last two pollen seasons; and (iii) positive skin prick tests (SPTs) for the relevant pollen extracts. Exclusion criteria were as follows: (i) previous immunotherapy for any pollen allergen; and (ii) any other severe chronic disease. To investigate the subset of the study population also affected by PFS, we used the occurrence of local oral symptoms induced by plant foods (OAS) as a secondary inclusion criterion. Parents or tutors of all participants provided informed written consent to clinical investigations. The study design and the procedures were approved by the ethical committee of each participating center.

### Questionnaire

Internationally validated questionnaires were devised to recruited children's parents: the International Study of Allergy

and Asthma in Childhood (ISAAC) (15), Allergic Rhinitis and its Impact on Asthma (ARIA) classification (16), and the Global Initiative for Asthma (GINA) (17). Additionally, demographic data, history of atopic disease, presence of PFS, and implicated foods were recorded. SAR was classified as intermittent or persistent, mild or moderate–severe according to the international guidelines 'ARIA classification' (16). In this population of children with pollen allergy, reported symptoms of OAS such as itching of the oral mucosa, with or without edema of the lips or tongue or respiratory symptoms, within 5 min of the ingestion of pollen-related foods, were diagnosed as pollen-food syndrome (PFS). The offending food(s) was/were defined by cross-checking a list of 33 plant foods (11 vegetables, 13 fruits, 4 nuts, and 5 legumes and seeds) included in the questionnaire. An informatics platform ('AllergyCARD™'; TPS Production, Rome, Italy) was used for data input.

### Skin prick tests

SPTs were performed with a panel of commercial extracts (ALK-Abelló, Italy) of airborne allergens such as timothy grass (*Phleum pratense*), Bermuda grass (*Cynodon dactylon*), olive tree, cypress, mugwort, pellitory, birch, ragweed, plane tree, Russian thistle, goosefoot, oak, and hazel. Histamine 0.1 mg/ml and glycerol solution were the positive and negative controls, respectively. Morrow Brown needles were used to prick the skin. Readings were taken at 15 min, and a wheal ≥3 mm was regarded as positive.

### IgE assays

IgE assays were performed to determine total IgE antibodies and IgE antibodies to pathogenesis-related class 10 protein from birch rBet v 1, profilin from timothy grass rPhl p 12, and nonspecific lipid transfer protein from peach rPru p 3 by ImmunoCAP FEIA (ThermoFisher Scientific, Sweden). Results were expressed in kU/l. Levels equal to or exceeding 0.35 kU/l were considered positive.

### Statistics

The average concentration of IgE antibodies to molecules in positive serum samples was calculated as geometric means. Data were summarized as numbers ($n$) and frequencies (%) if they were categorical and as mean and standard deviation (18) if quantitative. To evaluate the normal distribution of quantitative data, the Shapiro–Wilk test was applied. If the data were normally distributed a two-tailed unpaired t-test or otherwise a nonparametric Mann–Whitney $U$-test was applied to compare results between groups. Chi-square test ($\chi^2$) or Fisher's exact test was used to compare frequencies between groups. The Mantel–Haenszel linear-by-linear association chi-square test was used to compare trends over age category for male and female group separately supposing an alternative hypothesis of increasing trend. A $P$ value <0.05 was considered statistically significant. An unsupervised hierarchical agglomerative (bottom-up) clustering method was applied to identify potential groups within the PFS population ($n$ = 294). First, each patient began in its own cluster; thereafter, at every iteration, pairs of clusters were merged into a larger cluster until a unique cluster was formed (according to Ward's criterion) to minimize the total within-cluster variance. Gower standardization was used to calculate the dissimilarity matrix. All variables were equally weighted in the analysis. Variables with a number of missing values >40 were excluded from the analysis (SPT for hazelnut, goosefoot, ragweed, oak, and Russian thistle) and patients with at least one missing value for other variables were excluded from analysis ($n$ = 6). Cluster analysis considered offending foods with a frequency higher than 5%. The number of clusters was chosen according to the dendrogram and their interpretation. To compare the differences among the groups, chi-square test or Fisher's exact test was used for categorical variables, while the Mann–Whitney $U$-test (between two groups) or Kruskal–Wallis test (between more than two groups) was used for not-normally distributed continuous variables. Statistical analyses were performed with R (R Core Team, 2014) and IBM SPSS Statistics for Windows, version 21.0.

## Results

### Study population

A total of 1271 pediatric patients with SAR aged between 4 and 18 years (68% males) were included in the study (Table 1). Three hundred patients (23.6%) (180 males, mean age 10.9 ± 0.4 years (95% CI)) reported to have experienced typical PFS manifesting with symptoms restricted to the oral cavity (immediate oral itching, with or without angioedema of lips and/or tongue) following the ingestion of at least one plant-derived food. Two hundred and twenty-nine of them (73.6%) reported symptoms in the previous 12 months. Nine hundred and seventy-one (685 males, mean age 10.2 ± 0.2 years (95% CI)) did not report PFS.

### Characteristics and risk factors of PFS

PFS was observed starting from preschool-aged children (26/108 at 5 years of age, 24%) and its frequency increased progressively with age ($P$ for trend 0.023 in female gender, $P$ for trend 0.888 in male gender) (Fig. 1). Although males were observed more often than females (68%), this unbalance was less evident among children with SAR and PFS than among those without PFS (60% *vs* 71%, $P < 0.001$). PFS was associated with older age, parental atopy (at least one parent affected by one disease among SAR, asthma or atopic dermatitis), the mother affected by PFS, and environmental tobacco smoke (at least one parent smoking) ($P < 0.001$) (Table 1). Age at onset of SAR and severity of SAR were not statistically different between patients with PFS and without PFS. Patients with PFS showed a significantly longer SAR duration than patients without PFS (5.8 ± 0.3 years *vs* 5.0 ± 0.2 years (95% CI), $P < 0.001$). Patients with PFS manifesting as OAS were also more frequently affected by

**Table 1** Characteristics and risk factors of PFS in 1271 children with seasonal allergic rhinoconjunctivitis

| | With PFS | | Without PFS | | |
|---|---|---|---|---|---|
| Number | 300 | | 971 | | *P*-value* |
| Age (years) (mean, SD) | 10.9 | 3.5 | 10.2 | 3.4 | 0.004 |
| Males (*n*, %) | 180 | 60 | 685 | 71 | <0.001 |
| Atopy in the family | | | | | |
| Any atopic parent (*n*, %)† | 234 | 78 | 637 | 66 | <0.001 |
| Mother with PFS (*n*, %) | 31 | 10 | 35 | 4 | <0.001 |
| Father with PFS (*n*, %) | 10 | 3 | 19 | 2 | 0.240 |
| Geographic Area (*n*, %) | | | | | |
| North | 120 | 40 | 275 | 28 | |
| Centre | 133 | 44 | 465 | 48 | 0.017 |
| South and Islands | 47 | 16 | 231 | 24 | |
| Temporal characteristics of seasonal allergic rhinoconjunctivitis | | | | | |
| Age at onset of allergic rhinoconjunctivitis (years) (mean, SD) | 5.1 | 2.8 | 5.3 | 3.1 | 0.047 |
| Duration of allergic rhinoconjunctivitis (years) (mean, SD) | 5.8 | 3.3 | 5.0 | 3.3 | <0.001 |
| Moderate to severe allergic rhinconjunctivitis (*n*, %) | 116 | 39 | 398 | 41 | 0.516 |
| Environmental tobacco smoke‡ (*n*, %) | 164 | 55 | 440 | 45 | 0.007 |
| Allergic comorbidities | | | | | |
| Number of comorbidities (mean, SD) | 1.6 | 1.2 | 0.9 | 0.9 | <0.001 |
| Asthma (*n*, %) | 140 | 47 | 342 | 35 | <0.001 |
| Anaphylaxis (*n*, %) | 29 | 10 | 46 | 5 | 0.002 |
| Urticaria and/or angioedema (*n*, %) | 110 | 37 | 148 | 15 | <0.001 |
| Atopic dermatitis (*n*, %) | 144 | 48 | 321 | 33 | <0.001 |
| Gastrointestinal symptoms (*n*, %) | 44 | 15 | 43 | 4 | <0.001 |
| Atopic reactivity | | | | | |
| Overall SPT reactivity to pollens (mm) (mean, SD) | 60.0 | 2.1 | 41.0 | 1.0 | <0.001 |
| Levels of total IgE (kU/l) (mean, SD)§ | 2.64 | 0.03 | 2.55 | 0.02 | 0.001 |
| IgE reactivity to one or more group of panallergens (*n*, %) | 229 | 76 | 457 | 47 | <0.001 |
| IgE to Phi p 12 (>0.35 kU/l) (*n*, %) | 97 | 32 | 199 | 20 | <0.001 |
| IgE to Bet v 1 (>0.35 kU/l) (*n*, %) | 108 | 36 | 192 | 20 | <0.001 |
| IgE to Pru p 3 (>0.35 kU/l) (*n*, %) | 128 | 43 | 214 | 22 | <0.001 |

*Chi-square test, when condition was respected, or Fisher's exact test was used to compare frequencies: *T*-test for normally distributed independent samples and Mann–Whitney *U*-test for not normally distributed independent samples.
†At least one among hay fever, asthma, or atopic dermatitis.
‡At least one parent smoking.
§Log-transformed data was used.

allergic comorbidities (asthma, anaphylaxis, urticaria and/or angioedema, atopic dermatitis, gastrointestinal symptoms) with a mean number of comorbidities statistically higher ($1.6 \pm 0.14$ *vs* $0.9 \pm 0.06$ (95% CI)). We further observed a relevant north–south gradient in the frequency of PFS: 30.4% to in Northern Italy, 22.2% in Central Italy, and 16.9% in Southern Italy and Islands ($P < 0.001$).

### IgE sensitization to panallergens

We investigated the association between specific IgE sensitization to three panallergens (Phl p 12 as profilin, Bet v 1 as pathogenesis-related protein class 10, Pru p 3 as lipid transfer protein) and PFS. A total of 229 of 300 subjects (76.3%) with PFS showed the presence of specific IgE to at least one of these three panallergens, with a significantly higher frequency than patients without PFS ($P < 0.001$) (Table 1). Five possible combinations of IgE sensitization to the three panallergens were all well represented among 300 children with PFS: patients sensitized to one panallergen (profilin, nsLTP or PR-10), also called '*mono-panallergen*'; patients cosensitized to more than one panallergen ('*multi-panallergen*'); and patients not sensitized to any panallergen ('*no-panallergen*') (Fig. 2). Relevant and statistically significant differences were observed in the profile of the reported offending foods among the five groups (Fig. 3). The group of 46 children with SAR and PFS sensitized to Phl p 12 reported a significantly more frequent reactivity to the ingestion of *Cucurbitaceae* (melon, watermelon) and kiwifruit. The group of 39 patients with PFS sensitized to Bet v 1 reported more frequently reactions to ingestion of *Rosaceae* fruits (apple, peach), fennel, peanut, and walnut. Among the 59 patients with PFS sensitized to Pru p 3, hazelnut, peanut, peach, apple, and kiwifruit were the most frequent offending plant-derived foods. Among the 71 patients negative to all three panallergens, the mean number of reported offending foods was significantly lower than among the group of 75 patients cosensitized to at least two

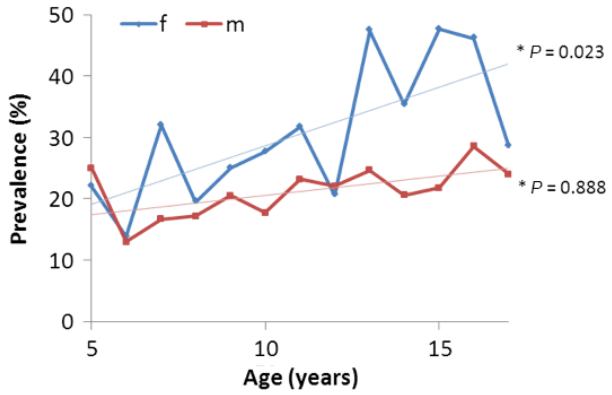


**Figure 1** Distribution of PFS frequency among 1271 children with SAR aged 4–18 years according to gender and age. *One-sided Mantel–Haenszel chi-square test for trend was used to compare trends over age category.

panallergens (mean 1.5, SD 1.9 *vs* mean 4.2, SD 3.5, $P < 0.001$); however, this group was characterized by a great reactivity to kiwifruit (42%).

### Sequential cluster analysis

To verify and identify endotypes of patients within the PFS population ($n = 294$), a hierarchical agglomerative cluster analysis was applied (Fig. 4). Two major clusters of patients were observed. The first cluster analysis defined patients (Cluster 1, $n = 85$) living in Northern Italy (84%) as having a very high frequency of comorbidities (in particular asthma, urticaria/angioedema, and atopic dermatitis), high total IgE levels, multiple pollen sensitization and cosensitization to >1 panallergen ('*multi-panallergen*'; Bet v 1 = 69%, Phl p 12 = 38%, Pru p 3 = 31%), and a high number of foods triggering oral symptoms (such as *Rosaceae*, *Apiaceae*, kiwifruit, peanut, hazelnut, walnut, and melon) (Table 2). The differences between the two major clusters dominated over differences within clusters (Fig. 4A); therefore, a second cluster analysis was applied using a sequential approach to the group of patients having less severe disease (Cluster 2, $n = 209$) (Fig. 4B). This step revealed four clusters of patients:

- The Cluster 2a ($n = 63$) included mostly children living in Central Italy, sensitized to profilin (Phl p 12), fagales, plantain, plane, and olive trees but not to pellitory, with high serum total IgE levels and oral symptoms elicited mainly by *Cucurbitaceae*, banana, peach, and kiwifruit; clinically, they were frequently characterized by a moderate–severe degree of SAR and asthma (Fig. 4B; Table 2);
- the Cluster 2b ($n = 36$) mainly included children living in Southern Italy, sensitized to nsLTP (Pru p 3) and to pellitory; their SAR started earlier in childhood, and oral symptoms were more frequently triggered by *Rosaceae,* banana, peanut, and hazelnut; clinically, cutaneous symptoms (urticaria/angioedema and atopic dermatitis) were recurrent (Fig. 4B; Table 2);
- the Cluster 2c ($n = 66$) included mostly patients living in Central Italy and not sensitized to any of the three panallergens ('*no-panallergen*' group), whose oral symptoms were induced very frequently only by kiwifruit (Fig. 4B; Table 2);
- the Cluster 2d ($n = 44$) included patients living in Central Italy mostly sensitized to PR-10, and whose oral symptoms were triggered mainly by apple, peach, and kiwifruit (Fig. 4B; Table 2).

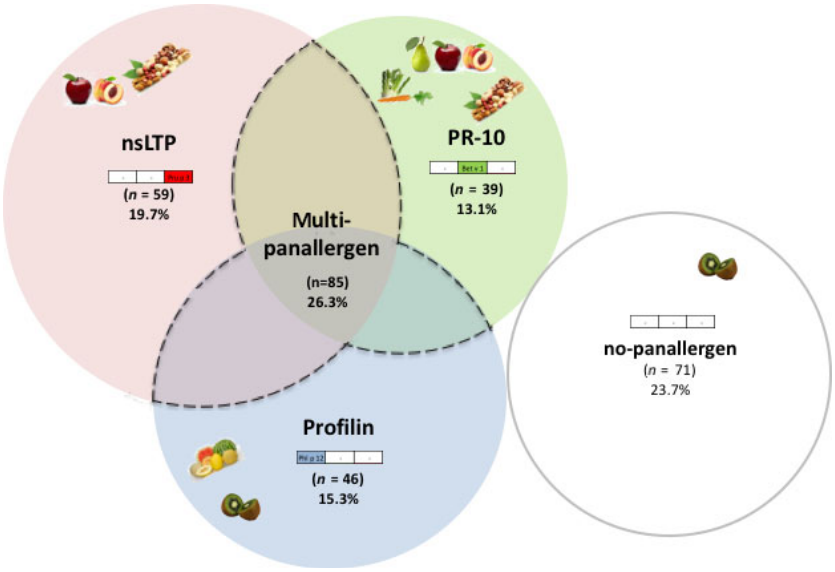


**Figure 2** Proportional Venn diagram of children with IgE sensitization profiles to three panallergens (Phl p 12, profilin; Bet v 1, PR-10; Pru p 3, nsLTP) generating five sensitization profiles in 300 Italian children with PFS. Frameworks represent diagnostic relevant foods triggering oral symptoms.

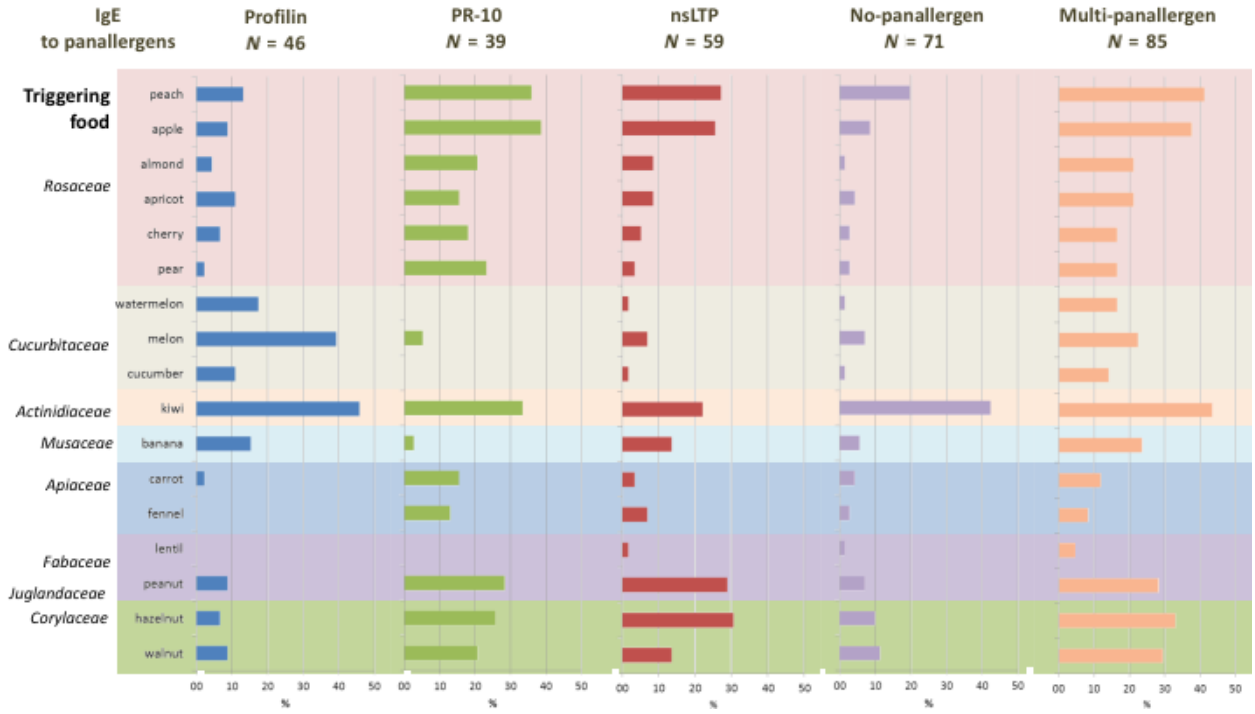


**Figure 3** Foods triggering oral symptoms in children with SAR and sensitized to one of three panallergens (Phl p 12, Bet v 1, and Pru p 3), not sensitized to any panallergen (no-panallergen), and cosensitized to more than one panallergen (multi-panallergen).

## Discussion

By testing IgE sensitization to three panallergens (PR-10, nsLTP, profilin) we found five endotypes of pollen-food syndrome in Italian children with Seasonal Allergic Rhinitis. These endotypes were recognized first with an inductive, descriptive statistical approach and then confirmed by an unsupervised hierarchical agglomerative cluster analysis. To our knowledge, this is the first report proposing such classification of PFS in childhood. In Northern European countries, birch pollen sensitization leads in a considerable part of the affected patients to PFS after contact with plant food (19). Conversely, polysensitization to a variety of pollens associated with food allergy manifesting as OAS is typical of Southern European countries (8, 10, 11). Thus, our findings not only confirm previous observation in adults (19), but also highlight that PFS is also in childhood very complex and more frequent than previously reported (20), occurring even at preschool age, with a prevalence steadily growing with age.

### 'Multi-panallergen PFS'

Among the five endotypes, Cluster 1, '*multi-panallergen*', encompasses children cosensitized to two or all three panallergens (PR-10, profilin, nsLTP). Most of these patients live in Northern Italy (84%), a region characterized by a continental climate, where hypersensitivity to birch (and alder) is more common than in Central and Southern Italy (18). Our children with '*multi-panallergen*' PFS tend to a more severe atopic disease, with high serum total IgE level, a broad spectrum of foods triggering oral symptoms and a higher frequency of allergic symptoms, which include asthma, atopic dermatitis, urticaria, and anaphylaxis. The characteristics of this endotype, combining IgE cosensitization to more allergic comorbidities, confirm the hypothesis posed by Bousquet et al. (21), proposing that multiple IgE reactivity to unrelated molecules are linked to co-occurrence of allergic diseases (allergic multimorbidities). In childhood, respiratory allergy starts by monosensitization followed by quick development of polysensitization (21) and, within the same allergenic source, it follows a 'molecular spreading' phenomenon (22). As many allergenic pollens and foods contain more than one panallergen, the pathway leading to a '*multi-panallergen*' PFS is probably a further example of 'molecular spreading' of IgE sensitization, involving in this case highly cross-reactive instead of species-specific molecules. Moreover, the identification of a severity-related PFS endotype will provide an important model for future studies targeted at understanding the biological mechanisms of IgE-mediated multisensitization and multimorbidities.

### 'Mono-panallergen PFS'

Clusters 2a, 2b, and 2d included predominant IgE sensitization to profilin, nsLTP, and PR-10, respectively ('*mono-panallergen*'). All these three endotypes were characterized by less severe disease, expressed by infrequent comorbidities and a relatively defined spectrum of triggering foods.

'Profilin PFS' – In '*profilin PFS*', oral symptoms are commonly triggered, as in adults (9, 23), by *Cucurbitaceae* (24)

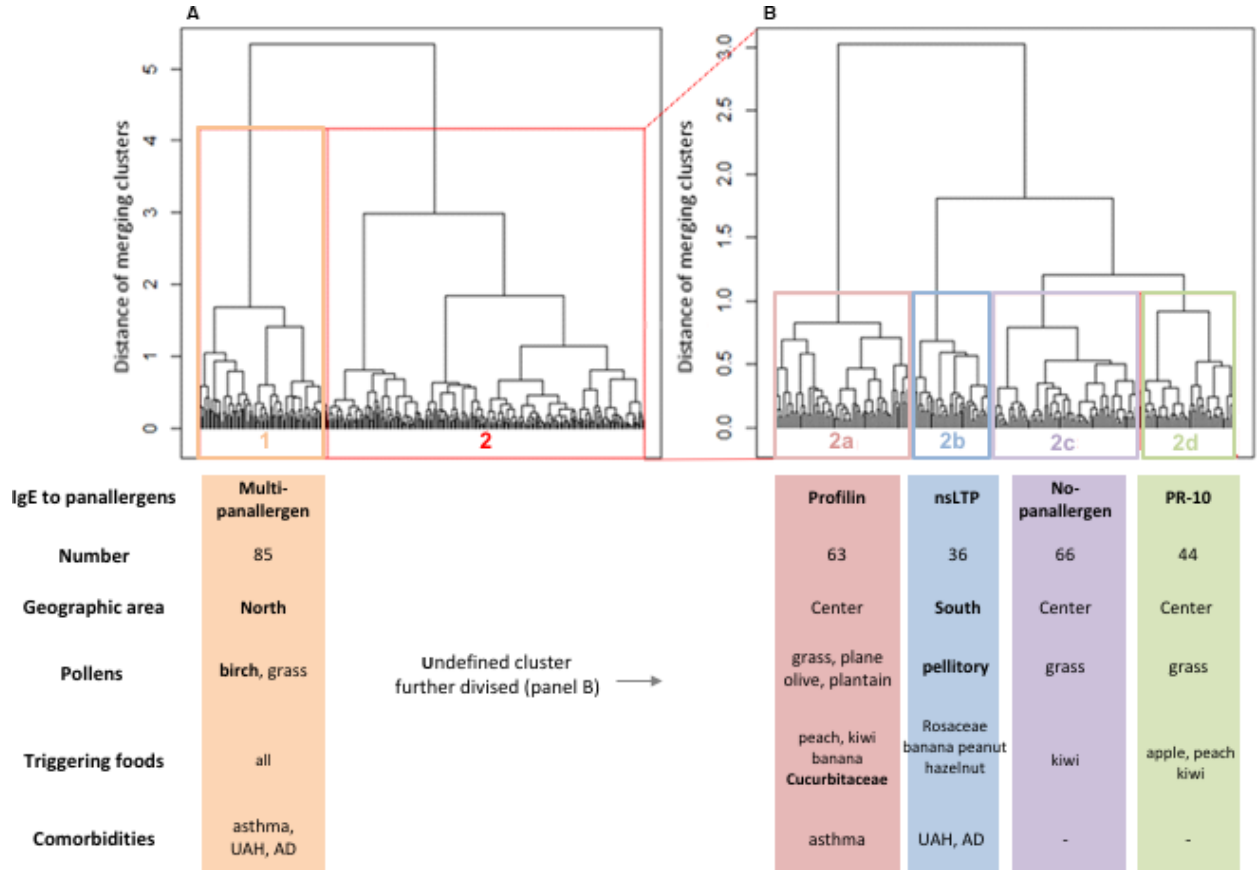


**Figure 4** Endotypes of PFS and their major characteristics defined by hierarchical cluster analysis: (A) A first cluster analysis was applied on the entire PFS population ($n$ = 294). The first cluster analysis defined patients (Cluster 1, $n$ = 85) living in Northern Italy (84%) as having a very high frequency of comorbidities (in particular asthma, urticaria/angioedema, and atopic dermatitis), high total IgE levels, multiple pollen sensitization and cosensitization to >1 panallergen ('*multi-panallergen*'), and a high number of foods triggering oral symptoms. Cluster 2 included 209 children with heterogeneous characteristics requiring further subclassification. (B) With a sequential approach, a second cluster analysis was applied to the 209 children belonging to the Cluster 2. This second step revealed four additional clusters of patients (clusters 2a, 2b, 2c, and 2d), this time with homogeneous and interesting characteristics. Labels of clusters are indicated for each group in the box. Main characteristics of clusters are explained in the table below the graphs (further details are in Table 2).

(melon, watermelon, and cucumber), peach, banana, and kiwifruit. This endotype was linked to a high serum level of total IgE, characterized by sensitization to birch, plantain, plane, and olive trees, and it was more common in Central Italy.

'LTP PFS' – The '*LTP PFS*' endotype is spread in Southern Italy (25), where sensitization to pellitory is very frequent, birch is rare, and oral symptoms are triggered mostly by *Rosaceae* (apple, peach, pear), banana, and nuts. This peculiar constellation confirms that PFS is strongly associated with sensitization to nsLTP in birch-free regions (26). Moreover, our results confirm that IgE sensitization to nsLTP, when secondary to pollen sensitization (class 2 food allergy), is frequently limited to oral symptoms (27), while it is more severe when generated by primary sensitization to food containing nsLTP (25).

'PR-10 PFS' – The '*PR-10 PFS*' affects children living in Central Italy, where birch is rare, and the PR-10 bearing pollens belong mostly to other *Fagales* (Quercus spp., Juglans spp., Carpinus spp., and others) (28). In *PR-10 PFS*, oral symptoms are triggered mainly by apple, peach, and kiwifruit. These findings confirm as well that also in a Mediterranean country, many patients with OAS to apple and peach are not sensitized to LTP but to PR-10s (29). However, the PR-10 molecules involved do not belong to birch, possibly explaining why no correlation between PR-10 sensitization and OAS triggered by hazelnut was found in this cluster.

### 'No-panallergen PFS'

The fifth, '*no-panallergen* PFS' endotype is a very interesting one. The PFS-affected children belonging to this endotype were sensitized to none of the three examined panallergenic molecules (PR-10, nsLTP, and profilin). They revealed a very mild disease, low frequency of comorbidities, and the highest frequency of mild SAR. Over 40% of these children reacted to kiwifruit (26, 30, 31). Our study highlights that kiwifruit

**Table 2** Characteristics of 294 Italian children with SAR and PFS according to hierarchical agglomerative cluster analysis[†,‡,§]

| | PFS patients ($n$ = 294) | | | | | PFS Cluster 2 patients ($n$ = 209) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cluster 1¶ $n$ = 85 | | Cluster 2 $n$ = 209 | | $P$* | Cluster 2a $n$ = 63 | | Cluster 2b $n$ = 36 | | Cluster 2c $n$ = 66 | | Cluster 2d $n$ = 44 | | $P$* |
| Male ($n$, %) | 54 | 64 | 121 | 58 | 0.446 | 35 | 56 | 17 | 47 | 45 | 68 | 24 | 55 | 0.178 |
| Age (median, IQR) | 11 | 8–14 | 11 | 9–14 | 0.857 | 12 | 10–15 | 9 | 7–12 | 11 | 7–13 | 12 | 11–14 | <0.001 |
| Parents with PFS ($n$, %) | | | | | | | | | | | | | | |
| 0 | 24 | 28 | 72 | 34 | | 21 | 33 | 15 | 42 | 15 | 23 | 21 | 48 | |
| 1 | 39 | 46 | 99 | 47 | 0.287 | 33 | 52 | 10 | 28 | 37 | 56 | 19 | 43 | 0.013 |
| 2 | 22 | 26 | 38 | 18 | | 9 | 14 | 11 | 31 | 14 | 21 | 4 | 9 | |
| Geographic area ($n$, %) | | | | | | | | | | | | | | |
| North | 71 | 84 | 44 | 21 | | 15 | 24 | 0 | 0 | 22 | 33 | 7 | 16 | |
| Center | 10 | 12 | 122 | 58 | <0.001 | 41 | 65 | 14 | 39 | 39 | 59 | 28 | 64 | <0.001 |
| South and Island | 4 | 5 | 43 | 21 | | 7 | 11 | 22 | 61 | 5 | 8 | 9 | 20 | |
| SAR onset age (median, IQR) | 4 | 3–6 | 4 | 3–7 | 0.420 | 6 | 3–6 | 3 | 3–4 | 4 | 3–6 | 6 | 3–8 | <0.001 |
| Mod/severe SAR ($n$, %) | 37 | 44 | 118 | 56 | 0.060 | 46 | 73 | 18 | 50 | 25 | 38 | 29 | 66 | <0.001 |
| Comorbidities ($n$, %) | | | | | | | | | | | | | | |
| Asthma | 65 | 76 | 70 | 33 | <0.001 | 28 | 44 | 10 | 28 | 16 | 24 | 16 | 36 | 0.089 |
| Anaphylaxis | 18 | 21 | 10 | 5 | <0.001 | 2 | 3 | 3 | 8 | 4 | 6 | 1 | 2 | 0.579 |
| Urticaria/angioedema | 48 | 56 | 58 | 28 | <0.001 | 12 | 19 | 21 | 58 | 19 | 29 | 6 | 14 | <0.001 |
| Atopic Dermatitis | 60 | 71 | 79 | 38 | <0.001 | 15 | 24 | 27 | 75 | 22 | 33 | 15 | 34 | <0.001 |
| Gastrointestinal symptoms | 26 | 31 | 18 | 9 | <0.001 | 4 | 6 | 7 | 19 | 6 | 9 | 1 | 2 | 0.063 |
| Offending foods ($n$, %) | | | | | | | | | | | | | | |
| *Rosaceae* | | | | | | | | | | | | | | |
| Apple | 36 | 42 | 35 | 17 | <0.001 | 5 | 8 | 18 | 50 | 0 | 0 | 12 | 27 | <0.001 |
| Peach | 37 | 44 | 47 | 22 | 0.001 | 14 | 22 | 11 | 31 | 4 | 6 | 18 | 41 | <0.001 |
| Almond | 32 | 38 | 2 | 1 | <0.001 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0.235 |
| Apricot | 27 | 32 | 10 | 5 | <0.001 | 4 | 6 | 3 | 8 | 1 | 2 | 2 | 5 | 0.369 |
| Cherry | 19 | 22 | 9 | 4 | <0.001 | 6 | 10 | 3 | 8 | 0 | 0 | 0 | 0 | 0.004 |
| Pear | 18 | 21 | 10 | 5 | <0.001 | 2 | 3 | 4 | 11.1 | 1 | 2 | 3 | 7 | 0.121 |
| *Cucurbitaceae* | | | | | | | | | | | | | | |
| Watermelon | 12 | 14 | 11 | 5 | 0.012 | 11 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | <0.001 |
| Melon | 19 | 22 | 29 | 14 | 0.108 | 24 | 38 | 1 | 3 | 3 | 5 | 1 | 2 | <0.001 |
| Cucumber | 8 | 9 | 10 | 5 | 0.177 | 9 | 14 | 1 | 3 | 0 | 0 | 0 | 0 | <0.001 |
| *Actinidaceae* | | | | | | | | | | | | | | |
| Kiwi | 48 | 56 | 66 | 32 | <0.001 | 28 | 44 | 2 | 6 | 28 | 42 | 8 | 18 | <0.001 |
| *Musaceae* | | | | | | | | | | | | | | |
| Banana | 21 | 25 | 18 | 9 | <0.001 | 10 | 16 | 4 | 11.1 | 4 | 6 | 0 | 0 | 0.014 |
| *Apiaceae* | | | | | | | | | | | | | | |
| Fennel | 13 | 15 | 4 | 2 | <0.001 | 1 | 2 | 2 | 6 | 1 | 2 | 0 | 0 | 0.381 |
| Carrot | 18 | 21 | 4 | 2 | <0.001 | 1 | 2 | 0 | 0 | 2 | 3 | 1 | 2 | 0.915 |

**Table 2** (continued)

| | PFS patients (*n* = 294) | | | | | PFS Cluster 2 patients (*n* = 209) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cluster 1¶ *n* = 85 | | Cluster 2 *n* = 209 | | *P** | Cluster 2a *n* = 63 | | Cluster 2b *n* = 36 | | Cluster 2c *n* = 66 | | Cluster 2d *n* = 44 | | *P** |
| *Fabaceae* | | | | | | | | | | | | | | |
| Peanut | 38 | 45 | 22 | 11 | <0.001 | 6 | 10 | 9 | 25 | 4 | 6 | 3 | 7 | 0.034 |
| *Corylaceae* | | | | | | | | | | | | | | |
| Hazelnut | 48 | 56 | 18 | 9 | <0.001 | 5 | 8 | 11 | 31 | 2 | 3 | 0 | 0 | <0.001 |
| *Juglandaceae* | | | | | | | | | | | | | | |
| Walnut | 40 | 47 | 13 | 6 | <0.001 | 6 | 10 | 0 | 0 | 4 | 6 | 3 | 7 | 0.282 |
| SPT (mm) (median, IQR) | | | | | | | | | | | | | | |
| Timothy grass | 6.0 | 4–8 | 7 | 5–9 | 0.576 | 8.0 | 6–10 | 5.0 | 1–7 | 6.8 | 4–9 | 6.5 | 4–8 | <0.001 |
| Olive tree | 4.5 | 2–8 | 4 | 0–6 | 0.055 | 5.0 | 2–8 | 0.5 | 0–5 | 2.8 | 0–6 | 4.5 | 0–6 | 0.003 |
| Pellitory | 2.0 | 0–4 | 0 | 0–4 | 0.481 | 0.0 | 0–4 | 3.3 | 0–6 | 0.0 | 0–3 | 2.0 | 0–5 | 0.002 |
| Plantain | 4.5 | 2–6 | 4 | 2–6 | 0.174 | 6.0 | 4–8 | 2.0 | 0–3 | 3.0 | 0–4 | 3.3 | 0–4 | <0.001 |
| Birch | 5.5 | 3–8 | 0 | 0–4 | <0.001 | 3.0 | 0–5 | 0.0 | 0–2 | 0.0 | 0–0 | 4.0 | 0–7 | <0.001 |
| Plane tree | 3.0 | 0–5 | 2 | 0–4 | 0.021 | 4.5 | 3–6 | 0.8 | 0–2 | 0.0 | 0–3 | 0.0 | 0–3 | <0.001 |
| T-IgE (kU/l) (mean ± %95 CI)** | 583 | ±1.3 | 384 | ±1.2 | 0.003 | 590 | ±1.2 | 330 | ±1.5 | 334 | ±1.3 | 288 | ±1.4 | 0.008 |
| IgE to Phl p 12 (*n*, %) | 32 | 38 | 63 | 30 | 0.267 | *57* | 90 | 3 | 8 | 2 | 3 | 1 | 2 | <0.001 |
| IgE to Bet v 1 (*n*, %) | 59 | 69 | 46 | 22 | <0.001 | 13 | 21 | 3 | 8 | 2 | 3 | 28 | 64 | <0.001 |
| IgE to Pru p 3 (*n*, %) | 26 | 31 | 42 | 20 | 0.075 | 7 | 11 | 23 | 64 | 2 | 3 | 10 | 23 | <0.001 |

*Chi-square test, when condition was respected, or Fisher's exact test was used to compare frequencies among groups and Mann–Whitney *U*-test or Kruskal–Wallis test was used to compare mean among two or four groups for their not.

†Variables included in the cluster analysis, but not included in the table: orange, lentil, pepper; SPTs for apple, peanut, hazelnut, peach, Bermuda grass, cypress, mugwort.

‡Continuous variables expressed as median and interquartile range (IQR); categorical variables expressed in absolute.

§Cut points for cluster characteristics: age <10 years; parents with PFS, geographic area >50%; SAR onset <4 years; severity SAR >50%; comorbidities >30%; offending foods 10%; pollens SPT = highest among clusters or >5 mm; T-IgE >500 kU/l; IgE to panallergens >30%.

¶Features that most distinguish one cluster from others highlighted with different colours for each cluster.

**Total IgE (T-IgE) represented as geometric mean and 95% CI.

can be not only the food most frequently triggering PFS in our study population, but also the only one triggering oral symptoms in children with no IgE to Phl p 12, Bet v 1 or Pru p 3. The kiwifruit's molecules responsible for this condition should be further investigated to ascertain whether they cross-react with pollen allergenic molecules or they are only kiwifruit-specific.

### Perspectives

The unsupervised cluster analysis identified the clusters without any *a priori* assignment. Moreover, the stability of the clusters was tested and it was rather robust. Thus, we have decided to use the definition 'endotype', previously used for asthma classification (32), to distinguish different types of PFS that may be generated with distinct pathophysiological mechanisms. The characteristics of patients with PFS sensitized to individual panallergens, corresponding to our '*mono-panallergen*' endotypes, had been already described. Interestingly, we describe here two novel PFS endotypes, namely the '*multi-panallergen*' and the '*no-panallergen*' ones, with peculiar clinical characteristics and triggers. The purely observational nature of our study prevents the formulation of diagnostic or interventional implications. Therefore, prospective studies in cohorts of patients with PFS are now required to test whether the high prevalence, the risk factors, and the new classification of PFS described here can be useful, respectively: (i) to raise awareness about PFS among pediatricians treating children with SAR; (ii) to predict which children, among those with SAR, will or will not develop a PFS during their disease course; and (iii) to help classifying patients with PFS in other countries (e.g., Mediterranean) and among adults. We are currently testing these hypotheses.

### Limitations

We have also to acknowledge some study limitations. The diagnosis of clinical allergy to foods was not based on oral food challenge tests. It is well known that <50% of the reported reactions to food in epidemiological studies are confirmed by a food challenge (33, 34). However, PFS symptoms immediately follow food ingestion and are promptly and specifically recognized by the patients. Accordingly, previous studies have shown that a positive clinical history detects PFS in a sensitive and specific way (19, 25, 35). Moreover, We did not test IgE to thaumatin-like proteins (TLPs) and other relevant cross-reacting molecules (36). However, sensitization to TLPs is quite infrequent and its clinical significance is still debated, and the molecules we tested are those most investigated in the diagnostics of PFS.

## Conclusions

In this study, we have shown that food allergy manifesting as OAS is, in children with pollen allergy, a frequent and complex syndrome that can be classified into five disease endotypes, characterized by sufficiently distinct hallmarks. These endotypes can easily be defined by testing the patients with serum IgE to Phl p 12, Bet v 1, and Pru p 3. A patient's classification based on these biomarkers may be instrumental to develop disease-modifying prevention and treatments, tailored to specific PFS endotypes. Therefore, our results support the integration of a molecular diagnosis in the clinical workup of food allergy manifesting as OAS in children with pollen allergy.

## Acknowledgments

We thank ALK-Abelló and TFS for providing the reagents for the study, TPS Production for the informatics platform AllergyCARD, and Mr. Charles Clawson for revising the English language.

## Conflict of interest

S. Tripodi has received a lecture fee from ThermoFisher (Phadia). A. Dondi has received consultancy fees from Charité University Hospital, Berlin, Germany. P. M. Matricardi has received research support from TFS and lecture fees from TFS and Allergopharma. The rest of the authors declare that they have no relevant conflicts of interest.

## Funding sources

Reagents for this study have been kindly provided by ALK-Abelló (Milan, Italy) (skin test reagents) and ThermoFisher Scientific (IgE assays); the informatics platform 'AllergyCARD™' has been kindly provided by TPS Production, Rome, Italy.

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