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Original article

Profilin sensitization and its clinical relevance to a population of atopic adults in Greece

Sensibilisation à la profiline et sa pertinence clinique dans une population d'adultes atopiques en Grèce

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Abstract

Background. – Profilin is a panallergen often responsible for cross-reactivity between pollen and plant foods. Oral allergy syndrome (OAS) consists a clinical expression of this cross-reaction, in some profilin-sensitized patients. The aim of this study was to determine the prevalence of sensitization to profilin in Greece and to examine the correlation of profilin-sensitization with OAS.

Methods. – Two hundred and sixty-four atopic adults were included in the study, with at least one positive SPT to airborne and/or food allergens. Sensitization to profilin was examined with the use of purified date palm profilin Pho d 2. SPT with our standard battery of commercial extracts and an additional panel with profilin-containing foods and pollen were conducted.

Results. – Twenty-nine of our atopic patients (11%) resulted positive to profilin and 79 (29.9%) patients referred symptoms of OAS. Patients with OAS reported peach, walnut, kiwi, banana and hazelnut, as the commonest plant foods causing OAS. OAS was correlated to positive SPT to apple, peach pulp (showing the highest correlation), and other plant foods. No statistically significant correlation was found between sensitization to profilin and OAS. A significant trend of profilin-sensitization was found in patients with allergic rhinitis, but not in asthmatics.

Conclusion. – Our results show a low prevalence of sensitization to profilin, in Greece. The in vivo sensitization to profilin's extract was not correlated to OAS and it is probable that another culprit panallergen is the cause for many OAS cases in our country.

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Keywords: Atopy; Profilin; Oral allergy syndrome; Cross-reaction; Epidemiology

Résumé

Introduction. – La profiline est un panallergène souvent responsable de réactivités croisées entre les pollens et les aliments végétaux. Le syndrome d'allergie orale (SAO) représente une expression clinique de ces réactions croisées chez certains patients sensibilisés à la profiline. Le but de cette étude était de préciser la prévalence des sensibilisations à la profiline en Grèce et d'étudier les corrélations entre la sensibilisation à la profiline et la SAO.

Méthodes. – Deux cent soixante quatre adultes atopiques ont été inclus dans cette étude, ayant au moins un prick test cutané (PTC) positif aux allergènes aéroportés et/ou alimentaires. La sensibilisation à la profiline a été étudiée à l'aide de la profiline de palmier dattier purifiée Pho d 2. Les PTC ont été effectués avec notre batterie standard d'extraits commerciaux et un panel supplémentaire comportant des aliments contenant de la profiline et du pollen.

Résultats. – Vingt-neuf de nos patients atopiques (11 %) se sont révélés positifs à la profiline et 79 (29,9 %) ont présenté des symptômes de la SAO. Les patients atteints de SAO ont signalé que la pêche, la noix, le kiwi, la banane et la noisette étaient les aliments végétaux les plus couramment

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à l'origine du SAO. Le SAO était corrélé à un PTC positif à la pomme, à la pulpe de pêche (présentant la plus forte corrélation) et à d'autres aliments végétaux. Aucune corrélation statistiquement significative n'a été observée entre la sensibilisation à la profiline et le SAO. Une tendance significative de la sensibilisation à la profiline a été observée chez les patients souffrant de rhinite allergique, mais pas chez les asthmatiques.

Conclusion. – Nos résultats montrent une faible prévalence de la sensibilisation à la profiline en Grèce. La sensibilisation in vivo à l'extrait de profiline n'était pas corrélée au SAO et il est probable qu'un autre panallergène soit la cause de nombreux cas de SAO dans notre pays.

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Mots clés : Atopie ; Profiline ; Syndrome d'allergie orale ; Réactions croisées ; Épidémiologie

1. Introduction

Panallergens are families of proteins ubiquitously distributed that share highly conserved sequences, structures and functions. The presence of panallergens with common conformational or linear epitopes in pollen and plant foods results in cross-reactivity, often translated to cross-allergic symptoms [1,2]. Profilins are 12–15 KDa proteins and belong in a family of plant panallergens that includes 44 identified molecules, acknowledged by the WHO/IUIS nomenclature subcommittee (<http://www.allergen.org>) [2].

Profilins are present in all eukaryotic cells and their main role appears to be the generation of cytoskeleton, the cell movement through the binding to actin, but also signal transduction and some more complicate ones [3–6]. The prevalence of sensitization to profilin ranges from 5 to 42% depending on the geographical exposure [2,7,8]. In Southern Europe its prevalence is reported to be less than 10%, however, in certain areas, sensitization rate to profilin of date palm has been shown to be > 50%, reaching proportions of major allergens [7–9].

Profilins are considered minor allergens of pollen, plant foods and latex, with an amino acid sequence similarity of at least 75% among profilins of different species [3]. Protein homology occurs also with other panallergens, comprising various protein families of plant as well as of animal origin and results in allergic cross-reactions between related and unrelated allergenic sources [2]. “Pollen-food allergy syndrome” or — most accurately — “oral allergy syndrome” (OAS) is a clinical expression of this cross-reaction [10]. However, studies from certain geographic regions indicate that profilins may also consist major allergens, inducing more severe reactions of respiratory or food allergy, which upgrades it to an important airborne and plant-food allergen [11–14].

The main aim of the present study was to determine the hitherto unknown frequency of profilin sensitization in Greece and its correlation to airborne and food allergens. For this purpose we implemented the most readily available method characterized by rapid results, low cost and high sensitivity, i.e. skin prick tests (SPT). In addition, we explored the correlation of profilin with atopic diseases (food and respiratory allergies, atopic dermatitis), focusing on OAS to plant foods and investigating the profile of Greek patients with this clinical entity.

2. Methods

2.1. Patients

This prospective study was conducted on a random sample of adult patients that visited a Greek Allergy Department of reference. Inclusion criteria were atopy and adulthood. Atopy was defined as at least one positive SPT to common food or respiratory allergens and a relative anamnesis. Exclusion criteria were ages under 18 years old, no history of allergy, or resulting negative to all SPT used.

Each participant was informed about the aim and the procedures of the study and provided a written consent before enrolment. The research was carried out in line with bioethical principles for humans and animals, according to the National Bioethics Committee and the Greek legislation, in accordance with the Declaration of Helsinki. The study was approved by Hospital's Ethics Committee.

2.2. SPT

Sensitization profile was assessed through SPT with pollen and plant food extracts. All patients underwent SPT with the standard panel of major airborne and food allergens for Greece (Stallergenes, France), in order to detect/confirm atopy. An additional panel (hereafter called “study's SPT panel”) was used for this study, consisting of plant foods, the three main pollens of our area (double-checking the ones of the Department's panel) and profilin allergen extracts. All SPT were usually performed on the same visit.

The additional study's SPT panel included: apple (Immuno-tek, Spain), hazelnut (Allergy Therapeutics, UK), peach pulp and peach peel separately (LETI, Spain), *Olea europaea* (olive), *Parietaria judaica* (Wall pellitory) and *Phleum pratense* (Timothy grass) (Allergy Therapeutics, UK) and purified date palm profilin Pho d 2 (ALK-Abello, Denmark). Positive (histamine 10 mg/mL) and negative controls were also used (Stallergenes, France).

Using *Parietaria judaica* and not *P. officinalis* was preferred due to the fact that its profilin Par j 3 has been recognized; therefore we assumed that its extract contains it. *Phleum pratense* was chosen as the representative species of the Poaceae Family,

containing the profilin *Phl p 2*, while *Olea europea* contains the profilin *Ole e 2*.

2.3. Statistical analysis

The number of patients to be included in our study was calculated based on the prevalence of sensitization to profilin in atopic patients in the Mediterranean area of Spain, which has been estimated at 6% [8]. As Greece is a Mediterranean country, with similar environmental conditions, we assumed that a similar prevalence of profilin-positive patients would be found. The recruitment of 241 subjects was calculated with a 3% margin of error and 95% confidence interval.

In order to investigate the correlation of profilin sensitivity with categorical variables (sex, OAS, anaphylaxis, allergic rhinitis, atopic dermatitis, asthma, sensitization in plant foods and pollen) the χ^2 test with Yates corrections was used. For small counts the Fisher exact test was used. Similar tests were used to evaluate associations between OAS and sensitivity in fruits and pollen. The extent of correlation between the statistically significant variables was calculated by odds ratio (OR) with 95% confidence interval (CI).

The association between SPT measurements and types of plant foods, pollen and profilin was evaluated using one-way analysis of variance (ANOVA). The sensitivity and specificity of SPT with foods of plant origin and profilin, as a prognostic marker for OAS, was investigated using ROC curves. The Pearson's correlation r was used to correlate the size of the wheal of profilin's SPT (after "normalized" against histamine) with the sizes of the wheal of plant foods' and pollens' SPTs. Statistical analysis was performed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). All tests were two-sided and statistical significance level was set at 5%.

3. Results

Two hundred sixty-four adult atopic patients, who visited the Allergology Department for investigation of rhinitis, asthma and food allergy, were recruited in the study from November 2014 to August 2015. Demographic and descriptive characteristics of the study's participants are presented in Table 1.

Sensitization to profilin was found in 29/264 (10.9%) patients and no correlation was detected with gender or age ($P > 0.05$). All profilin-positive patients had a history of allergic rhinitis,

which was a very common problem of our sample, reported by 237 (89.8%) patients, most of them (90.2%) sensitized to pollen of study's SPT panel. Profilin-sensitization tended to be associated with higher likelihood of allergic rhinitis (OR = 1.1, 95% CI [1.0–1.1], $P = 0.05$), but not correlated with asthma (75 patients) or atopic dermatitis (17 patients) ($P = 0.45$ and $P > 0.99$, respectively). Regarding anamnesis of food allergy, 29.9% of the participants reported OAS after consuming fruits, vegetables or tree nuts, while 18.9% had a history of systemic reaction or anaphylaxis.

As shown in Table 2, 15.7% of the patients sensitized to profilin had also sensitivity to Olive and 15.0% of them to Timothy grass, suggesting that presenting positive SPT to profilin is more likely connected to sensitization to *Olea europea* (OR = 3.9, 95% CI [1.4–10.6]) or *Phleum pratense* (OR = 3.1, 95% CI [1.2–7.8]). The identification of a possible predictive marker for profilin sensitization, using combinations of SPT results, was further explored. Double positivity to Timothy and Olive pollen extracts was significantly ($P = 0.006$) correlated to profilin-sensitization and could be used as a marker of sensitization to profilin, but with low sensitivity (69.0%) and specificity (59.1%).

Although the size of SPT wheals is not clearly related to the strength of sensitization, we tried to detect any possible correlations between profilin wheals' sizes and the relative ones of the other SPT (normalized to histamine's wheal), using Pearson's r coefficient. A significant correlation was noticed between the SPT wheals of profilin and peach pulp ($r = 0.53$, $P = 0.003$) as well as between the SPT wheals of profilin and Phleum ($r = 0.62$, $P = 0.008$), while no correlation of profilin with Olea ($r = 0.43$, $P = 0.09$) or Parietaria SPT ($r = 0.30$, $P = 0.24$) was recorded.

Seventy-nine (29.9%) of our patients reported OAS to plant foods as shown in Table 3. Most patients (70/79) that reported OAS were SPT-negative to profilin and only 9 profilin-positive patients reported a relative anamnesis, suggesting that in our study sensitization to profilin was not connected to OAS symptomatology ($P > 0.99$). Sensitization to profilin was neither correlated with more severe types of food allergy (systemic reactions/anaphylaxis) ($P > 0.99$).

It was noticed that patients with sensitivities to peach peel or to peach pulp, were more likely to develop OAS compared to non-sensitized ones (OR = 6.2, 95% CI [3.2–12.0]) and (OR = 5.7, 95% CI [2.7–12.1]), respectively. Similar was the outcome for patients with apple (OR = 8.8, 95% CI [2.3–32.9]) and hazelnut (OR = 5.5, 95% CI [2.7–11.2]) sensitizations. Although apple was not recognized and reported as a culprit food for OAS (Table 2), most (76.9%) of the participants with OAS were SPT-positive to it, followed by sensitization to peach peel (64.9%), hazelnut (63.4%) and peach pulp (64.3%).

Subjects suffering from OAS were more likely to present positive SPTs to any plant food (OR = 8.2, 95% CI [4.5–14.9], $P < 0.001$). Moreover, those positive to more than 3 plant foods had higher likelihood of OAS (OR = 2.8, 95% CI [1.6–3.9], $P < 0.001$), than those sensitized to less than 3 plant foods (OR = 0.2, 95% CI [0.1–0.3], $P < 0.001$). However, using ROC curve analysis, sensitization to any plant food and/or profilin was unable to predict OAS.

Table 1
Demographic and clinical characteristics of the participants.

	Number	%
Age (years): 37.7 ± 1	264	
Male	101	38.3
Oral allergy syndrome	79	29.9
Food anaphylaxis	50	18.9
Seasonal allergic rhinitis	237	89.8
Asthma	75	28.4
Atopic dermatitis	17	6.4
Sensitized to profilin ^a	29	10.9

^a SPT positive to purified date palm profiling Phod 2 (ALK-Abellö, Denmark).

Table 2

Results of profilin SPT in comparison with the extracts of the study's SPT panel.

		Sensitized to profilin (%)	Non-sensitized to profilin (%)	P values
Parietaria	Positive	10.4	89.6	0.868
	Negative	11.8	88.0	
<i>Olea europea</i>	Positive	15.7	84.3	0.008
	Negative	4.5	95.5	
<i>Phleum pratense</i>	Positive	15.0	85.0	0.023
	Negative	5.4	94.6	
Peach peel	Positive	15.4	84.6	0.376
	Negative	9.9	90.1	
Peach pulp	Positive	16.2	83.8	0.416
	Negative	10.1	89.9	
Apple	Positive	7.7	92.3	>0.99
	Negative	11.2	88.8	
Hazelnut	Positive	14.6	85.4	0.588
	Negative	10.3	89.7	

Table 3

Frequency of reported culprit food allergens causing Oral allergy syndrome.

Food of plant origin	Oral allergy syndrome patients (%)
Peach	29
Walnut	26.50
Kiwi	21.50
Banana	17.70
Hazelnut	11.40
Peanut	10
Eggplant/apricot	8
Melon	7.50
Strawberry/almond/cherry	6
Cabbage/tomato/watermelon/apple/ pineapple/grape/orange/peanut/spinach	5–3
Sesame seed/sunflower	<3
seed/pear/cashew nut/brazilnut/lemon/ cucumber/pepper/mustard/chestnut/fig/ mango/papaya/plum/onion/lentil/peas	

4. Discussion

According to our findings, the prevalence of sensitization to profilin in Greek atopic adults is 11%, similar or a little higher than those of European areas located almost at the same latitude with Greece [8,15]. Regarding the correlation among sensitizations, it was noticed that sensitization to profilin was more correlated with the relevant one to airborne- than to food-allergens.

Sensitization to profilin is mainly induced by the relative protein contained in pollen of the local flora, so its prevalence varies in the various geographical areas [7,12]. Grass is considered the most potent inducer of IgE-sensitization to profilin, while Birch, Olive and Ragweed pollen may also cause the primary sensitization to it, with sensitization patterns varying in different regions [12,16,17]. The strong connection of Grass and Olive to profilin was confirmed by our study.

Profilin is present in the three main pollen allergens of our country, however sensitization to it was related to *Olea europea*

and *Phleum pratense* but not to *Parietaria judaica*. The reason for this contradiction is the lack of cross-reaction between the profilin of *Parietaria* with the relative one in other pollen, no matter the almost identical amino acid sequence [18–19]. Profilin consists of a conformational epitope so even slight differences in the sequences are enough for mismatch with “cross-reacting” antibodies.

The fact that we discovered a profilin-monosensitive patient, with no other positive SPT was surprising. In a previous study, conducted in Portugal, four monosensitized to profilin patients have also been described [15]. A possible explanation to the absence of a pollen-sensitivity is the failure to include the culprit pollen in the SPT panel, since only commercially available extracts of pollen, that are implicated in more than 95% of allergic respiratory diseases in our area, were examined. In order to exclude a false positive result to profilin, the relative SPT was repeated to the other upper extremity of the patient, with the same result.

OAS can be clinically described as a complex syndrome of allergic symptoms restricted in the oral cavity, elicited promptly by the ingestion of fresh fruits and non-cooked vegetables, in patients with seasonal allergic rhinoconjunctivitis [20]. As confirmed by our study, all patients reporting OAS had a history of allergic rhinitis and most of them resulted SPT-positive to plant foods. A complexity of sensitizations has been underlined in Mediterranean patients, and discreet endotypes of OAS have been described, following sensitization to multiple, one or no panallergen [21,22].

In our study, no correlation with severe types of food allergy was shown, while only few cases of OAS presented IgE-sensitization to profilin, leading to the conclusion that in most Greek patients with OAS this is not the culprit allergen. Focusing on profilin may be regarded as a limitation of our study because it did not include the investigation of other panallergens like nsLTP and PR-10. It can be assumed that in our patients with OAS symptoms, PR-10 (Bet v 1-like), PR-5 (Thaumatococcus-like), nsLTPs or other allergens are the culprit allergenic proteins [16,23].

The prevalence of OAS in Europe ranges 30–70% [3]. As mentioned before, prevalence of OAS depends on the sensitization to pollen allergens of each geographical area and certainly on the local dietary habits, in particular the consumption of non-cooked plant foods. In a Greek online survey that attempted to define the prevalence of self-reported food allergy, 3,673 adults were enrolled and 25.9% of them reported symptoms of OAS, which is close to our findings [24].

In the present study two Rosaceae (apple and peach), followed by hazelnut, were the most common food-sensitizations in participants reporting OAS. This result agrees with other studies reporting Rosaceae as the main fruits that cause OAS and food allergies in general [25,26]. Based on similar patterns and although remaining to be proved, the Greek sensitization pattern that results in OAS, starts due to Grass and/or Olive pollinosis with sensitization to profilin, or to another panallergen and is expressed with outbreaks mainly after ingestion of Rosaceae [16].

In conclusion, according to our results, the prevalence of profilin-sensitization in atopic Greek adults is 11%. This outcome is a little higher than expected; however it remains at the same relatively low levels of the Mediterranean area. Profilin-sensitization is noticed only in patients with allergic rhinitis, while no correlation of profilin with OAS was noticed, which means that another (or other) panallergen(s) might be the cause of this clinical entity in our country.

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