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SUPPORTING INFORMATION

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Cosensitization to profilin is associated with less severe reactions to foods in nsLTPs and storage proteins reactors and with less severe respiratory allergy

To the Editor,

Profilin was originally identified as an allergenic component in birch pollen and as a relevant pan-allergen in both plant and vegetable food.¹ 48 profilins are currently registered in the WHO/IUIS database. Despite their well-known relevance as allergens, profilin's precise role in eliciting allergic symptoms from a clinical point of view is still a matter of debate.² Previous studies showed that profilin sensitization may underlie severe food-induced allergic reactions in some patients³ but also exert a protective effect against systemic reactions associated with the sensitization to a stable allergen such as nsLTP.⁴ In view of these diverse findings, we used a micro-arrayed detection system to identify and characterize profilin IgE reactivity in a large cohort of unselected patients, with the aim to study the influence of profilin hypersensitivity on the clinical expression of allergic disease.

The study was carried out on 4753 unselected persons born in Central or Southern Italy, attending the outpatient Allergy Clinic of IDI-IRCCS, Rome, between September 2015 and December 2017. Inclusion criteria were as follows: signed written informed consent, history of allergic rhinitis, bronchial asthma, or adverse reactions to foods, including oral allergic syndrome and urticaria. A total of 626 individuals (291 women; age 29 ± 17 years, range 1-82 years) sensitized to at least one of Bet v 2, Hev b 8, Mer a 1, and Phl p 12 were identified. Subjects not sensitized to profilin (4127 patients), acted as allergic controls, to investigate the different behavior of both species-specific and pan-allergen (ie, nsLTP, seed-storage protein [SSP], or polcalcin) hyper-reactors in the presence or absence of profilin sensitization. Demographic details and clinical data (food-related reactions, respiratory, and dermatologic symptoms) were recorded using the TD-Synergy[®] Laboratory Information System (Siemens Healthcare Diagnostics, Muenchen, Germany) and a customized electronic database. IgE reactivity of sera collected after the first visit was analyzed using the 112 ImmunoCAP-ISAC platform (Thermo

Abbreviations: BA, bronchial asthma; ISAC, immuno solid-phase allergen chip; nsLTP, nonspecific lipid transfer protein; OAS, oral allergic syndrome; SR, systemic reaction to food; SSP, seed-storage proteins.

Fisher Scientific, Uppsala, Sweden). All data were analyzed using the IBM SPSS Statistics Package (SPSS, version 21, Chicago, IL).

The clinical and molecular characteristics of participants are summarized in Table 1. Annual mercury profilin (Mer a 1) represented the most commonly recognized profilin, closely followed by the Latex profilin (Hev b 8.0204). Four hundred and four (64.5%) patients recognized all four profilins, whereas 174 (27.8%) were negative for timothy grass profilin (Phl p 12), and 130 (20.8%) for the birch profilin, suggesting that Bet v 2, which is generally viewed as the archetypal profilin, might not contain all the potential IgE-binding epitopes of profilins. Seventy-seven (12.3%) patients recognized only one profilin, further confirming the presence of individual, species-specific epitopes.⁵

High degree of IgE co-recognition was observed, with correlation coefficients ranging between .78 and .96 (Table S1, in the repository); nonetheless, none of the profilins was recognized by all individuals, demonstrating that detecting several homologous molecules at the same time is an added value.⁶ Supervised two-way hierarchical cluster analysis produced a single main cluster of reactivity including Mer a 1, Hev b 8, and Bet v 2 (Figure S1, in the repository), while a minor subset of patients was Phl p 12-positive, showing a different IgE recognition pattern.

Some profilins are involved not only in pollen sensitization but also in food allergy.⁷ As a general rule, as food allergens, profilins elicit mild reactions, such as the oral allergy syndrome,² although severe adverse reactions in subjects overexposed to grass pollen have been recently reported.³ In our cohort, a history of oral allergic syndrome (OAS) was significantly associated with all profilins studied, while profilin IgE recognition was always negatively associated with a history of SR to foods (Figure 1 and Figure S2 repository). Multiple logistic regression analysis, when simultaneously adjusting for all the molecules studied, age, and sex, showed a significant relationship only between a history of OAS and Bet v 2 reactivity ($P < .0001$).

Persons allergic to nsLTP, a stable food allergen, co-sensitized to profilin and/or PR-10 show a lower frequency of severe adverse reaction.⁴ In our large Mediterranean population, we demonstrated that the "protective" effect of profilin sensitization in reducing the occurrence of severe adverse reaction to food is not limited to nsLTP reactors, but is also exerted in patients sensitized to SSP (Figure 1). nsLTP reactivity was detected in 262 (41.9%) profilin-sensitized individuals, showing a lower prevalence of food-induced SR if compared to the 1118 nsLTP-hypersensitive patients not co-sensitized to profilins (44.3% vs 59.6%; $P < .0001$). Similarly, 274 (43.8%) participants co-sensitized to profilin and SSP showed a reduced

TABLE 1 Clinical, demographic, profilin recognition profiles, and symptoms spreading of participants

		O.A.S.		Systemic reaction		Respiratory symptoms	
		Profilin sensitization					
		Absent	Present	Absent	Present	Absent	Present
Sex (M F)	335 291	252 403	147 131	656 1023	105 108	1118 1608	250 218
Age (y $\pm$ STD)	29 $\pm$ 17	30 $\pm$ 17	28 $\pm$ 15*	34 $\pm$ 19	32 $\pm$ 18*	33 $\pm$ 18	30 $\pm$ 16
Bet v 2	496 (79.2%)	16.1%	50.0%*	40.6%	33.1%*	66.1%	76.4%*
Hev b 8	562 (89.8%)	16.0%	46.6%*	40.6%	34.2%*	66.1%	75.4%*
Mer a 1	565 (90.3%)	16.0%	46.9%*	40.7%	33.1%*	66.1%	75.0%*
Phl p 12	452 (72.2%)	16.8%	46.5%*	40.5%	33.6%*	66.4%	74.6%*

OAS, oral allergic syndrome.

* $P < .01$.

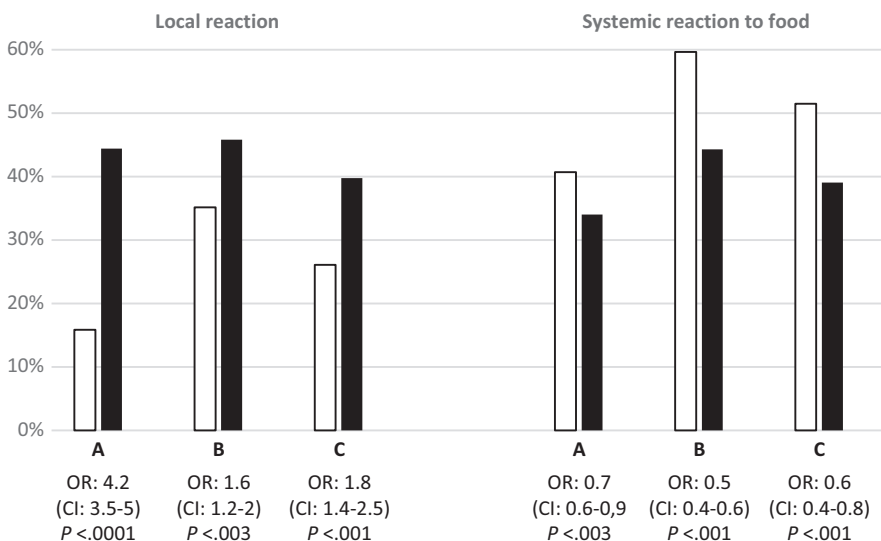


FIGURE 1 Prevalence (%) of specific symptoms (local or systemic reaction to food) among (i) patients sensitized (black bars) or not sensitized (white bars) to profilins in total population A, nsLTP reactors B, and SSP reactors C. The crude odds ratios and the 95% confidence intervals are reported where significant direct (>1) or inverse (<1) associations were observed

prevalence of SR than the 874 SSP reactors not co-sensitized to profilins (39.1% vs 51.5%; $P < .0001$) (Figure 1). Such observation was mainly recorded in the case 2S albumins from peanut (Ara h 2 and Ara h 6), Brazil nut (Ber e 1), and sesame (Ses i 1) (Figure S3, in the repository).

We confirm that the grass pollen profilin is probably the most potent sensitizer to this family of allergens,⁸ as >94% of cases were timothy grass hypersensitive, followed by cypress (Cup a 1; 72%) and olive tree (Ole e 1; 55%) pollen allergies. A significant relationship was observed between the occurrence of rhinoconjunctivitis, and the profilins studied ($P < .001$), while bronchial asthma was significantly lower in profilin reactors co-sensitized to pellitory (232 participants) or grass pollen (589 patients, $P < .001$; Figure S4 in the repository). Interestingly, profilin co-recognition did not affect the association of polcalcin hypersensitivity with asthma (not shown)⁹

Altogether, our data on a large Italian population show that profilin sensitization should not be considered only as a confounding factor when addressing the IgE sensitization profile in polysensitized patients. A previous study has reported that profilin may be a potentially harmful food allergen in heavily sensitized patients challenged with low amounts of purified profilin.³ Our results, however, are more in accordance with other previous observations,⁴ proposing that profilin might be regarded as a possible “protective factor” in both food and respiratory allergies. These observations are potentially valuable for the clinically oriented allergologist, as they could indicate the clinical outcome of allergic patients identifying a subset of patients with a lower risk of both systemic food reactions and bronchial asthma.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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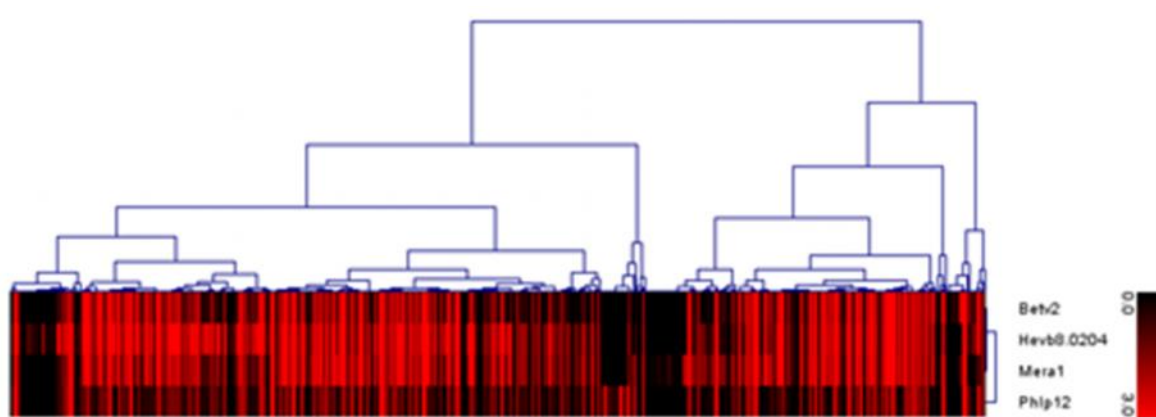


Figure E1 repository. Supervised two-way hierarchical clustering analysis of 626 subjects with IgE reactivity to Bet v 2, Mer a 1, Hev b 8 and Phl p 12 profilins. Logarithmic IgE value distribution was used to generate the heat map. Subjects were IgE-positive to at least one of the four profilins studied. Patients are reported on the x-axis, with profilins on the y-axis. The black to bright red scale corresponds to IgE values from negative to highly positive.

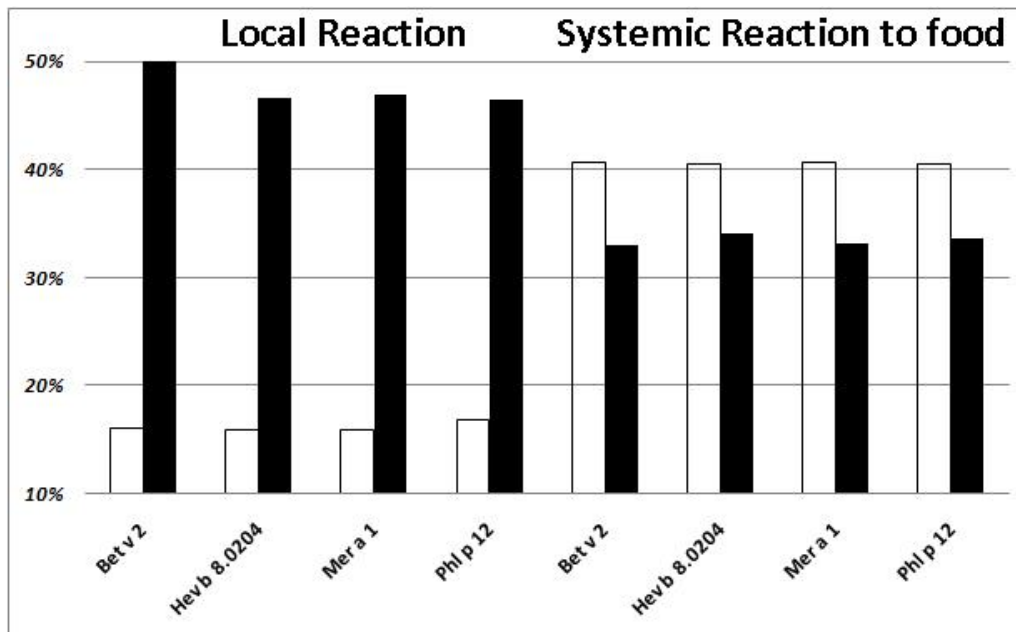


Figure E2 repository. Prevalence (%) of specific symptoms (Local or Systemic Reaction to food) among patients sensitized (black bars) or not sensitized (white bars) to each tested profilin. (Local Reaction: Bet v 2: ORa= 5.2, 95% CI= 4.3-6.3, $p < 0.001$; Hev b 8.0204: ORa= 4.6, 95% CI= 3.8-5.5, $p < 0.0001$; Mer a 1: ORa= 4.6, 95% CI= 3.8-5.6, $p < 0.001$; and Phl p 12: ORa= 4.3, 95% CI= 3.5-5.2, $p < 0.001$; Systemic reaction: (Bet v 2: ORa= 0.7, 95% CI= 0.6-0.8, $p < 0.001$; Hev b 8.0204: ORa= 0.7, 95% CI= 0.6-0.9, $p < 0.005$; Mer a 1: ORa= 0.7, 95% CI= 0.6-0.8, $p < 0.001$; and Phl p 12: ORa= 0.7, 95% CI= 0.6-0.9, $p < 0.005$)

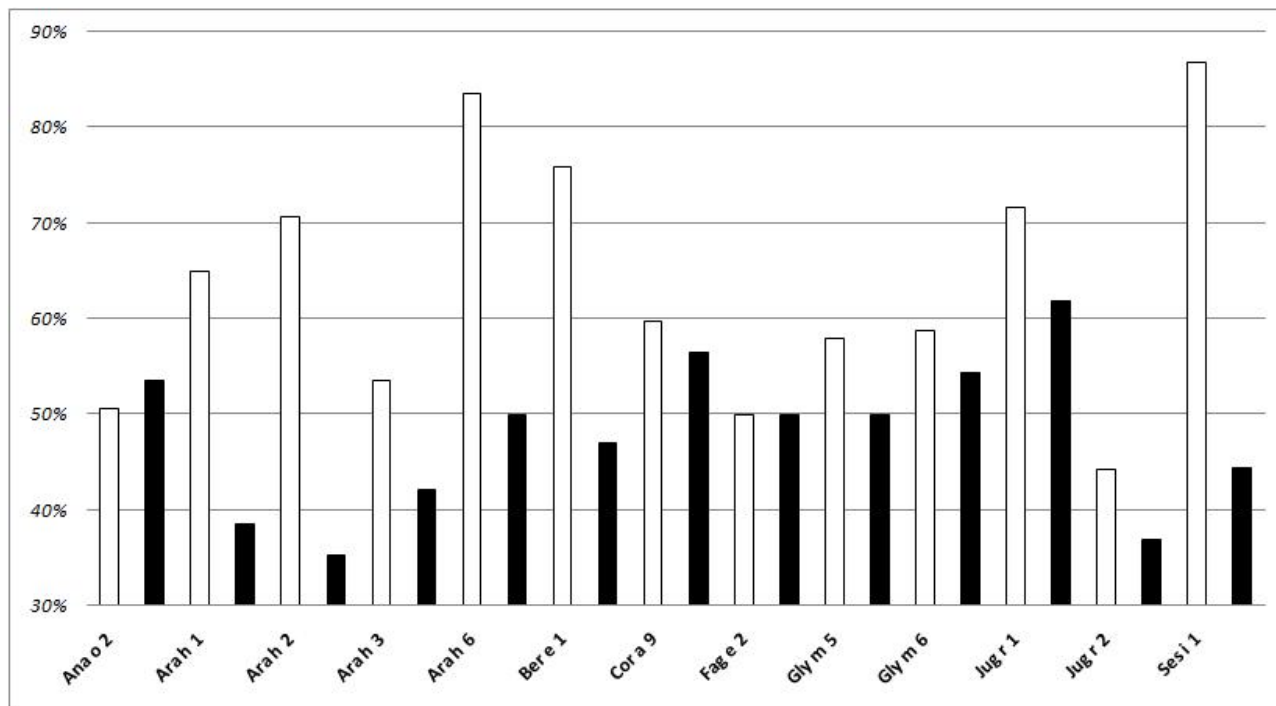


Figure E3 repository. Systemic reaction prevalence in seed storage proteins reactors with (black bar) or without (white bar) profilin co-recognition. The following molecules were tested: 2s Albumins (Ara h 2, Ara h 6, Ber e 1, Fag e 2, Jug r 1, Ses i 1); Vicilins (Ara h 1, Gly m 5, Jug r 2) and Legumins (Ana o 2, Ara h 3, Cor a 9, Gly m 6). A significant reduction in SR occurrence was found in the case of Ara h 2 (Fisher exact test, P-value = 0.017), Ara h 6 (P = 0.002), Ber e 1 (P = 0.028), Jug r 2 (P = 0.036), and Ses i 1 (P = 0.003) reactors.

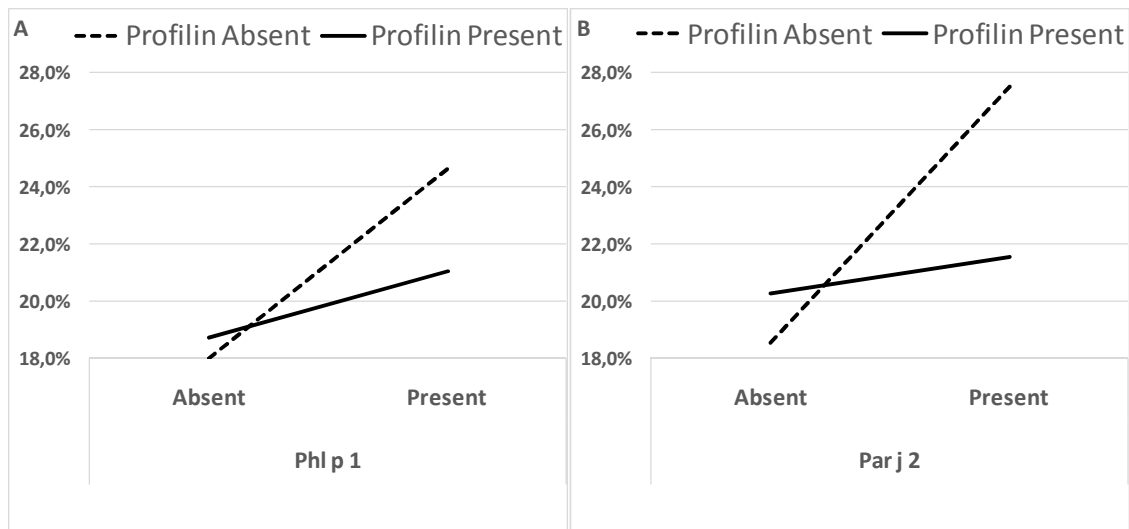


Figure E4 repository. Bronchial Asthma occurrence in timothy grass Phl p1 and pellitory Par j 2 reactors with (solid black line) or without (dashed black line) profilin co-recognition.

Table I repository. Bivariate analysis of reciprocal relationships between the profilin studied.

Spearman coefficient values and the absolute number of IgE reactive subjects are shown for paired allergens. Correlation coefficient from 0.7 to 1.0 indicates a strong positive association (bold) in all cases.

Bet v 2 (496)	Hev b 8.0204 (562)	Mer a 1 (565)	Phlp12 (452)	
Bet v 2	0,95 (479)	0,95 (482)	0,81 (408)	<i>rho</i> <i>no.</i>
	Hev b 8.0204	0,96 (525)	0,78 (429)	<i>rho</i> <i>no.</i>
		Mer a 1	0,85 (430)	<i>rho</i> <i>no.</i>