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Co-sensitizations to gibberellin regulated proteins (GRPs) in Italy: results of a polycentric study

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Key words

Gibberellin regulated proteins (GRPs); co-sensitization; PR-10; nsLTP; profilin.

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Impact statement

A high percentage of co-sensitizations to PR-10, Profilin, and nsLTP is present in patients sensitized to GRPs, and PR-10 appears to be a protective factor against anaphylaxis.

Summary

Background. *Gibberellin regulated proteins (GRPs) are small glycoproteins that induce allergy to various types of fruit. This study aimed to evaluate co-sensitization to cypress pollen and other molecules responsible for fruit allergy, such as nsLTP (Pru p 3), PR-10 (Bet v1), and Profilin (Bet v2).* ***Methods.*** *Sixty subjects sensitized to peach GRP (Pru p 7) were consecutively recruited from four Italian centers: 28 males and 32 females (mean age 37.9 years; range 11-79). Specific IgE for Pru p 7, Pru p 3, Bet v 1, Bet v 2, cypress pollen extract (Cup s), and Cup a 1 were determined in all subjects.* ***Results.*** *Sensitization rates to Cup s, Cup a 1, Pru p 3, Bet v 1, and Bet v 2 in the entire studied population were 90.0%, 83.3%, 45.8%, 40.0%, and 30.0%, respectively. In subjects residing in Northern Italy, the respective sensitization rates were 96.4%, 80.0%, 50.0%, 73.3%, and 40.0%, while in those residing in Southern Italy they were 83.3%, 86.7%, 40.0%, 6.7%, and 20.0%. The only significant difference was observed for PR-10 ($p < 0.0001$). Co-sensitization to PR-10 was found to be associated with a reduced risk of anaphylaxis (OR: 0.125). Allergic reactions were most commonly triggered by peach (26/40), followed by orange (12/40), with other foods being less frequently implicated.* ***Conclusions.*** *This study confirms a high association between sensitization to Pru p 7 and cypress pollen and highlights a high percentage of co-sensitization to nsLTP, PR-10, and profilin. PR-10 emerged as a protective factor against anaphylaxis.*

Introduction

Gibberellin regulated proteins (GRPs) are a class of small glycoproteins consisting of 63 amino acids with a low molecular weight of 7 kDa. They possess an isoelectric point (pI) around 9, are water-soluble, and are characterized by a high cysteine content, resulting in the formation of 6 disulfide bridges. This structural feature confers high stability to GRPs, rendering them resistant to proteolysis and heat. Consequently, they have the potential to induce systemic allergic reactions, whether consumed in raw or heat-treated forms. The name of this protein family derives from their regulation by gibberellin, a phytohormone produced by plants in response to various environmental stresses. Gibberellin is also applied exogenously in agriculture to modulate plant growth and fruit ripening. However, the impact of such treatments on the content of GRPs and their allergenic properties remains only partially elucidated.

Although the first identified molecule belonging to GRPs was potato snakin-1 (1), it never demonstrated allergenicity. Thus, the first true allergen belonging to GRPs was identified in peach (*Prunus persica*) and named peamaclein (Pru p 7) (2). Subsequently, allergens belonging to GRPs were identified in other fruits: apricot (*Prunus mume*, Pru m 7) (3), pomegranate (*Punica granatum*, Pun g 7) (4), orange (*Citrus sinensis*, Cit s 7) (5), cherry (*Prunus avium*, Pru av 7) (6), and more recently in pepper (*Capsicum annuum*, Cap a 7) (7). Allergens belonging to this family have also been described in the pollen of *Cupressaceae*, particularly in *Cupressus sempervirens* (Cup s 7) (8), *Juniperus ashei* (Jun a 7) (9), and *Cryptomeria japonica* (Cry j 7) (10). Considering the ubiquity of GRPs, it is highly probable that the list of proteins belonging to this family is destined to expand.

A high sequence identity of amino acids among GRPs has been demonstrated (11), responsible for a high probability of IgE cross-reactivity towards such molecules. In particular, cross-reactivity between Cup s 7 and Pru p 7 (12) and between Cup s 7 and Cit s 7 (13) has been well established. This lead to the hypothesis that GRPs allergy is a pollen/fruit allergy syndrome (PFAS), wherein primary sensitization occurs via the respiratory route through *Cupressaceae* pollen. This hypothesis has been corroborated by epidemiological studies and experimental cross-inhibition studies (14). However, not all individuals with symptoms attributable to sensitization to GRPs exhibit concurrent sensitization to *Cupressaceae*, showing that primary sensitization in some cases occurs not through the respiratory route but directly through dietary intake.

The aim of this multicenter study was to evaluate, in an Italian population sensitized to Pru p 7 (the only GRP currently commercially available), the percentage of co-sensitization to cypress pollen, particularly to the major molecular allergen Cup a 1, which defines its role as the primary sensitizer. Furthermore, co-sensitizations to non-specific Lipid Transfer Protein (nsLTP) of peach (Pru p 3), birch pollen PR-10 (Bet v1), and profilin (Bet v 2) were evaluated to assess whether different profiles exist between Northern and Southern Italy and whether different co-sensitizations may modulate the severity of symptoms in individuals sensitized to GRPs.

Materials and methods

Patients

This study employed a consecutive enrolment design across four Italian centers from Northern (Udine, Pordenone, Florence-Prato) and Southern Italy (Palermo) to recruit subjects sensitized to Pru p 7, identified based on clinical suspicion of fruit allergy. Recruitment occurred from January 2002 to December 2023. Sixty subjects were enrolled, comprised of 28 males and 32 females, with a mean age of 37.9 years (range 11-79). There were no significant demographic differences between Northern Italy (30 subjects, 13 males, and 17 females, mean age 35.7 years, range 11-79) and Southern Italy (30 subjects, 15 males, and 15 females, mean age 40.2 years, range 11-77). Clinical history data were collected from 40 subjects, including foods associated with symptoms and their severity. Symptoms were categorized as "anaphylaxis", defined as the presence of urticaria combined with at least one other symptom affecting the respiratory, gastrointestinal, or cardiovascular systems, or "non-anaphylaxis" (oral allergy syndrome, urticaria, gastrointestinal symptoms, or isolated respiratory symptoms).

Methods

Specific IgE (sIgE) levels for Pru p 7, Pru p 3, Bet v 1, Bet v 2, Cup a 1, and cypress pollen extract (*Cupressus sempervirens*, Cup s) were measured using the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden) following the manufacturer's instructions. Data were expressed in kUA/L, with a cut-off set at 0.1 kUA/L.

Statistics

Statistical analysis was performed using MedCalc version 9.3.9.0 (Mariakerke, Belgium). Percentages of positivity for specific allergens were calculated for all subjects sensitized to Pru p 7 and separately for those from Northern and Southern Italy. Quantitative variables, such as sIgE levels, were analyzed using Student's t-test for unpaired data. Odds ratios (OR) were calculated for the risk of anaphylaxis in relation to co-sensitization towards nsLTP, PR-10, and profilin, individually and collectively. Significance was set at $p < 0.05$.

Ethics

This is a retrospective observational study, therefore no statement regarding studies conducted on humans and animals is required. Informed consent was obtained from all patients included in the study.

Results

The mean sIgE level for Pru p 7 in the entire studied population was 4.3 ± 5.43 kUA/L, with no significant difference between Northern (3.17 ± 6.21 kUA/L) and Southern Italy (5.43 ± 6.7 kUA/L) (p = ns). Sensitization percentages to Cup s, Cup a 1, nsLTP (Pru p 3), PR-10 (Bet v 1), and profilin (Bet v 2) are detailed in **table I**. Significant differences between Northern and Southern Italy were observed for PR-10 co-sensitization (73.3% *vs* 6.7%; $p < 0.0001$).

Among Pru p 7 sensitized individuals, 83.3% showed primary sensitization to *Cupressaceae* pollen, with 90.0% testing positive for the extract. The associations between Pru p 7 and Cup s/Cup

Table I - IgE values for Pru p 7 (mean and range) in all tested samples, divided by origin (Northern and Southern Italy), and percentage of co-sensitization towards cypress extract (Cup s), major cypress allergen (Cup a 1), peach nonspecific lipid transfer protein (Pru p 3), birch PR-10 (Bet v 1), and Profilin (Bet v 2).

	Pru p 7 (GRP) Mean (range)	Cup s n (%)	Cup a 1 n (%)	Pru p 3 (nsLTP) n (%)	Bet v1 (PR-10) n (%)	Bet v 2 (Profilin) n (%)
All (n = 60)	4.3 kUA/L (0.15-27.6)	54/60 (90%)	50/60 (83.3%)	27/60 (45.8%)	24/60 (40%)	18/60 (30%)
Nord (n = 30)	3.17 kUA/L (0.15-27.6)	29/30 (96.7%)	24/30 (80%)	15/30 (50%)	22/30 (73.3%)*	12/30 (40%)
Sud (n = 30)	5.43 kUA/L (0.41-24.5)	25/30 (83.3%)	26/30 (86.7%)	12/30 (40.0%)	2/30 (6.7%)*	6/30 (20%)

*p < 0.0001.

a 1 positivity/negativity are shown in **table II**. Clinical reactions to Pru p 7-associated foods are illustrated in **figure 1**. Peach was the most common trigger for clinical reactions (26/40), followed by orange (12/40), kiwi, lemon, tomato (5/40), strawberry (3/40), and other foods. Anaphylaxis occurred in 55.0% of patients, with no significant difference in sIgE levels between those with and without anaphylaxis. Patients co-sensitized to PR-10 had significantly lower anaphylaxis frequency (p = 0.0278; OR 0.125). Conversely, those with anaphylaxis showed a higher prevalence of Pru p 3 co-sensitization (45.5% *vs* 22.2%; OR 2.916), although not reaching significance (**table III**).

Table II - IgE profile (mono-positive and double-positive) towards cypress extract (Cup s) and its major allergen (Cup a 1) in subjects sensitized to Pru p 7.

		n	%
	Cup s^{Pos}	54	90
	Cup a 1^{Pos}	50	83.3
Cup s^{Pos}	Cup a 1^{Pos}	49	81.6
	Cup a 1^{Neg}	5	8.3
Cup s^{Neg}	Cup a 1^{Pos}	1	1.7
	Cup a 1^{Neg}	5	8.3

Figure 1 - Foods associated with allergic reactions in subjects sensitized to Pru p 7.

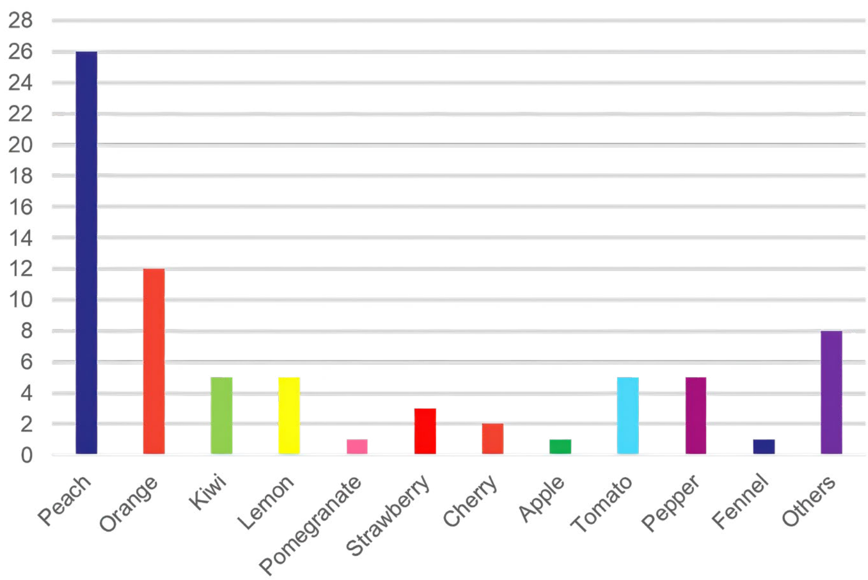

***Table III** - Percentage of patients with anaphylaxis and non-anaphylaxis in co-sensitized to nsLTP (Pru p 3), Bet v 1 (PR-10), profilin (Bet v 2), individually and in combination.*

Co-sensitizations	Anaphylaxis (n = 22)	Non-anaphylaxis (n = 18)	OR (95%CI)	Statistical significance (P)
Pru p 3 (nsLTP)	10/22 (45.5%)	4/18 (22.2%)	2.916 (0.724-11.739)	ns
Bet v 1 (PR-10)	2/22 (9.1%)	8/18 (44.4%)	0.125 (0.022-0.702)	**< 0.03**
Bet v 2 (Profilin)	6/22 (27.2%)	6/18 (33.3%)	0.750 (0.193-2.913)	ns
Bet v 1 and Bet v 2	1/22 (4.5%)	6/18 (33,3%)	0.095 (0.010-0.888)	**< 0.05**
Pru p 3 and Bet v 1	1/22 (4.5%)	1/18 (5.5%)	0.809 (0.047-13.92)	ns
Pru p 3 and Bet v 2	3/22 (13.6%)	1/18 (5.5%)	2.684 (0.254-28.31)	ns

OR: odds ratio; ns: not significant.

Discussion and conclusions

In accordance with previous studies (14, 15), our investigation also revealed that sensitization to Pru p 7 is predominantly associated (83.3%) with primary cypress pollen sensitization, with no significant differences between Northern and Southern Italy, thus confirming the pollen-fruit allergy syndrome (PFAS) hypothesis. However, it remains a 16.7% subset of patients sensitized to Pru p 7, but not to Cup a 1, likely representing cases where the primary sensitizing agent is not cypress pollen but directly the food. Among the 10 negative Cup a 1 sera, 5 tested positive for cypress extract. This sIgE profile may be explained by the fact that cypress contains GRPs cross-reactive with those present in the fruits that act as a primary sensitizer. Another 5 subjects were negative for both Cup a 1 and cypress extract. In these cases, the lack of cross-reactivity with cypress GRPs may be due to either low sIgE values towards GRPs (4 cases had values < 2 kUA/L of Pru p 7) or IgE reactivity towards epitopes not shared between Pru p 7 and Cup s 7.
The remarkably high number of patients co-sensitized to peach non-specific Lipid Transfer Protein (nsLTP) (Pru p 3), with no significant difference between Northern and Southern Italy, is unexpected considering the higher prevalence of nsLTP sensitization in Southern Italy (16). Whether this is a bias related to the selected population and the small sample size, or a genuine finding, needs to be evaluated in future studies on larger populations. Despite the higher percentage of subjects co-sensitized to Pru p 3 in the anaphylaxis group (45.5%) compared to those without (22.2%) and the OR was 2.916, statistical significance was not achieved, likely due to the wide confidence interval (95%CI 0.724-11.739). Thus, further studies with larger samples are necessary to assess the real role of Pru p 3 co-sensitization in GRP-sensitized individuals.
In contrast, IgE positivity for PR-10 was significantly higher in patients enrolled from the North, which is expected given the higher prevalence of *Fagales* sensitization in Northern Italy. Similarly to previous studies on nsLTP (17-18), PR-10 co-sensitization was found to be protective against anaphylaxis (OR 0.125), whereas profilin co-sensitization alone was not consistent with findings by Pastorello *et al.* (17). Therefore, PR-10 co-sensitization should be considered a prognostic factor for less severe reactions in clinical practice, although it cannot entirely exclude the risk of anaphylaxis.
Lastly, as demonstrated in a recent study conducted in Italy on 23 GRP-sensitized subjects, peach was the most associated food with GRPs sensitization, followed by citrus fruits. This may be explained by the dietary habits in our country, where these fruits are commonly consumed. However, it is noteworthy that only 3 subjects (7.5%) presented symptoms with a single food (peach, strawberry, and orange, respectively). This percentage is lower compared to the 30% reported in the recent study by Cecchi *et al.* (19), which may be due to the high number of subjects co-sensitized to nsLTP in our study compared to the study in question, where cases were selected for Pru p 3 negativity. The peculiarity of our study lies in consecutively evaluating all GRP-sensitized cases, not just those selected for monosensitization, making the data more reflective of what is observed in everyday clinical practice. However, a limitation of the study is that Pru p 3 co-sensi-

tized subjects were not subjected to oral challenges with peach pulp, which theoretically contains only GRPs, to assess the different roles of GRPs and nsLTP in symptom genesis.

In conclusion, this study, conducted on a consecutive case series of Pru p 7-sensitized subjects, confirmed the high association with cypress sensitization, supporting the PFAS hypothesis, and highlighted high percentages of co-sensitizations to nsLTP, PR-10, and profilin. Only PR-10 co-sensitization differed between Northern and Southern Italy. Although a higher prevalence of Pru p 3 co-sensitization was observed in subjects with anaphylaxis, significance was not reached, while PR-10 co-sensitization was found to be protective against anaphylaxis. Peach and citrus fruits are the most associated foods with GRPs sensitization in Italy.

Although our study provides valuable insights into GRPs sensitization, the findings should be cautiously interpreted due to the study's limitations and the need for confirmation in larger, more diverse populations. The study's consecutive enrolment approach enhances its applicability to real-world clinical practice, but further research is warranted to validate these findings.

Fundings

None.

Contributions

DanVil, DanVis, VG, LC, DM, IB, MB, PF, OR: methodology, data curation. DanVil, ES: formal analysis. DanVil, IB: conceptualization, data curation. DanVil, ES: writing – original draft. All authors: writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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