Profilin, a change in the paradigm

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Abstract

Profilin is a protein present in all eukaryotic cells, and is responsible for the cross-reactivity between pollen, latex and plant foods. It has been classically acknowledged as a minor or nearly irrelevant allergen, although recent data are changing this conception. The objective of this manuscript is to comprehensively review the published data on the role of this ubiquitous allergen in pollen, latex and plant food allergy.

The patterns of recognition of this minor allergen follow a north-south gradient, and although present in all pollens and vegetables, it is significantly associated with grass pollen and *Cucurbitaceae* fruits. Heb v 8, the latex profilin, is usually a marker of profilin allergy in plant food allergic patients, but has no clinical relevance in latex allergy. Profilin sensitization jeopardizes pollen allergy diagnosis and immunotherapy selection, and although component resolved diagnosis can identify its impact, there are no tailored treatments available. In recent years, several new publications have shown how profilin should be taken into account and, under certain circumstances, considered as a severity marker, an allergen capable of inducing respiratory symptoms and, in its natural purified form, a potential candidate for etiological food allergy treatment.

Current data on profilin strongly support the need for a shift in the previously accepted concept of this allergen. More research should be done to assess the real clinical impact of this sensitization in specific populations and to develop therapeutic strategies.

Key words: Profilin. Phl p 12. Bet v 2. Allergy. Food Allergy. Asthma. Immunotherapy. Allergen.

Resumen

La profilina es una proteína presente en todas las células eucariotas, siendo responsable de la reactividad cruzada entre polen, látex y alimentos vegetales. Ha sido reconocida clásicamente como un alérgeno menor o irrelevante; sin embargo, datos recientemente publicados están modificando esta interpretación. El objetivo de este manuscrito es realizar una revisión comprensiva de la literatura sobre el papel de este ubicuo alérgeno en el polen, látex y los alimentos vegetales.

El patrón de reconocimiento de este alérgeno menor sigue un gradiente de norte a sur, y a pesar de estar presente en todos los pólenes y vegetales, está significativamente asociado al polen de gramíneas y a las frutas de la familia *Cucurbitaceae*. Heb v 8, la profilina del látex, es habitualmente un marcador de alergia a profilina en pacientes alérgicos a vegetales pero sin relevancia clínica en la alergia a látex. La presencia de la sensibilización a profilina dificulta el diagnóstico de alergia a pólenes y la selección de la inmunoterapia, y a pesar de que el diagnóstico por componentes puede identificar su impacto, no existen tratamientos personalizados disponibles. En los últimos años, diversas publicaciones nuevas han demostrado como la profilina debe ser tenida en cuenta y considerada bajo determinadas circunstancias, como un marcador de gravedad, como un alérgeno capaz de inducir síntomas respiratorios, y en su forma natural purificada, como un potencial candidato para realizar un tratamiento etiológico para tratar la alergia a alimentos.

El conocimiento actual sobre la profilina impulsa la necesidad de cambiar el concepto que previamente se tenía sobre este alérgeno. Sería preciso investigar más para valorar el impacto clínico real de esta sensibilización en poblaciones específicas y desarrollar estrategias terapéuticas.

Palabras clave: Profilina. Phl p 12. Bet v 2. Alergia. Alergia a alimentos. Asma. Inmunoterapia. Alérgeno.

Introduction

Profilins are 12–16 kDa, actin(–monomer)-binding proteins expressed in all eukaryotic cells and certain viruses, with the exception of some protists [1,2]. Profilins promote polymerization of actin filaments and monomers and are thus involved in the generation of the cytoskeleton and in movement [1]. Their role in such essential processes explains their ubiquitous expression and high levels of conservation [3] (**Supplementary 1**). The identification of 50 additional profilin ligands suggests an important role in many more complex molecular processes as well as in signal transduction [2,4]. The first allergenic profilin described, Bet v 2 from birch pollen, was identified in 1991 [5], and since then, many allergenic members have been identified in pollen, plant foods and latex [6], showing a high degree of cross-reactivity due to their common epitopes.

Some profilin B cell epitopes, both sequential and conformational, have been described using different approaches. The actin-binding site and the adjacent plant-specific pocket were found to comprise an immunogenic region responsible for a cross-reacting response in the *Arabidopsis* profilin [7]. Two regions overlapping with the actin-binding site were identified as major cross-reactive epitopes, and a third site, consisting of residues 30-50, was found to be a likely cause of extensive cross-reactivity in birch profilin [8]. Several epitopes, which in fact cover most of the surface, have been identified in model structures of several profilins. Radauer *et al*. highlighted three main candidates: epitope 1 SWQTYVDDHQYQGL; epitope 7, PGAMVIQGEPGARGKPNE; and epitope 8, MKDEPGHVIQGEPGARKE [9]. Leitner *et al.* found that the circular peptide CAISGGYPVC inhibited IgE binding to mugwort pollen, birch pollen and celery tuber profilin and speculated that this epitope might be an important epitope in plant profilins [10]. In the case of Cuc m 2, the most important watermelon allergen, the sequence S2W3A5Y6D9H10T111P112G113Q114 N116M117R121L122 [11] was described as the main epitope. This IgE binding region was implicated in cross-reactivity with most plant profilins, such as Phl p 12 and Bet v 2, due to the high identity observed (**Table 1**). The

identification of this sequence allows it to be used as a diagnostic marker for cross-reactivity mediated by this family, as well as for future strategies in immunotherapy.

The established concept of profilin

Prevalence:

In order to establish profilin prevalence, it is extremely important to clarify whether the selected population is first chosen for their pollen allergy or based on plant food allergy. Among pollen-allergic patients, profilin sensitization ratios across Europe can vary widely, especially for different primary sensitizers: from 5% in a Swedish birch pollen allergic cohort [12] to 51% in a subset of a *Mercurialis annua* allergic population in Spain [13]. This geographical variability and north-south gradient is due to the predominance of different pollens across Europe [14] that has been previously identified [3].

Profilin sensitization is assumed to be always preceded by sensitization to a major allergen, although cross-sectional studies have failed to identify the primary sensitizer in most panallergen-sensitized subjects because the vast majority of subjects are sensitized to 2 or more different pollen sources [15]. Grass allergy has been pointed out by several studies [16] as one of the more robustly associated profilin sensitizers. Barber et al.[17] studied 891 pollen allergic patients from southern Spain with a panel of 13 purified allergens, and 15% of them were sensitized to the apple profilin (rMal d 4). More interestingly, a geographical variation according to seasonal grass pollen load was perceived, revealing areas containing 50% of profilin-sensitized patients. These descriptive results were explained by a logistic multivariate analysis showing that profilin sensitivity was associated with major grass allergens (Phl p 1: OR= 3.16; 95% CI: 1.71–5.83, and Phl p 5: OR=6.19; 95% CI: 3.86–9.91). With a similar design, but in 1329 patients from northern Spain, the same authors [18] detected that 18.8% of the population was profilin-sensitive, and this sensitivity was again significantly associated with Phl p 5 (OR= 5, CI not provided). As a consequence, profilin seems to play a relevant role in

areas with predominance of grass allergy. These areas can be identified by epidemiological studies mapping the sensitization clusters per region, as has been performed in Spain [19].

Understanding the role of pollens other than grass in profilin sensitization can be undertaken with the olive pollen allergy model, as this pollen reaches maximum known exposure levels in some areas of Andalucia [17]. For the olive profilin (Ole e 2) we can find contradictory results regarding the prevalence and sensitization impact. It is usually acknowledged as a minor allergen [20] and was not found to be associated with Ole 1 in one of the aforementioned studies [17]. Moreover, this lack of association has already been the subject of commentary by other authors [21]. However, in the study by Quiralte et al. [22], 54% of 146 olive allergic patients displayed sIgE (specific IgE) to Ole e 2. The authors speculate that due to the extremely heavy load of olive pollen in the studied area (Jaen, Spain, average 500 to 1000 grains/m^3 with peaks of 10000 grains/m^3), the patients may have become sensitized to more olive allergens than in other places, underscoring the relevance of the area in the patients' molecular recognition patterns. One potential weakness of this study is the lack of information regarding the patients' sensitization to other pollen sources. This heavy pollen load was also used to explain relevant sensitization to other minor olive allergens, especially Ole e 7, which was found to be linked to an increase in asthma prevalence and allergic disease severity [17,23].

On the other hand, if patients are selected based on plant food allergy as the main criterion, the profilin-sensitization geographical distribution displays a similar north-south gradient. In an interesting study by Fernandez-Rivas et al. [24], a component resolved diagnosis was run to assess the area-dependent recognition patterns to Mal d 1 (major apple allergen, Bet v 1 homologue), Mal d 2 (TLP), Mal d 3 (LTP), and Mal d 4 (profilin) in a group of 389 apple allergic subjects from four different European countries: Austria, Italy, Netherlands and Spain. Their results showed that apple allergy in individuals from the Netherlands, Austria and Italy was associated with Mal d 1 and milder symptoms, whereas in Spain apple allergy was linked to Mal d 3 and severe manifestations. Regarding profilin, both sensitization and sIgE levels were higher in Spain and Italy (around 40% and 30% of subjects, respectively) compared to the Netherlands or Austria, where it was recognized in no more than 15% of the population. This

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study provides further evidence of the higher prevalence of profilin sensitization in southern countries than in northern countries, a trend that is also supported by Andersen et al. [25] in their review of the panallergens involved in *Rosaceae* fruit allergy. After including 38 European studies with determination of several isolated allergens, they state that in western Mediterranean areas, PR-10 sensitization is almost absent, with LTP being the first cause of *Rosaceae* fruit allergy, followed by profilin, which is also linked to non-*Rosaecae* fruits. To the contrary, in northern and central Europe, *Rosaceae* fruit allergy is mostly due to class 2 fruit allergy and cross reactivity with PR-10 (Bet v 1 homologues) with poorer profilin recognition.

Profilin sensitization diagnosis

Profilin allergy diagnosis can be performed either *in vitro* or, in the countries were purified profilin extract is available, *in vivo*. *In vivo* diagnosis with purified palm tree profilin, nPho d 2, at 50 µg/ml, has been proven to have a high diagnostic efficiency [18,26–29]. For *in vitro* profilin diagnosis, a single profilin (either Bet v 2 or Phl p 12) is sufficient [26]. Profilin recognition variability of *in vitro* diagnosis is more related to specific isoform selection and protein folding rather than to real recognition differences of the various allergenic sources [26]. Recently, a consensus document on the use of molecular diagnosis in allergy in daily practice, including a chapter on profilin and its characteristics, has been issued [30].

The role of profilin in respiratory allergy

Profilin has been accepted as a minor aeroallergen in most pollen sources [31], having little or no clinical impact and a prevalence below 50% in most cases. There are some exceptions, however, such as the case of Che a 2, the *Chenopodium album* profilin, which was recognized by 55% (n=104) of a Spanish *Chenopodium* allergic population [32]. However, its clinical impact was not fully addressed since the same group was also sensitized to Che a 3 (46%) and displayed bands for several other different molecular weights in Western blots of a sample of 12 patients. Its role as a major allergen in this pollen was later supported in a different population

of 32 *Chenopodium* allergic patients from Iran, where 81% displayed IgE to rChe a 2 [33]. Another example of profilin as a major allergen is the case of Pho d 2, which triggered 56% and 64% of positive skin prick tests (SPT) and ELISA, respectively, in a population of 25 date palm allergic patients [34]. As in the *Chenopodium* population mentioned above, the results of Western blotsexhibited several other bands in addition to their reactivity to a 14.4kDa band (supposedly profilin), compromising the real clinical impact of profilin.

There is only one classical report considering the impact of profilin sensitization using a purified rBet v 2 extract for nasal challenge [35]. In a population of 24 tree and/or grass pollen allergic patients, 10 showed sIgE to rBet v 2, and 8 also presented symptoms in the specific nasal provocation challenge with rBet v 2. Despite this hint of evidence, it is generally accepted that profilin is not a relevant respiratory allergen.

The role of profilin in food allergy

Food allergy to profilin-containing foodstuffs is due to primary sensitization to profilin through inhalation and a subsequent development of the so-called pollen-food syndrome (PFS) [36] according to a type II food allergy mechanism. Most syndromes involve weed (*Ambrosia*, *Chenopodium*, *Artemisia*, etc.) and grass and birch pollens (see **Table 2**). Although performing an extensive review of the literature in search of profilin-linked pollen-food syndromes is beyond the scope of this manuscript, it should be borne in mind that some of the classical references supporting such syndromes might only provide hints of the association and not proper evidence of it, either because there is no proper identification of the causative allergen or because the patients' clinical background is missing.

The most frequent scenario is profilin recognition with little or no clinical relevance [37,38]. Due to its pepsin digestion lability [39] and thermal sensitivity [40], profilin triggers oral allergy syndrome (OAS), where symptoms involving the lips, tongue and mouth, as well as throat itchiness are self limited both in time and extension, and appear immediately after the intake of

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raw plant foods. Nevertheless, there are 2 reports of systemic reactions to lychee fruit [41] and zucchini [42], with profilin being the putative allergen that creates an exception to this rule.

Despite being considered a minor allergen, profilin is the major allergen of some plant foods, and such is the case of melon (Cuc m 2) [43], orange [44] and soybean (Gly m 3) [45,46]. It can induce symptoms to virtually every plant food; however, allergy to melon, watermelon, citrus fruits, tomato and banana have been pointed out as "clinical markers" of profilin hypersensitivity in a population of patients with OAS after the ingestion of vegetables [38,47].

The role of profilin in latex allergy

To assess the role of Hev b 8 in latex allergy and its associated syndromes, it is of utmost importance to clarify whether a patient became sensitized to latex in the first place, or if initial sensitization was due to other sources (pollen or plant foods). At the same time, it is also useful to bear in mind that Hev b 8 is present in very low amounts or even absent in natural latex rubber gloves [48].

In primarily latex-sensitized subjects, Hev b 8 recognition has been seen to reach 40% when a purified recombinant form of rHev b 8 is used in a spina bifida and latex-allergic selected population [49]. In a recent work by Vandenplas et al. [50] including 82 natural rubber induced occupational asthma patients studied with a panel of 12 latex allergens, Hev b 8 was only recognized in 4 (4.8%) subjects; however, in 2 of these, it was the only putative allergen, although the authors still resist consider it a clinically relevant sensitization. Usually, the presence of sIgE to Heb v 8 is a marker of non-relevant sensitization. Schuller et al.[51], with a 9 latex allergen platform, detected monosensitization to latex profilin in 2 (14.2%) out of 14 latex allergic and in 19 (67.8%) out of 28 non-allergic latex-sensitive patients. Overall, Hev b 8 is not considered to have clinical impact in latex allergy and, according to guidelines [52], subjects sensitized to this allergen alone do not need a latex-free setting during surgical procedures.

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Around 30 to 50% of latex allergic patients have IgE mediated symptoms to many plant foods [53], most frequently to avocado, banana, kiwi, chestnut and papaya, which was first described as the latex-fruit syndrome by Blanco et al. [54]. Latex class I chitinases (Hev b 6) [55] and patatin like proteins (Hev b 7) [53] have been directly involved, and despite other allergens also being potentially involved, the role of latex profilin is questionable [56]. Generally, the Hev b 8 sensitization found in pollen and/or fruit allergic patients is not based upon a primary sensitization to latex, but most likely a cross reactive phenomenon due to the high similarity of its sequence with profilins from those other sources, ranging from 93.9% to 89.3% with Ole e 2 (olive profilin) and Hel a 2 (sunflower profilin) or 95.5% to 88.6% with Pyr c 4 (pear profilin) and Ara h 5 (peanut profilin), respectively [57]. Garnier et al. [58] reported 130 patients with positive sIgE to natural rubber latex, 97 of whom were latex allergic. Among the 33 non-latex allergic subjects, 30 had either food or pollen allergy or both, and 26 were monosensitized to rHev b 8. In contrast, in a subset of 46 latex allergic patients without pollen allergy, only one displayed sIgE for rHev b 8, although he was food allergic. All this evidence reinforces the lack of impact of rHev b 8 positivity among fruit/pollen allergics regarding latex allergy.

The shift in the perception of profilin

Previously, profilin was shown to be a prevalent pan-allergen that is seemingly unable to unleash remarkable food allergic reactions, and does not induce respiratory symptoms or latex allergy. However, within the last years, several publications have raised significant doubts on some of these concepts, leading to the belief that profilin is an allergen that should no longer be overlooked.

Profilin, a severity and evolution marker

In previous large epidemiological studies analyzing the pollen-molecular recognition pattern in different areas of Spain, profilin was found to be both a disease-severity and polysensitization marker in grass allergy [17–19]. In the aforementioned population of 146 olive allergic patients, Ole e 2 sensitization was statistically associated with asthma (p= 0.04, OR: 2.2, CI: 0.9–5.1),

although the confidence interval includes the null effect [22]. Similarly, in a cohort of 1271 pollen allergic children where 296 (23%) were profilin-sensitized subjects, rPhl p 12 sensitization was statistically associated with longer disease duration and OAS to plant foods but not to more severe disease [21].

Due to the cross-sectional design of the previous studies, stronger evidence of profilin as a marker of long-evolution allergic disease could only be provided by longitudinal studies, such as the one performed by Hatzler et al. [59], where a group of 820 newborns were followed up until the age of 13 years. Serum samples and clinical evaluation of all subjects were performed regularly, and the authors found that among the 177 subjects who finally developed seasonal allergic rhinitis to grass, profilin sensitization invariably appeared in the latter stages of evolution and never as an early marker, thus supporting its role as an indicator of longer-term disease.

Taking an opposite approach, that is, from clinical behavior to molecular recognition, a subgroup of pediatric patients [21] was assessed to establish their molecular characterization according to a predefined clinical profile. Three hundred pollen allergic children were selected due to reported OAS within 5 minutes of ingestion of pollen-related foods, and diagnosed with PFS [60]. IgE antibodies to PR-10 (rBet v 1), LTP (rPru p 3), and timothy grass profilin (rPhl p 12) were determined to classify the patients. Two profilin-related clinical endotypes arose following a cluster analysis approach. A group of 63 profilin monosensitized children was identified whose main distinctive characteristics were grass, plane, olive and plantain pollen sensitization; OAS caused by peach, kiwi, banana and fruits from the *Cucurbitaceae* family; and a high frequency of asthma. A more interesting group included 85 children, in whom more than one pan-allergen (38% profilin sensitized) was detected; these patients recognized birch and grass pollen, suffered symptoms with all the studied plant foods and experienced several comorbidities such as asthma, urticaria/angioedema and atopic dermatitis. This study provides compelling evidence that profilin sensitization is itself associated with higher presence of

asthma, and when accompanied by other pan-allergens, is associated with more severe allergic disease.

A symptomatic aeroallergen

The impact of profilin in respiratory allergy has traditionally been considered low or non-existent [3], and there are recent clinical studies supporting this statement [61]. However, in recent years, some isolated case reports suggested that profilin may be the culprit allergen in patients with pollinosis. Favré et al. [62] report the case of a grass pollen allergic patient who later developed symptoms in birch pollen season with negative sIgE to rBet v 1, rBet v 4 and rBet v 6 but with a positive rBet v 2 who also had a positive nasal challenge with nBet v 2, suggesting that Birch pollen symptoms were produced by the single sensitization to Bet v 2. Asero et al. [63] describe the case of a 32-year-old female with allergic rhinoconjunctivitis and positive SPT to all whole-pollen extracts and a profilin (nPho 2, purified natural date palm pollen profilin) extract. Surprisingly, in both ImmunoCAP and ISAC platforms, all major pollen allergens were only weakly recognized or not recognized at all, although a strong positivity for profilin was detected (rPhl p 12: 12.6 kUA/L), leading the authors to the conclusion that profilin was the most probable culprit allergen for her respiratory symptoms.

The evidence suggested in these scarce case reports is reinforced by a few very well-designed studies with *in vivo* and *ex vivo* provocation tests. Núñez et al. [64] demonstrated that profilin can induce ocular symptoms, performing conjunctival challenges with nPho d 2 by exploring two groups of pollen allergic patients: one profilin sensitized (n=17) and a control group comprising individuals not sensitized to profilin (n=14). None of the non-profilin sensitized patients reacted, while 65% (11/17) of the profilin sensitized patients had a positive response. Two different dilutions were used (50 and 5 µg/ml), and most of the reacting patients needed the highest dose to produce positive test results (8/11). Ruiz et al. [65] showed how profilin (nPho d 2) induced nasal and bronchial positive challenges in 43% and 77% of a profilin sensitized

cohort (n=23), respectively, but not in 5 non-profilin sensitized pollinic controls, providing evidence that profilin can trigger nasal and bronchial symptoms in sensitized patients. A recent publication [66] demonstrated how in 40 Bet v 2 sensitized birch allergic patients, stimulation with Bet v 2 and Phl p 12 induced a dose dependent basophil activation.

The above-mentioned data support the notion that despite the misguided perception of clinical irrelevance, profilin acts as a clinically relevant aeroallergen. Moreover, due to its ubiquity in different pollens and plants, sensitized subjects might react clinically to multiple allergen sources, presenting perennial symptoms and potentially a more severe allergic phenotype.

A not so mild food allergen

Profilin is thought to be a clinically irrelevant food allergen that mostly elicits mild symptoms, although some exceptions have been reported [41,42]. No other cases of systemic profilin allergy appeared until recently, when 9 out of a cohort of 26 grass pollen profilin-sensitized adults from a high grass pollen pressure area reported systemic reactions after the ingestion of plant foods [67]. In the study, only 18 subjects (8 with previous reported systemic reaction) agreed to undergo a double blind, placebo controlled food challenge (DBPCFC) with nPho d 2. The DBPCFC maximum cumulative dose was 822.2 µg, equivalent to the profilin in 283 g of melon. All patients reacted in the challenge (median 81.24 µg, range 0.074-821.24), and in 61.1% (11/18) subjects systemic symptoms were elicited, with adrenaline being used in 5 cases. The authors speculate that the very high levels of grass in the atmosphere during pollen season (peaks of 2000 grains/m^3 and sustained levels above 300 grains/m^3) and the high degree of sensitization to grass allergens in the patients in this geographic area are critical determinants of their severe profilin reactivity phenotype. In two recent presentations delivered at the 2016 annual EAACI meeting in Vienna [68,69], the authors described extensive oral mucosa remodeling together with a 10 fold increase in effector cell sensitivity associated with severe food profilin mediated reactions. This is the first evidence that the oral mucosa can be an

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effective route for eliciting severe food reactions, with potential impact in sublingual immunotherapy mechanisms and evolution from respiratory to food allergy.

As previously mentioned, melon and watermelon are the foods most frequently involved in profilin food allergy [63]. The explanation for this seems to be the higher pH of melon compared to other fruits and vegetables [67], which increases profilin stability and allows for a more efficient mucosal interaction. Why patients who previously tolerated and ingested profilin daily develop such a severe allergic phenotype after presenting with severe grass respiratory allergy is yet to be elucidated. The study of this particular population, representing a unique clinical model, may pose an opportunity to understand allergic disease evolution and the increasing allergy pandemic [70] and to explore new biomarker strategies in allergy.

Profilin sensitization impact in the selection of allergen immunotherapy (AIT)

Profilin sensitization jeopardizes the diagnosis and treatment in pollen polysensitized patients. Moreno et al. [71] describe a discrepancy in 56% of AIT prescriptions when 1263 pollen allergic subjects are diagnosed in terms of SPT to whole extracts, compared to the use of component resolved diagnosis (CRD) to the major grass and olive pollen allergens. Previously, and using a similar approach, Sastre et al. [72] had described a change in the composition in up to 54% AIT prescriptions after CRD was applied in a group of 141 adults previously assessed only by SPT to whole extract, pointing to profilin and polcalcin sensitization as one of the main confounding factors. Some other authors have reported similar findings [73,74]. Nonetheless, all these reports state how AIT prescription might change after CRD and the assessment of major and minor allergens, but there is no data as to whether CRD applied in patient AIT selection improves the efficacy or not.

Although CRD seems to be helpful in assessing the presence of major and cross reactive allergens, it does not provide information on the clinical relevance, and might be of limited utility if more than one primary source allergen arises. In fact, it is not uncommon to detect

profilin sensitization in patients who are primarily sensitized to 2 or more pollens [15]. It should also be borne in mind that profilin sensitization has been associated with a higher prevalence of sensitization to "genuine" allergens from other pollen sources such as Phl p1/p5, Cup a 1, Art v 1 and Ole e 1 [21], so its presence might be considered a marker of an advanced sensitization to the source, rather than just a mere finding to be ignored. Organ-specific challenges may be used to solve this issue and assess clinical relevance [75,76] in polysensitized patients. However, the content of profilin in whole extracts might also obscure the real meaning of a positive test in organ challenges, as has been suggested by some authors [64]. The amount of profilin in extracts used in organ-specific challenges is usually disregarded [76]; however, Ruiz et al. [65] analyzed Pho d 2 content in 8 different pollen diagnostic extracts (ALK-Abelló, Madrid, Spain), and only grass preparations (*Lolium* and *Phelum)* seemed to have larger amounts of this protein (75 and 46.1 µg/vial of freeze-dried extract, respectively), whereas *Betula, Chenopodium, Olea, Plantago* and *Salsola* profilin content remained far below 5 µg/vial. Compared to the major allergen content of each source per vial, profilin percentage ranges from 0.8% for *Lolium* to 0.01% for *Plantago*. Profilin might be even less represented than as shown by Ruiz et al., as seen in the results of Focke et al, [77] who analyzed the qualitative and quantitative allergen composition in 4 different timothy grass pollen extracts, and found out that Phl p 12 could not be detected in any of them. In conclusion, it seems unlikely that the profilin content in challenge extracts might bias its results, although better knowledge of this issue would be desirable.

Treating profilin allergy

The possibility of tailoring AIT on a molecular level has been speculated for many years [78], and this approach has been given the name of component resolved immunotherapy. A recombinant form of Phl p 12 [79] and a mutant form of Cuc m 2 (melon profilin) [80] have been developed and proposed as candidates for profilin allergy immunotherapy. However, there are few double blind, placebo controlled aeroallergen trials [81,82] with rPhl p 1, rPhl p 2, rPhl

p 5a, rPhl p 5b and rPhl p 6 showing that using recombinant forms in respiratory allergy can be effective and safe, although recombinants have yet to be approved for use in humans. Despite these optimistic reports, Tripodi et al. [83] describe 39 different recognition patterns for the 8 phleum allergens studied in a population of 200 grass allergic children, and even after ignoring polcalcin and profilin, their results still led to a significant degree of mismatch in the potential AIT composition when compared to a previously employed recombinant vaccine [81]. Considering both the minor impact of profilin in respiratory allergy and the low prevalence of sensitization compared to other allergens, it seems unlikely that a rPhl p 12 AIT product will be developed, although exploratory research is under way in the field [84].

Taking a more viable approach, profilin as it is currently used in AIT products could be the best chance to target this allergen. Very recently, Asero et al. [85] performed profilin-inhibition assays with the sera of 18 pollen allergic profilin-sensitized subjects with commercially available birch, grass, ragweed and olive pollen AIT extracts, and concluded that due to the high level of inhibition (80%-90%), these products contained large amounts of profilin and were potentially able to desensitize patients to this allergen. Despite this recent report, several reasons discourage the use of current AIT products to specifically treat profilin allergy: AIT products are only standardized for major allergens [86], the differences in protein content are very wide [87] and the profilin content in allergen extracts is low [65] or undetectable [77]. Supporting these considerations, in a cohort of 33 grass allergic patients (51% profilin-sensitized), the levels of IgG4 for Phl p 12 were undetectable after 16 weeks of grass SCIT (65 µg of Phl p 5 cumulative dose, Alutard SQ®, Alk-Abello, Hørsholm, Denmark). Also, Phl p 1, Phl p 2 and Phl p 11 IgG4 levels were low, leading the authors to suggest that the vaccine content of all these four allergens was also so low that it was unable to elicit the induction of sIgG4 [88].

Considering both scenarios, and in accordance with suggestions by other authors [21,59], the best way to treat profilin sensitization/allergy may be to use preventive administration of regular AIT in early stages of pollen sensitization, halting the expected progression towards higher sIgE levels and a wider recognition of other allergens from the same source. As profilin sensitization is mainly associated with grass pollen, evidencing an increase of prevalence at higher intensities

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in the grass pollen gradient, and grass extracts proving to have the highest profilin content [65], once the primary sensitization to Phl p 1 and or Phl p 5 is confirmed, grass monotherapy is likely the best therapeutic option to treat profilin sensitized patients in the absence of a specific profilin therapy. Unfortunately, due to the lack of specificity of whole-extract based diagnosis and the underuse of component resolved diagnosis [89], the correct identification of profilin-positive grass monoallergic patients is limited and thus are treated with less efficacious extracts in the best case or might even be at risk of de novo sensitizations in the worst scenario.

In type II food allergy it has been hypothesized that by administering pollen AIT with the primary allergen, symptoms due to cross-reactivity to its homologues in plant foods will also be reduced. There are some interesting publications in birch pollen allergic patients with vegetable allergy due to the cross-reactivity of PR-10 proteins (Bet v 1 homologues), showing both beneficial effects [90,91] and no effect [92,93] in the associated food allergy despite a good response of the respiratory symptoms. The experience with profilin is far more limited, with only two reports of food allergy being successfully treated with AIT, [94,95], suggesting that pollen AIT is unable or underpowered to treat the secondary food allergy.

Another route that has been explored to minimize the impact of profilin allergy in these patients is the production of plant foods with reduced allergenicity [96], although these proposals have not led to any real-life implementation yet. So far, the best option to treat profilin food allergy is the one appearing in the recent publication by Nucera et al. [97]. In their article, 7 patients with profilin-PFS and OAS to a wide array of foods (median number of foods triggering symptoms: 9) are treated with a nPho 2 extract (50 µg/ml) following a sublingual protocol with incremental doses up to a maximum dose of approximately 75 µg of profilin per week. The duration of the treatment was 9 to 10 months, it was very well tolerated, and in the exit DBPCFC with each of the offending foods, patients increased the number of vegetables they could eat from 23% to 92.9%. This new approach needs further optimization, and although profilin usually induces only mild symptoms, the high number of implicated foods produces a significant burden for the patients and represents an important therapeutic target.

Conclusions

Profilin plays a relevant role as sensitizer and as a confounding factor in both diagnosis and treatment of pollen and plant food allergic subjects. Its relevance in latex allergic individuals remains low or non-existent according to several available publications. In controlled settings it has shown its ability to induce symptoms in all respiratory organs, although it still has to be elucidated whether it can induce respiratory symptoms in real life exposure and to which extent it contributes to the patients' symptoms. Its role in the bothersome OAS to several fruits has been acknowledged in pollen allergic patients, and how in selected populations suffering seasons with heavy loads of grass pollen, can trigger systemic reactions to plant foods. From a more holistic perspective, profilin sensitization has been significantly linked to more severe allergic disease presentation and its presence should be seriously considered by allergists, beyond the classical concept of profilin as merely a confounding factor in patient evaluation. Despite the aforementioned relevance, there is no solid therapeutic approach to treat this allergy, and currently available AIT products are most probably underpowered and food immunotherapy insufficiently explored. Primary prevention strategies could be the best option for these patients if candidates are identified in early stages of their disease.

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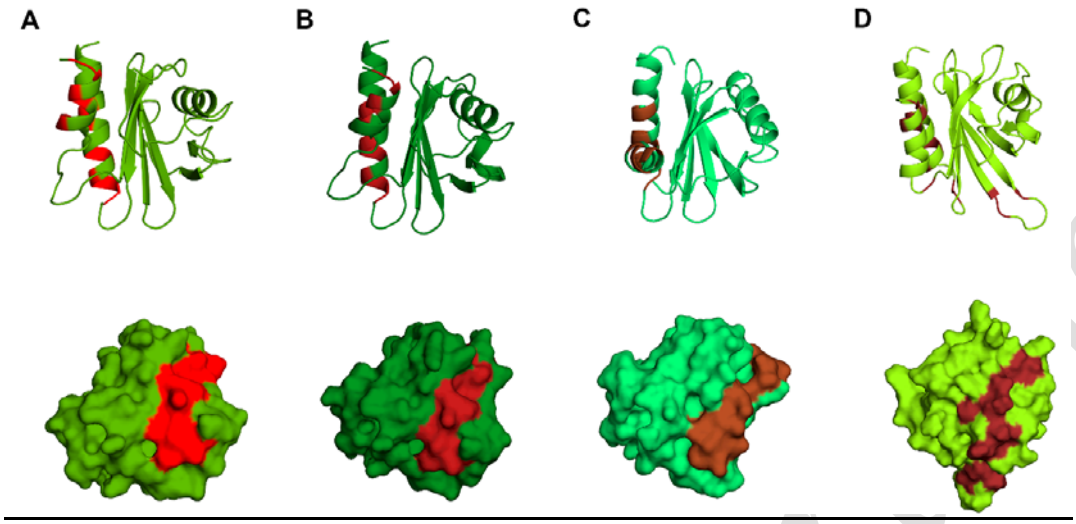
FIGURES

Figure 1



TABLES

Table I

Members of the profilin family identified as allergens. Sequence identity to Cuc m 2 is indicated

Plant species	Allergen name	Id (%)	UNIPROT
Cucumis melo (Muskmelon)	Cuc m 2	100	Q5FX67
Actinidia deliciosa (Kiwi fruit)	Act d 9	68	FG438715*
Ambrosia artemisiifolia (Short ragweed)	Amb a 8	69	Q2KN24
Ananas comosus (Pineapple)	Ana c 1	76	Q94JN2
Apium graveolens (Celery)	Api g 4	78	Q9XF37
Arachis hypogaea (Peanut)	Ara h 5	79	Q9SQI9
Artemisia vulgaris (Mugwort)	Art v 4	69	Q8H2C9
Betula verrucosa (Betula pendula) (European white birch)	Bet v 2	74	P25816
Capsicum annuum (Bell pepper)	Cap a 2	85	Q93YI9
Chenopodium album (Pigweed)	Che a 2	77	Q84V37
Citrus sinensis (Sweet orange)	Cit s 2	76	P84177
Corylus avellana (Hazel)	Cor a 2	83	Q9AXH5
Daucus carota (Carrot)	Dau c 4	75	Q8SAE6
Glycine max (Soybean)	Gly m 3	83	O65809
Helianthus annuus (Sunflower)	Hel a 2	71	O81982
Hevea brasiliensis (Para rubber tree (latex))	Hev b 8	82	O65812
Malus domestica (Apple)	Mal d 4	77	Q9XF42
Olea europea (Olive)	Ole e 2	74	O24169
Phleum pratense (Timothy)	Phl p 12	76	P35079
Phoenix dactylifera (Date palm)	Pho d 2	77	Q8L5D8
Prunus persica (Peach)	Pru p 4	79	Q8GT40
Pyrus communis (Pear)	Pyr c 4	77	Q9XF38
Salsola kali (Russian thistle)	Sal k 4	76	C6JWH0
Sinapis alba (Yellow mustard)	Sin a 4	81	E6Y2M0
Solanum lycopersicum (Tomato)	Sola l 1	85	Q93YG7

Table 2. Plant food syndromes where profilin is involved

Syndrome	Pollen name	Plant food name
USA, *profilin sensitization prevalence (15%*[98]***)**		
Ragweed-melon-banana[99]†	Ragweed (*Ambrosia*)	*Cucurbitaceae* (melon) *Musaceae* (banana)
CENTRAL EUROPE, *profilin sensitization prevalence* (15-26%) [100,101]		
Mugwort-birch-celery[41,102–106]	Birch *(Betula)*, Mugwort (*Artemisia*)	*Apiaceae* (celery), lychee, carrot. Anise, fennel, coriander and cumin
Birch-fruit [107]	Birch (*Betula*)	Banana, pinapple
Ragweed-melon-banana [42,108]	Ragweed (*Ambrosia*)	*Cucurbitaceae* (zuchini), *Musaceae* (banana)
Compositae-fruit [41]	Compositae (A*mbrosia*, *Artemisia…*)	Lychee
SOUTHERN EUROPE, *profilin sensitization prevalence* (15-50%)[17]		
Goosefoot-fruit [109,110]	*Chenopodium*	*Cucurbitaceae* (melon), *musaceae* (banana), *rosaceae* (peach), *liliaeae* (garlic)*
Mugwort-spice [111]	Mugwort (*Artemisa*)	*Liliaceae* (garlick)
Mugwort-peach []	Mugwort (*Artemisa*)	*Rosaceae* (peach)
Ragweed-melon [111]	Grass, weeds and trees	*Cucurbitaceae* (melón, zuchini)
Grass/Olive-Rosaceae and several fruits [17,18,27,38,113,114]	Grass and olive	Peach, Banana, Fig, Kiwi, Melon, Orange, Peach, Pineapple, Watermelon
Plane Tree-fruit [115]	Plant Tree (*Platanus*)	Rosaceae, other fruits, peanut, treenuts and vegetables

Syndromes are included in areas where they are most frequently described for didactic purposes, although some of them were also described in different areas, which are also included in the references. It should be taken into account that in most cases, other allergens such as Bet v 1, CCDs or others, might play a relevant role, and in most cases it is not possible to clarify the culprit of clinical reactivity.

† identifies studies where profilin is suspected due to molecular weight, but without proper identification