

Molecular Recognition Profiles and Clinical Patterns of PR-10 Sensitization in a Birch-Free Mediterranean Area

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Keywords

Bet v 1 · Food allergy · IgE-mediated allergy · Mal d 1 · Oral allergy syndrome · Rhinitis · Respiratory diseases · Urticaria · PR-10 · ImmunoCAP-ISAC

Abstract

Background: The order Fagales represents an important cause of tree-pollen allergy in northern countries. We investigated the IgE recognition profiles, mutual relationships, and association with clinical symptoms of a panel of allergens belonging to the PR-10 family, the main proteins responsible for Fagales allergy (Act d 8, Aln g 1, Api g 1, Ara h 8, Bet v 1, Cor a 1.0101, Cor a 1.0401, Gly m 4, Mal d 1, and Pru p 1). **Methods:** A total of 526 PR-10-reactive subjects living in central and southern Italy were studied by ImmunoCAP-ISAC-112 microarray analysis. **Results:** Overall, Bet v 1 reactivity was the most commonly (74%) observed among PR-10 proteins, but Cor a 1.0101 was the most prevalent in participants aged <6 years, and between 15 and 65 years. Overall, 26% of the PR-10-reactive persons were Bet v 1 negative, whilst 93.6% of the PR-10 polyreactive individuals were Bet v 1 positive. Among the 10 PR-10s evaluated, 100 combina-

tions were recorded. The strongest association was observed between molecules with the highest sequence identities (Bet v 1 and Cor a 1.0101, Cor a 1.0401 or Aln g 1; Mal d 1 and Pru p 1). Bet v 1-, Cor a 1.0101-, and Aln g 1-specific IgE recognition was associated with respiratory symptoms, whilst Ara h 8, Cor a 1.0401, Gly m 4, Mal d 1, and Pru p 1 were selectively linked to an oral allergic syndrome. **Conclusions:** Testing IgE reactivity to a panel of PR-10s in a birch-free area discloses peculiar relationships between clinical phenotypes and sensitization profiles, allowing the identification of novel cluster patterns.

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Introduction

Allergy to pollen and associated plant food proteins represents an important health issue in Western countries [1]. Recently, considerable research effort has been expended in identifying the molecules responsible for IgE sensitization, and 873 allergens are currently included in the WHO/IUIS database (www.allergen.org), 24 of which are classified as PR-10 (pathogenesis-related protein

group 10). PR-10 molecules are 16–18 kDa and defend plants against fungi and other microorganisms, and they share a common tertiary structure [2] (<http://pfam.xfam.org/ clan/CL0209>). The levels of these proteins found in pollen from early flowering Fagales trees, seeds, nuts, vegetable, and fruits are influenced by abiotic and biotic stress [3, 4] to such an extent that different storage conditions can affect their concentration [5]. Cross-reactivity among PR-10s has been demonstrated but, when comparing Bet v 1.0101 and Ost c 1.0101 as representative members of the Betuloideae and Coryloideae botanical subfamilies, at least 25% of IgE epitopes seem to differ [6]. About 38% identity is found by comparing the sequences of PR-10 pollen from trees belonging either to the Betulaceae or the Fagaceae family [7]. PR-10 homologues are also present in a wide range of plant foods [8], where they are localized homogeneously throughout peel and pulp [9, 10]. Such molecules can induce oropharyngeal symptoms (oral allergy syndrome; OAS) in up to 70% of PR-10-reactive individuals, following the ingestion of various fruits or vegetables, such as Rosaceae (Mal d 1 from apple, Pru p 1 from peach), Actinidiaceae (Act d 8 from kiwifruit), or Corylaceae (Cor a 1 from hazelnut) fruits, Fabaceae (Gly m 4 from soybean, Ara h 8 from peanut) legumes, and Apiaceae vegetables (Api g 1 from celery, Dau c 1 from carrot). PR-10s from plant foods are thermolabile, but studies on purified allergens, such as Mal d 1, seem to indicate that other processing techniques, such as pulsed electric field or high-pressure treatment, could have little or no effect on their secondary structure [11]. Severe reactions to soybean have been reported due to stability to the moderate heating of Gly m 4 [12]. Some patients may have food-associated allergic symptoms without rhinoconjunctivitis during the tree-pollen season, with the so-called silent Bet v 1 sensitization [13].

Despite the vast knowledge of PR-10 allergy and its diagnosis, several issues remain unanswered. These include the following: (i) the relationship between PR-10 molecules, IgE reactivity profiles, and clinical symptoms, (ii) the relative diagnostic values of measuring IgE responses to single versus multiple PR-10 molecules, and (iii) the link between pollen and/or food PR-10 reactivity and clinical outcome.

In this cross-sectional study, we aimed to assess the following in a population that is not exposed to Birch but that is PR-10 reactive: (i) the correlation between age and PR-10 sensitization, (ii) the IgE recognition profile, using a series of PR-10 molecules when the Bet v 1 is not the main sensitizer, (iii) whether PR-10 IgE recognition patterns could be associated with specific clinical pheno-

types, and (iv) the possibility of using only a few molecules for a precise diagnosis and for clinical outcome. To this end, unselected Italian patients attending an allergy clinic were screened by IgE microarray for sensitization profiles to food and/or pollen PR-10. Here, we report these sensitization profiles and their association with clinical parameters.

Methods

Recruitment and Characterization of Study Subjects

At the outpatient allergy unit of the dermatological institute IDI-IRCCS, Rome, 3,225 unselected participants born in central or southern Italy presenting between September 2013 and August 2016 were recruited for the study. Their demographic details and clinical data (food-related reactions, allergic rhinitis, bronchial asthma, and dermatological symptoms) were recorded using the TD-Synergy[®] Laboratory Information System (Siemens Healthcare Diagnostics, Munich, Germany) and a customized electronic database. Participants showing sensitization to at least 1 of the 10 currently available PR-10 molecules spotted on the IgE microarray (Act d 8, Aln g 1, Api g 1, Ara h 8, Bet v 1, Cor a 1.0101, Cor a 1.0401, Gly m 4, Mal d 1, and Pru p 1) were included in the study. The study was approved by the Institutional Ethical Committee of IDI-IRCCS (106/CE/2005), and written, informed consent was obtained from patients or caregivers before participation, in accordance with the Declaration of Helsinki.

Study Design

In our cross-sectional study, participants were categorized on the basis of clinical symptoms at the time of the first visit. This information was collected before IgE testing so that clinicians were at that time blinded to PR-10 data. The clinical presentation was categorized as follows: group 1, symptoms unequivocally suggestive of adverse reactions to food, localized to the oral mucosa characterized by itching, burning sensation, or angioedema in the lips, mouth, and/or pharynx (indicative of OAS) due to the ingestion of fruits, vegetables, tree nuts or peanuts; group 2, symptoms of rhinoconjunctivitis and/or bronchial asthma only (respiratory symptoms; RS) with tolerance of all plant-derived foods. Sera from all participants were collected and stored at the time of the first visit.

IgE Antibody Measurements

Serum IgE reactivity was analyzed using the latest commercially available ImmunoCAP-ISAC platform (ThermoFisher Scientific, Uppsala, Sweden) as per the manufacturer's instructions. In brief, ImmunoCAP-ISAC 112 slides were washed, rinsed, and dried at room temperature. Undiluted serum (30 µL) from each patient was pipetted onto the slide and, after 120 min of incubation at room temperature in a humid chamber, the slides were washed, rinsed, and dried. IgE binding was detected by the addition of an Alexa Fluor[®] 532-labeled anti-human IgE antibody (ThermoFisher Scientific). The slides were then washed, rinsed, dried, and stored in the dark until scanning. Images were acquired by scanning allergen biochips with a CapitalBio LuxScan[™] 10K microarray scanner. IgE values were expressed as ISU arbitrary units (ISAC standardized units) corresponding to IgE antibody levels (in the ng/mL range, detection limit 0.1 ISU-E) [14–16].

Table 1. Clinical, demographic, and PR-10 recognition profiles of the participants

	Total (n = 526)	RS (n = 405)	OAS (n = 270)
Sex (M/F)	253/273	183/222	121/149
Age, years	30 ± 17	30.4 ± 16.8	30.1 ± 16.5
Act d 8	0.13 ± 0.52 (15)	0.13 ± 0.55 (14)	0.14 ± 0.47 (18)
Aln g 1	2.33 ± 5.5 (54)	2.5 ± 5.91 (57)	3.14 ± 6.27* (65.9)
Api g 1	0.31 ± 1.33 (17)	0.32 ± 1.32 (16)	0.31 ± 1.24 (19)
Ara h 8	1 ± 5.27 (31)	1.17 ± 5.96 (33)	1.17 ± 4.47 (42.6)
Bet v 1	9.21 ± 17.5 (74)	9.52 ± 16.58 (78)	12 ± 19* (86)
Cor a 1.0101	4 ± 9.63 (71)	4.19 ± 9.75 (76)	5.17 ± 9.69* (80.0)
Cor a 1.0401	2.81 ± 7.37 (57)	2.97 ± 7.18 (58)	3.73 ± 8.65* (67)
Gly m 4	0.59 ± 2.2 (24)	0.61 ± 2.28 (25)	0.73 ± 2.1 (30)
Mal d 1	4.57 ± 12 (62)	4.63 ± 11.66 (63)	5.72 ± 13.4* (75)
Pru p 1	2.54 ± 8.5 (52)	2.68 ± 9.29 (53)	3.4 ± 10.23* (63)

Data are mean ISU-E levels ± standard deviation for each molecule with the prevalence of reactivity in parentheses (%). * $p < 0.05$.

Statistical Analysis

All analyses were performed using the statistical package IBM SPSS, version 23. The TD-Synergy Laboratory Information System was used to collect and search the demographic (age and gender), clinical, and laboratory data of all patients who attended the outpatient allergy clinic and underwent specific IgE testing. The degree of relationship between the quantitative variables studied was analyzed using the Spearman correlation (r) test, given the nonparametric distribution of the observed values.

The association between IgE reactivity to PR-10 and clinical symptoms was studied in univariate and multivariate analyses. In univariate analysis, the Mann-Whitney U test (2 groups) was first used to compare PR-10 continuous IgE values in subjects with or without a given clinical involvement. In addition, each variable of interest was dichotomized (as negative or positive) to study the proportion in the 2 groups thus obtained of subjects with organ involvement. Categorical variables were analyzed using the Pearson χ^2 test. Finally, multiple logistic regression was performed for the clinical variables with dichotomous scores (present/absent) to investigate whether associations between clinical symptoms and reactivity to different PR-10s were present after simultaneously adjusting for other variables of interest. Separate models were produced for each condition, which included all study molecules in addition to sex and age. p values < 0.05 were considered significant.

The relatedness of IgE recognition profiles was examined with the Genesis 1.7.2 program by component analysis [17]. To better depict the relationships among the PR-10s studied, we performed a principal correspondence analysis, which is a multivariate statistical technique that is capable of identifying systematic relationships between the pattern of similarity of the molecules tested and patient's IgE response, by displaying these as points on graphs. The entire data set comprised Act d 8, Aln g 1, Api g 1, Ara h 8, Bet v 1, Cor a 1.0101, Cor a 1.0401, Gly m 4, Mal d 1 and Pru p 1, across all the 526 samples positive for at least 1 PR-10.

Results

Study Population

A total of 526 PR-10-positive individuals (273 females and 253 males, mean age 30 ± 17 years, range 6 months to 82 years) were detected out of the unselected population of 3,225 subjects not exposed to Birch. IgE reactivity to Ara h 8 (36.65 vs. 25.7%) and Cor a 1.0101 (77.7 vs. 64.8%) was significantly more frequent in women than in men ($p < 0.05$). The clinical and molecular characteristics of study patients and the IgE recognition profile for each of the 10 PR-10s tested are summarized in Table 1.

Bet v 1 represented the most commonly recognized PR-10 (74% of PR-10-positive participants), followed by Cor a 1.0101 (71%). Aln g 1, Cor a 1.0401, Mal d 1, and Pru p 1 were positive in more than half of the patients.

When we verified the IgE recognition profile using the 10 PR-10 molecules, we recorded 100 distinct combinations. The top-ranking were Act d 8(–), Aln g 1(+), Api g 1(–), Ara h 8(–), Bet v 1(+), Cor a 1.0101(+), Cor a 1.0401(+), Gly m 4(–), Mal d 1(+), and Pru p 1(+), which were found in 38 patients (7.2%). The second most frequent was monoreactivity to Cor a 1.0101, which was detected in 36 patients (6.8%), and the third was the recognition of all 10 molecules tested, which was recorded in 29 subjects (5.5%; Table 2).

Cor a 1.0101 IgE reactivity was the most prevalent among children under 6 years of age (54.2%), and in patients between 15 and 65 years old (from 75 to 79%;

Table 2. PR-10 molecule recognition profile

	Patients		Act d 8	Aln g 1	Api g 1	Ara h 8	Bet v 1	Cor a 1.0101	Cor a 1.0401	Gly m 4	Mal d 1	Pru p 1
	n	%										
1	38	7.2		+			+	+	+		+	+
2	36	6.8						+				
3	29	5.5	+	+	+	+	+	+	+	+	+	+
4	27	5.1		+	+	+	+	+	+	+	+	+
5	24	4.6					+	+				
6	23	4.4					+					
7	22	4.2										+
8	22	4.2		+		+	+	+	+	+	+	+
9	20	3.8					+	+			+	
10	20	3.8		+		+	+	+	+		+	+
11	16	3.0						+				
12	16	3.0		+			+	+	+		+	
13	15	2.9	+	+		+	+	+	+	+	+	+
14	12	2.3									+	
15	10	1.9		+			+	+			+	

The most frequent PR-10 molecule recognition combination (15/100), encompassing 62.7% of the patients studied, is reported. +, reactive.

Fig. 1). Of 139 patients (26.4%) negative for Bet v 1, 46 (33.1%) were Cor a 1.0101 positive and 30 (21.6%) were Pru p 1 positive.

One hundred and thirty-seven participants (26%) reacted to only 1 of the PR-10s studied, of which 26.3% reacted only to Cor a 1.0101. Of the “PR-10 poly-reactive” population ($n = 389$), 93.6% were Bet v 1 positive, 87.4% Cor a 1.0101 positive, and 80.4% Mal d 1 reactive. Interestingly, 29 of these patients (5.5%) reacted to the entire set of PR-10s tested, and patients positive to more than 7 PR-10s (about one-third of the entire population), were always Bet v 1, Cor a 1.0101, and Mal d 1 positive.

Molecule IgE Recognition Relationship

Amino acid sequence identities between the PR-10 pollen and plant-food allergens studied ranged between 33.5% (among Api g 1 and Ara h 8) and 88% (Pru p 1 and Mal d 1; Table 3b). In keeping with this, after the bivariate analysis of reciprocal relationships of IgE to PR-10s, the strongest positive association was observed among molecules bearing the highest amino acid sequence identities (i.e., among Bet v 1, and Cor a 1.0101, Cor a 1.0401 or Aln g 1, and among Mal d 1 and Pru p 1; Table 3a).

Figure 2 summarizes the results of the principal correspondence analysis, 3-dimensionally showing the relationship of the PR-10s in terms of IgE corecognition. Since this technique places variables in a 3-dimensional

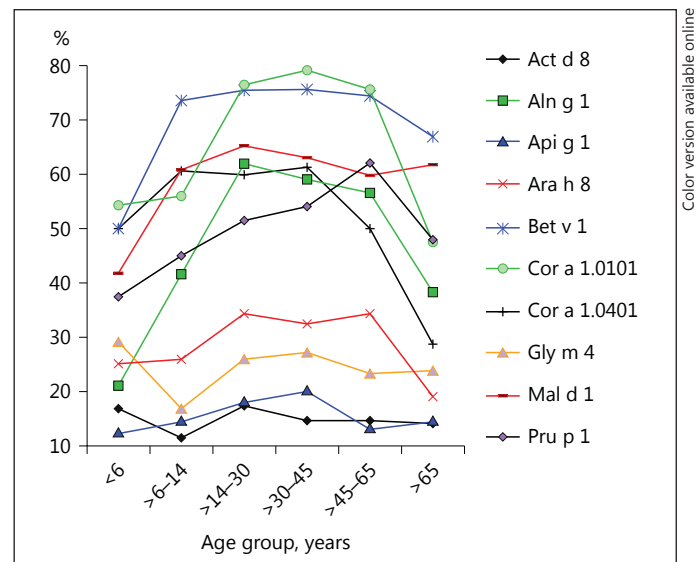


Fig. 1. Age-distribution profiles of PR-10s as a whole, summarizing the IgE recognition trend for each distinct component in a single line. A different pattern of IgE reactivity for each age group was observed. The percentages of each single molecule indicate the proportion of patients positive to that molecule within the category of reactivity.

space based on their levels of reciprocal correlation (i.e., highly correlated variables are close to each other, while uncorrelated variables are placed far away from each other), we observe here that the behavior of pollen-derived

Table 3. Bivariate analysis of reciprocal relationships between the PR-10s studied ($n = 526$ positive samples)

a Spearman rank correlation (rho coefficient, ρ) values and the absolute number of IgE-positive subjects (in parentheses) for paired allergens

	Act d 8	Aln g 1	Api g 1	Ara h 8	Bet v 1	Cor a 1.0101	Cor a 1.0401	Gly m 4	Mal d 1	Pru p 1
Act d 8	(79)									
Aln g 1	0.396 (70)	(284)								
Api g 1	0.358 (37)	0.408 (75)	(89)							
Ara h 8	0.45 (59)	0.614 (146)	0.443 (66)	(165)						
Bet v 1	0.367 (71)	0.819 (265)	0.361 (78)	0.554 (149)	(387)					
Cor a 1.0101	0.322 (70)	0.773 (262)	0.334 (77)	0.502 (151)	0.847 (330)	(376)				
Cor a 1.0401	0.43 (72)	0.779 (239)	0.432 (80)	0.637 (150)	0.784 (272)	0.689 (257)	(300)			
Gly m 4	0.428 (51)	0.594 (117)	0.487 (61)	0.686 (107)	0.54 (120)	0.498 (120)	0.578 (118)	(128)		
Mal d 1	0.411 (73)	0.729 (242)	0.408 (82)	0.568 (146)	0.822 (303)	0.707 (278)	0.779 (252)	0.548 (118)	(325)	
Pru p 1	0.407 (70)	0.706 (212)	0.432 (78)	0.613 (141)	0.707 (243)	0.594 (224)	0.746 (225)	0.576 (114)	0.782 (240)	(273)

b Amino acid identities (%) among the PR-10s studied

	Act d 8	Aln g 1	Api g 1	Ara h 8	Bet v 1	Cor a 1.0101	Cor a 1.0401	Gly m 4	Mal d 1	Pru p 1
Act d 8	Uniprot: D1YSM5									
Aln g 1	50	Uniprot: P38948								
Api g 1	46.2	40	Uniprot: P49372							
Ara h 8	38.3	43.7	33.5	Uniprot: Q6VT83						
Bet v 1	49.3	<i>81.2</i>	40	46.2	Uniprot: P15494					
Cor a 1.0101	48.7	76.2	37.5	42.5	72.5	Uniprot: Q08407				
Cor a 1.0401	52.1	65.8	36.6	52.8	<i>67.1</i>	63.3	Uniprot: Q9SWR4			
Gly m 4	44	47.5	35.4	<i>70.2</i>	46.2	46.2	56.5	Uniprot: C6T1G1		
Mal d 1	50	55.6	38.1	49.3	56.8	56.2	62.1	50.6	Uniprot: Q40280	
Pru p 1	53.7	57.5	40	49.3	59.3	58.7	63.9	52.5	86.8	Uniprot: Q2I6V8

Rho coefficient values from 0.7 to 1.0 indicate a strong positive association, indicated in bold in **a**. Values above 55% are indicated in italics in **b**. The Uniprot accession number is indicated for each molecule (<http://www.uniprot.org/align/>).

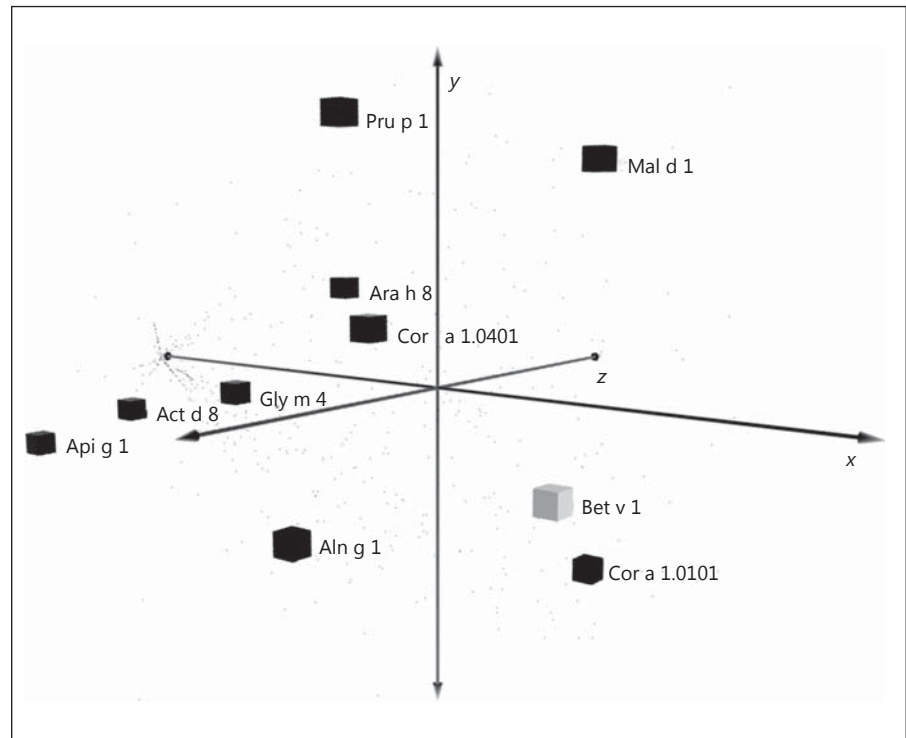


Fig. 2. Multiple correspondence analysis plot, showing the mutual interrelationships between PR-10s (each molecule is represented by 1 dot) in terms of IgE reactivity. The plot illustrates the divergent behavior of IgE recognition in pollen and plant-food PR10s.

molecules (i.e., Bet v 1 and Cor a 1.0101) and plant-food allergens (i.e. Mal d 1 and Pru p 1) is significantly divergent.

IgE Reactivity and Clinical Symptoms

The association between PR-10 reactivity and the clinical outcome documented at the time of sample collection is shown in Figure 3. A history of RS was clearly associated with Bet v 1, Cor a 1.0101, and Aln g 1 reactivity, but not with reactivity to any of the plant-food allergens studied (Fig. 3a). On the other hand, all of the molecules, except for Act d 8 and Api g 1, were significantly associated with a history of food allergy, and accordingly Ara h 8, Cor a 1.0401, Gly m 4, Mal d 1, and Pru p 1 were selectively linked to OAS occurrence (Fig. 3b). Furthermore, ISU-E values of specific IgE to Aln g 1, Bet v 1, Cor a 1.0101, Cor a 1.0401, Mal d 1, and Pru p 1 were also significantly higher in patients with OAS than in PR-10-reactive persons without a food allergy (Table 1).

Aln g 1 ($\chi^2 = 10.962$, $p < 0.001$, OR 1.977, 95% CI 1.316–2.969) and Cor a 1.0101 ($\chi^2 = 7.950$, $p < 0.005$, OR 1.972, 95% CI 1.223–3.180) reactivity was associated with the presence of bronchial asthma, while Aln g 1 ($\chi^2 = 3.924$, $p < 0.05$, OR 1.486, 95% CI 1.003–2.201), Bet v 1

($\chi^2 = 23.308$, $p < 0.0001$, OR 2.342, 95% CI 1.549–3.540), and Cor a 1.0101 ($\chi^2 = 16.734$, $p < 0.0001$, OR 2.754, 95% CI 1.810–4.189) were significantly associated with the presence of rhinoconjunctivitis.

Taking into consideration all the molecules studied along with the factors of age and sex, multiple logistic regression analysis demonstrated a significant relationship between a history of OAS and Ara h 8 ($p < 0.001$, OR 2.77, 95% CI 1.547–4.961), Mal d 1 ($p < 0.03$, OR 1.72, 95% CI 1.029–2.902), and Bet v 1 reactivity ($p < 0.003$, OR 2.298, 95% CI 1.319–4.006). IgE to Bet v 1 ($p < 0.001$, OR 2.892, 95% CI 1.497–5.349) was also linked to a history of RS. Finally, an IgE reactivity to more than 4 PR-10 molecules was significantly associated with OAS ($\chi^2 = 44.163$, $p < 0.0001$, OR 3.302, 95% CI 2.309–4.772).

Discussion

In this study, we used a microarray-based detection system [18] to identify and characterize PR-10 IgE reactivity in a large cohort of unselected patients attending central and southern Italian allergy clinics, and correlated the different PR-10 reactivity profiles with the clinical expression of allergic disease. Notably, this investigation

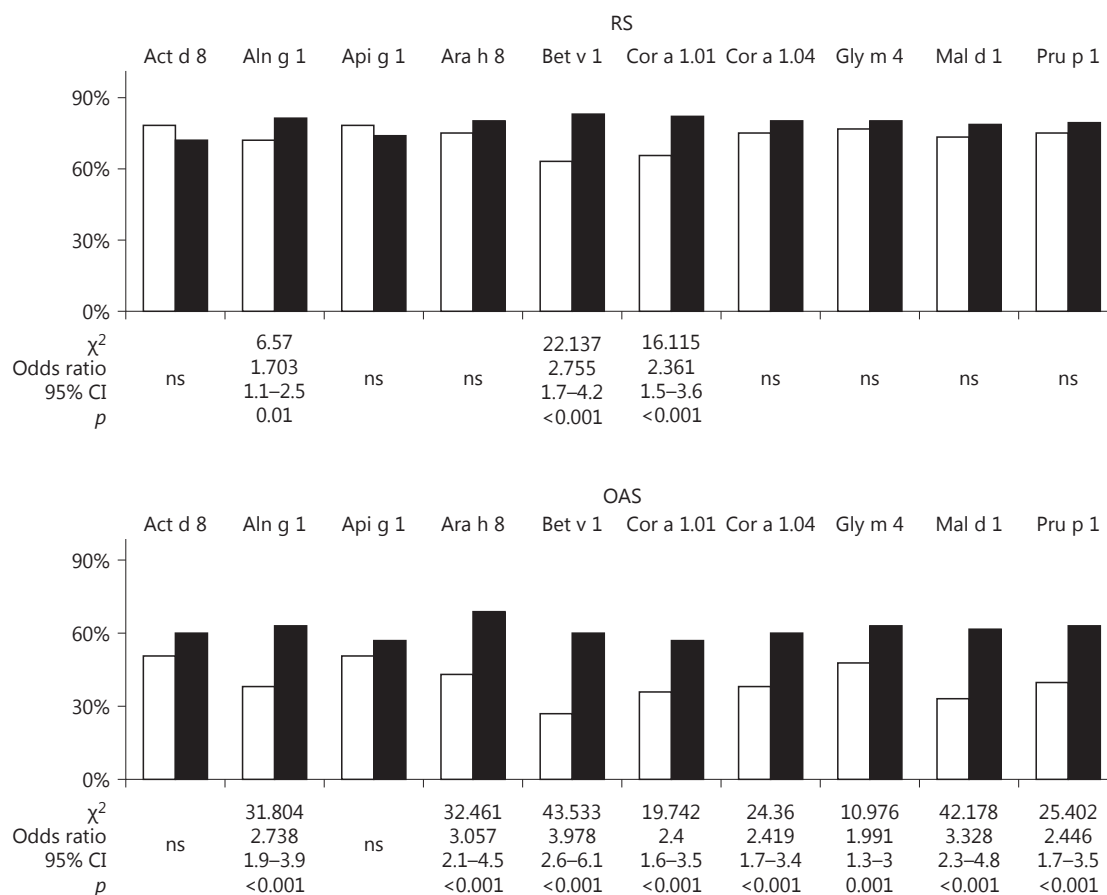


Fig. 3. Frequencies (%) of specific symptoms (RS or OAS) among patients sensitized (black bars) or not sensitized (white bars) to the respective PR-10s. The crude odds ratios and the 95% CIs are reported where significant direct (>1) associations were observed.

was carried out in a Mediterranean area free of birches (and therefore without Bet v 1) but rich in other Fagales sources where PR-10 reactivity is recorded in about 15% of allergic people [19, 20]. It therefore led to a number of novel observations.

The data confirm Bet v 1 as the most commonly recognized PR-10 molecule among those tested (74% of PR-10-positive participants), despite the fact that this molecule is virtually absent in the patients' environment, thus suggesting that Bet v 1 is the molecule that works best to identify PR-10-reactive persons even when it is not the primary sensitizer. Thus, 93% of patients who scored positive on more than 2 PR-10s were Bet v 1 positive. Cor a 1.0101 recognition was more prevalent among children under 6 years of age, as already observed [21], thus sug-

gesting its possible role as a primary sensitizer in the absence of "environmental Bet v 1."

Of our central and southern Italian PR-10-sensitized cohort, 26% were Bet v 1 negative, with about a third of them being Cor a 1.0101 positive, thus suggesting that, although Bet v 1 is generally considered the most representative PR-10 molecule, it might not contain all potential IgE-binding epitopes, and a wider assessment of IgE reactivity through quite a few PR-10 molecules may be useful for the diagnosis of PR-10 reactivity, particularly in populations not exposed to birch pollen [6].

The combined measurement of Bet v 1, Cor a 1.0101, and Mal d 1 identified 86% of the study subjects as PR-10 positive, while only 5.5% of this cohort were immuno-reactive to all 10 PR-10 molecules studied, suggesting a

variable degree of shared epitope recognition. It could be hypothesized that, as mentioned, some patients could be sensitized by either hornbeam or oak pollen. Since those molecules are not present on the chip, we can speculate that more Fagales pollen allergens could be useful for a more accurate diagnostic workup, especially in birch-free areas. In effect, it has been demonstrated that Dau c 1 from carrot [22] and Cor a 1 from hazelnut can sensitize patients in the absence of Bet v 1 sensitization [21]; thus, it is also possible to hypothesize that other PR-10s are able to act as primary sensitizers in areas such as the Mediterranean, where birch pollen levels are low or absent [6].

Our data confirm the heterogeneity of a Bet v 1-specific IgE response, in which the IgE epitopes are dispersed across the entire surface of the molecule; therefore, the recognition profile might differ from 1 patient to another [23]. In addition, the pattern of exposure to pollen might play a key role. The study was performed in an area where patients are mainly exposed to pollen of hornbeam, alder, and oak, with the major allergens Car b 1 [6], Aln g 1, and Que a 1 [24], respectively, belonging to the Bet v 1 family [25]. As shown by Hauser et al. [6], exposure to “non-birch” Fagales pollens is associated with a more heterogeneous pattern of sensitization to PR-10s than in subjects exposed to birch pollen. Accordingly, exactly 100 combinations of hypersensitivity to the 10 currently available PR-10 molecules tested were observed in this study. One limitation to this observation is that our assessment was restricted to the molecular isoforms represented on the ISAC array. Nevertheless, this finding highlights the added value of testing an extended series of homologous molecules when assessing panallergen immunoreactivity [19].

It is believed that a similar protein structure, rather than a similar linear sequence, is the reason for cross-reactivity among pollen (i.e., Bet v 1) and plant food [26], but it has also been demonstrated that isolated Api g 1-specific antibodies could not recognize Bet v 1 [27]. In keeping with that observation, we report significant associations of IgE-recognizing PR-10 molecules bearing the highest amino acid sequence identities, i.e., Bet v 1, Cor a 1.0101, and Aln g 1 on the one hand, and Mal d 1 and Pru p 1 on the other hand, as was observed on a more limited scale [28].

An increased occurrence of allergic rhinitis with elevated Bet v 1-specific IgE levels or higher numbers of PR-10 molecule recognition proteins has been found in a pediatric population [29], and a strict relationship has been detected between the occurrence of OAS and higher Bet v 1 IgE levels [30, 31]. Accordingly, we found higher Aln g 1, Bet v 1, Cor a 1.0101, Cor a 1.0401, Mal d 1, and Pru p 1

IgE levels in patients affected by OAS when compared with PR-10-reactive individuals without food allergy, as also suggested by previous observations with an extract-based diagnostic approach [32, 33], thus demonstrating that a broader range of PR-10 detection can be useful to unveil more aggressive clinical profiles. A history of RS was significantly associated with sensitization to Bet v 1, Cor a 1.0101, and Aln g 1, whilst Ara h 8, Cor a 1.0401, Gly m 4, Mal d 1, and Pru p 1 sensitization, or the immunoreactivity to 4 or more PR-10s, was specifically related to OAS. Interestingly, despite the statistically significant relationship of every single molecule with the respective symptoms, there is also a high percentage of IgE-positive but asymptomatic patients for each of the molecules tested. Such an observation underlines the need for a comprehensive panel of molecules in order to associate the occurrence of a given clinical phenotype with a profile of IgE sensitization, as is also confirmed by multiple logistic regression analysis, where all the analytes were considered as a whole.

In conclusion, important associations between clinical presentation and specific PR-10 sensitization profiles were observed, as well as novel cluster patterns that likely indicate cross-reactivity. In brief, we found that: (i) in a Bet v 1-free area, Bet v 1 is still the most IgE-reactive PR-10 protein, but IgE against Cor a 1.0101 is the most prevalent in patients aged <6 years, (ii) IgE recognition relationships are in agreement with sequence identities, and (iii) Bet v 1, Cor a 1.0101, and Aln g 1 reactivity are associated with RS, whilst Ara h 8, Cor a 1.0401, Gly m 4, Mal d 1, and Pru p 1 are selectively linked to OAS occurrence. Overall, our data demonstrate that multi-molecule evaluation leads to a better assessment of PR-10 diagnosis in a birch-free area.

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Disclosure Statement

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria, educational grants, participation in speakers' bureaus, membership, employment, consultancies, stock ownership, or other equity interest, and expert testimony or patent licensing arrangements), or nonfinancial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this paper.

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