

Profilins: Mimickers of Allergy or Relevant Allergens?

Alexandra Santos^{a, b} Ronald Van Ree^a

^aDepartments of Experimental Immunology and Otorhinolaryngology, Amsterdam Medical Center, University of Amsterdam, Amsterdam, The Netherlands; ^bImmunoallergy Department, Coimbra University Hospital, Coimbra, Portugal

Key Words

Asthma • Component-resolved diagnosis • Cross-reactivity • Food allergy • Immunoblot • Pan-allergen • Profiling • Recombinant allergen • Rhinitis

Abstract

Profilins are ubiquitous proteins, present in all eukaryotic cells and identified as allergens in pollen, latex and plant foods. The highly conserved structure justifies the cross-reactive nature of IgE antibodies against plant profilins and their designation as pan-allergens. Primary sensitization to profilin seems to arise from pollen sensitization with later development of cross-reactive IgE antibodies against plant food (and possibly latex) profilins. The role of profilin in inducing allergic symptoms needs to be evaluated and raises important issues in allergy diagnosis due to cross-reactivity. IgE cross-reactivity among profilins is associated with multiple pollen sensitization and with various pollen-food syndromes. In respiratory allergy, sensitization to pollen to which the patient has virtually no environmental exposure has been identified as a manifestation of profilin sensitization. As a food allergen, profilin usually elicits mild reactions, such as oral allergy syndrome, is not modified by processing and is especially important in allergy to some fruits, such as melon, watermelon, banana, tomato, citrus fruit and persim-

mon. Purified natural and recombinant profilins for in vitro and in vivo allergy tests are helpful in the diagnostic work-up. Herein we review the current state of knowledge about the allergen profilin and its implications in the diagnosis and treatment of allergic diseases. We conclude that, although its role in triggering allergic symptoms is still controversial, profilin is undoubtedly a relevant allergen. As a pan-allergen, profilin is associated with multiple pollen sensitization and pollen-food-latex syndromes that the allergist has to be aware of in order to accomplish an accurate diagnosis and successful treatment of allergic diseases.

Copyright © 2011 S. Karger AG, Basel

Introduction

Allergy has very much evolved in recent years. The knowledge about specific allergens in various sources has increased. Genes of different allergens have been cloned and sequenced and the corresponding proteins expressed as recombinant molecules that can easily be obtained in high quantities with reproducible quality. As a result of these developments, a more molecular approach is now emerging in clinical practice. A clinician cannot only characterize clinical presentations and allergenic sources that trigger the allergic symptoms, but can

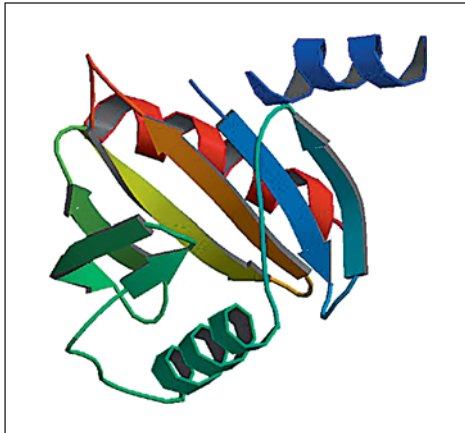


Fig. 1. Ribbon diagram of birch pollen profilin [5]. Available online at <http://www.rcsb.org/pdb/explore/images.do?structureId=1CQA> (accessed October 2009).

now also identify specific allergen molecules that may explain different clinical presentations and risk profiles. This molecular approach allows a more specific diagnosis and better targeted treatment of allergic diseases.

In this paper, we will review the current state of knowledge about the allergen profilin and its implications in diagnosis and treatment of allergic diseases.

Profilin: Involvement in Cytoskeleton Organization and Signal Transduction

Profilins constitute a family of highly conserved proteins, which are present in all eukaryotic cells, including plants, fungi, protozoa, animals and viruses. Profilin was first described by Carlsson et al. [1] as a profilamentous complex of actin-associated low-molecular-weight protein. In the cell, profilin is one of the most abundant proteins. Immunofluorescence [2] and electron microscopy [3] studies suggest that profilin has a peripheric localization in the cytoplasm, sometimes associated with the inner surface of the cell plasma membrane. Primary sequences of profilins are composed of 125–153 amino acids, with molecular masses ranging from 12 to 15 kDa and isoelectric points between 4.3 and 9.2. For some species multiple isoforms have been described, which may slightly differ in physicochemical properties.

The three-dimensional structures of some allergenic profilins have been elucidated [4, 5]. As an example, birch pollen profilin is constituted by a central 6-stranded antiparallel β -sheet and two α -helices, one at the amino-

terminal side perpendicular to the sheet and another parallel at the carboxy-terminal side [5] (fig. 1). The overall fold of the protein was found to be similar to previously described profilins, for example, mammalian and amoeba profilins [6, 7]. Although the amino acid sequence of these taxonomically distant profilins is quite different, their overall fold is highly conserved [8].

Profilin binds actin and other ligands, such as phosphatidylinositol-4,5-bisphosphate and poly-L-proline. Binding to actin signifies its role in the regulation of actin polymerization in the cytoskeleton, catalyzing nucleotide exchange and the addition of actin monomers to preformed actin filaments [9–12]. Organisms that do not express profilin have a disorganized actin cytoskeleton and abnormal morphology [13, 14]. Profilins also act as mediators of communication between membranes and the cytoskeleton, interacting with the phosphatidylinositol-bisphosphate pathway of signal transduction [15]. The ability to bind to poly-L-proline is a feature of profilin shared with prolyl-hydroxylase. As a matter of fact, profilin was discovered as a contaminant during purification of this enzyme using poly-L-proline, a reagent that is now also used to purify profilin [16, 17].

Profilin: Its Discovery as an Allergen

In 1991, profilin was first identified as an allergen in birch pollen. Valenta et al. [18] cloned and sequenced cDNA encoding a pollen protein distinct from Bet v 1 from a birch pollen cDNA library using IgE from birch pollen-allergic patients selected on the basis of clinical history and sensitization to birch pollen as assessed by RAST and immunoblot assays using birch pollen extracts. The cloned allergen was identified as birch profilin by sequence homology to known profilins, and designated Bet v 2. Purified natural profilin and recombinant allergen were both able to bind IgE from birch pollen-allergic patients on immunoblot. Subsequently, Valenta et al. [19] identified profilin as an allergen in grass (*Phleum pratensis*) and weed (*Artemisia vulgaris*) pollen and Van Ree et al. [20] and Vallier et al. [21] found it in plant food. Other allergenic profilins have been identified in many other types of pollen and latex, but more importantly also in plant foods (table 1). Its ubiquitous presence and conserved structure in the plant kingdom are at the basis of the acknowledged cross-reactive nature of IgE antibodies against plant profilins from pollen, plant foods and latex. This characteristic is the reason for the designation of profilin as a pan-allergen.

Table 1. Allergenic profilins described to date [97, 111, 112]

Allergen	Isoforms	Plant species		References
		scientific name	common name	
<i>Pollen</i>				
Amb a 8	Amb a 8.0101 Amb a 8.0102	<i>Ambrosia artemisiifolia</i>	short ragweed	[96]
Ara t 8		<i>Arabidopsis thaliana</i>	mouse-ear cress	[97]
Art v 4		<i>Artemisia vulgaris</i>	mugwort	[98]
Bet v 2		<i>Betula verrucosa</i>	birch	[18]
Che a 2		<i>Chenopodium album</i>	pigweed	[27]
Cro s 2		<i>Crocus sativus</i>	saffron	[99]
Cyn d 12		<i>Cynodon dactylon</i>	bermuda grass	[100]
Hel a 2		<i>Helianthus annuus</i>	sunflower	[26]
Mer a 1		<i>Mercurialis annua</i>	annual mercury	[52]
Ole e 2		<i>Olea europaea</i>	olive tree	[101]
Par j 3	Par j 3.0101 Par j 3.0102	<i>Parietaria judaica</i>	pellitory	[102]
Phl p 12	Phl p 12.0101 Phl p 12.0102 Phl p 12.0103	<i>Phleum pratense</i>	timothy	[94]
Pho d 2		<i>Phoenix dactylifera</i>	date palm	[29]
<i>Plant foods</i>				
Ana c 1		<i>Ananas comosus</i>	pineapple	[33]
Api g 4		<i>Apium graveolens</i>	celery	[103]
Ara h 5		<i>Arachis hypogaea</i>	peanut	[30]
Cap a 2		<i>Capsicum annuum</i>	bell pepper	[104]
Cit s 2		<i>Citrus sinensis</i>	orange	[35]
Cor a 2		<i>Corylus avellana</i>	hazel	[105]
Cuc m 2		<i>Cucumis melo</i>	muskmelon	[50]
Dau c 4		<i>Daucus carota</i>	carrot	[31]
Fra a 4		<i>Fragaria ananassa</i>	strawberry	[95]
Gly m 3	Gly m 3.0101 Gly m 3.0102	<i>Glycine max</i>	soybean	[36]
Lit c 1		<i>Litchi chinensis</i>	litchi	[73]
Lyc e 1		<i>Lycopersicon esculentum</i>	tomato	[104]
Mal d 4	Mal d 4.0101 Mal d 4.0102 Mal d 4.0201 Mal d 4.0202 Mal d 4.0301 Mal d 4.0302	<i>Malus domestica</i>	apple	[106]
Mus xp 1		<i>Musa x paradisiaca</i>	banana	[33]
Pru av 4		<i>Prunus avium</i>	sweet cherry	[107]
Pru p 4	Pru p 4.0101 Pru p 4.0201	<i>Prunus persica</i>	peach	[108]
Pyr c 4		<i>Pyrus communis</i>	pear	[66]
Tri a 12	Tri a 12.0101 Tri a 12.0102 Tri a 12.0103 Tri a 12.0104	<i>Triticum aestivum</i>	wheat	[109, 110]
<i>Latex</i>				
Hev b 8		<i>Hevea brasiliensis</i>	latex	[38]

Profilin: Prevalence of Sensitization

Profilin is a minor allergen in most pollen. Sensitization to profilin was observed in about 20% of patients allergic to birch pollen in different studies as assessed by IgE immunoblot [19], IgE immuno-adsorption [22], skin prick testing (SPT) [23] or ImmunoCAP [24] with rBet v 2. Of note, different profiles of sensitization to rBet v 2 have been described in diverse geographical locations [25], with 5–7% of birch pollen-allergic patients from Scandinavian centers recognizing rBet v 2 as opposed to 20–38% of birch pollen-allergic patients from Central and Southern European centers. The prevalence of sensitization to profilin was similar among grass (30%) and mugwort (33%) pollen allergic patients [19]. In another study, IgE binding to profilin from sunflower pollen was also about 30% in a population of sunflower-allergic patients from sunflower-growing areas [26]. Profilin seems to give rise to a higher frequency of sensitization among patients allergic to *Chenopodium album* [27], *Mercurialis annua* [28] and *Phoenix dactylifera* [29] pollen, but in some of these studies sensitization to profilin was only determined by IgE binding on immunoblot at the level of profilin's molecular mass. Therefore, possibly other allergens with similar apparent mass could be involved and the potential role of profilin as a major allergen in these pollens should therefore still be confirmed using natural purified or recombinant profilins from the respective sources.

In patients with food allergy, the prevalence of sensitization to profilin greatly varies in different studies. For patients with allergy to certain foods, such as peanut [30], carrot [31], celery [32] and pineapple [33], profilin has been reported as a minor allergen, whereas for patients with allergy to melon [34], orange [35] and soybean [36], profilin has been claimed to be a major allergen, with 71, 78–87 and 69% of sensitization, respectively.

In latex-allergic patients, studies have shown slightly different data on prevalence of sensitization to latex profilin. Nieto et al. [37] reported 42% of sensitization to natural and recombinant Hev b 8 as assessed by EAST (6/17 spina bifida patients and 7/14 other latex-allergic patients, 9 of whom were healthcare workers). Rihs et al. [38] reported lower prevalences, with 12% of spina bifida patients and 20% of healthcare workers presenting IgE antibodies to rHev b 8 on ImmunoCAP and immunoblot. This heterogeneity could be explained by differences in recombinant Hev b 8 molecules and/or patients' selection. Nevertheless, both studies describe Hev b 8 as a minor allergen in latex allergic patients.

Profilin: The Etiology of Cross-Reactivity

Sensitization to plant food profilins is exclusively seen in patients with IgE antibodies against pollen profilins, usually in the context of a broad spectrum of pollen sensitization. The interpretation of this observation is that profilin sensitization is induced by pollen profilins. This is documented by IgE-binding assays, where higher IgE responses were observed to pollen profilins than to plant food profilins, and by inhibition assays, where pollen profilins were potent inhibitors of IgE binding to plant food profilins, whereas the reverse was not observed [20]. Support for pollen as the source of profilin sensitization has also been found in an occupational setting, for example in the production process of saffron where profilin from *Crocus sativa* pollen induced sensitization [39]. However, with respect to plant foods, there is no real evidence of primary sensitization to food profilins. Whether latex Hev b 8 can directly sensitize as an inhalant allergen has not been established, but some data suggest that some latex-sensitized patients may present negative SPT to tree pollen and have no specific IgE to Bet v 2, indicating that some IgE-binding epitopes on latex profilin do not cross-react with birch pollen profilin [38]. However, cross-reactivity with profilin from other pollen was not excluded and could therefore be responsible for sensitization to latex profilin.

Among pollen profilins, grass pollen profilins seem to be the most potent inducers of IgE against profilin. This is suggested by the gradient of prevalence of sensitization to profilin from the North to the South of Europe, with greater prevalence in Southern European countries [40]. Different factors may contribute to potency differences between grass and birch pollen profilins. One factor may be a higher profilin content of grass pollen, another factor a higher inherent allergenicity of grass profilin compared to birch profilin. Additionally, exposure to grass pollen may be higher, either due to greater quantity of pollen per season or to a more concentrated exposure in a shorter pollen season. In accordance with the prevalence of sensitization to grass and birch pollen in Northern, Central and Southern Europe, we may assume that in these different areas, primary sensitization to profilin is caused by Bet v 2 alone, Bet v2 and/or Phl p 12, and Phl p 12 alone, respectively (fig. 2). The variable mix of recognition of slightly different epitope spectra on birch and/or grass profilins will have impact on the resulting cross-reactivity patterns to plant foods (table 2). Exposure to other tree and weed pollen profilins may make the situation even more complex.

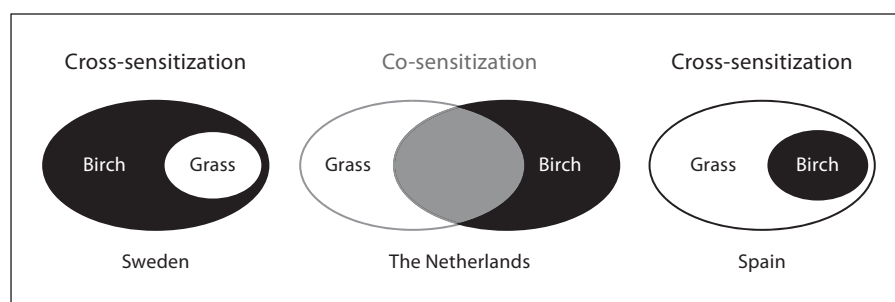


Fig. 2. Patterns of sensitization to pollen profilin in different geographical areas.

Table 2. Pollen-food allergy syndromes involving profilin

Syndrome	Pollens	Pollen allergens	Plant foods	Geographical location	References
Birch-celery	Birch (<i>Betula verrucosa</i>)	Bet v 1 Bet v 2	Apiaceae (Umbelliferae) Rosaceae	Northern Europe Central Europe	[54, 55, 74, 113]
Celery-mugwort-spice	Mugwort (<i>Artemisia vulgaris</i>)	Art v 4 CCD High MW Germin-like Osmotin-like	Apiaceae Solanaceae Piperaceae Anacardiaceae Liliaceae	Central Europe	[60, 114–118]
Mugwort-mustard		Art v 4 Art v 3 High MW	Cruciferae (Brassicaceae) Leguminosae Rosaceae	Southern Europe	[119]
Mugwort-peach		Art v 4 Art v 3	Prunoideae	Southern Europe	[120]
Ragweed-melon-banana	Ragweed (<i>Ambrosia artemisiifolia</i>)	Amb a 8 Amb a 6 CCD High MW	Cucurbitaceae Musaceae	USA	[121, 122]
Goosefoot-fruit	Goosefoot (<i>Chenopodium album</i>)	Che a 2 Che a 3 Polcalcins	Rosaceae Cucurbitaceae Musaceae Liliaceae	Southern Europe	[123]
Pollen-exotic fruit	Asteraceae (Compositae)	Profilin	banana, pineapple, litchi		[33, 73]
Grass-fruit	Grass	Profilin CCD	Rosaceae	Southern Europe	[57]
Plane-fruit	Plane tree (<i>Platanus acerifolia</i>)	Profilin? Pla a 3	hazelnut, peanut, chickpea, peach, apple, lettuce, maize	Southern Europe	[124, 125]

Profilins are in bold. CCD = Cross-reactive carbohydrate determinants; MW = molecular weight.

Olive pollen profilin has also been identified as an allergen cross-reactive with profilins from other pollen [41], as opposed to the major olive pollen allergen Ole e 1 which is a marker of primary sensitization to olive pollen [42]. With respect to weed pollen, mugwort pollen pro-

filin showed cross-reactivity not only with grass and birch but also with ragweed pollen profilins [19, 43]. On the contrary, IgE against *Parietaria* profilin shows limited cross-reactivity with grass and birch pollen profilins [44]. In a study by Stumvoll et al. [45], the major allergens

of *Parietaria* pollen, Par j 1 and Par j 2, were identified as the main sensitizers in patients from Southern Europe whilst sensitization to Par j 3, the pellitory profilin, was more common in weed-sensitized patients from non-Mediterranean areas, where pellitory pollen is virtually absent. Pre-incubation of Austrian patients' sera with mugwort pollen extract strongly inhibited IgE binding to nitrocellulose-blotted *Parietaria* pollen extract. Therefore, IgE recognition of pellitory profilin in some patients from non-Mediterranean areas is most likely the result of cross-reactivity to profilin from other weed pollen, such as ragweed and mugwort.

Several studies have addressed the location and characterization of IgE epitopes on profilins [46–48]. Fedorov et al. [49] mapped Bet v 2 IgE epitopes on the N- and C-terminal α -helices and on a segment of the protein containing two strands of the β -sheet. These epitopes reside in evolutionarily conserved sequences, thus offering an explanation for cross-reactivity. The epitopes overlapped the binding sites for natural profilin ligands, suggesting that native, ligand-free profilin is possibly the original sensitizing agent.

Profilin IgE epitopes are conformational rather than linear [4, 36, 47, 50, 51]. Overall, there is a correlation between the degree of sequence homology and the degree of cross-reactivity, but it depends on whether the homology is surface exposed or not. Shared surface topology is the basis for the extensive IgE cross-reactivity observed between botanically unrelated profilins. This also explains why profilins from phylogenetically related sources do not always cross-react, as it is the case of profilins from *Hevea brasiliensis* (latex) and *Mercurialis annua* pollen, both species belonging to the Euphorbiaceae family [52]. Combining a computer-modeled structure method with IgE ELISA and ELISA inhibition experiments using sera from profilin-sensitized subjects, Radauer et al. [51] showed that the molecular surface of profilin was a mosaic of conserved and variable residues. The presence of species-specific profilin IgE epitopes may contribute to the diverse profiles of sensitization to profilin-containing sources.

Profilin: Cross-Reactivity Patterns

The extensive cross-reactivity of IgE antibodies directed to profilins has been observed between pollen from botanically unrelated plant species (trees, grasses and weeds), plant foods (fruits, vegetables, nuts and spices) and latex.

Cross-Reactivity between Pollen from Different Plant Species

Valenta et al. [19] elegantly showed that natural and recombinant profilins from birch, grass and mugwort pollen were able to bind IgE from selected patients sensitized to profilin and to pollen from the three plant species. Separately analyzing IgE reactivity to grass and mugwort pollen, 30% of grass pollen-allergic and 33% of mugwort pollen-allergic patients showed IgE reactivity to profilin from grass and mugwort pollen, respectively, similarly to recombinant profilin from birch pollen (rBet v 2). The presence of common IgE-binding epitopes among the studied profilins was confirmed by IgE immunoblot inhibition experiments. IgE reactivity of patients' sera on immunoblot correlated well with the capacity of their basophils to induce histamine release using purified profilins. The cross-reactivity between birch, grass and mugwort profilin observed in IgE immunoblot inhibition assays was further supported by the fact that a rabbit polyclonal anticelery profilin antibody bound to purified profilins from birch, grass and mugwort pollens on IgG Western blot. Other studies followed documenting cross-reactivity between pollen from different plant species [26, 29, 53].

Cross-Reactivity between Pollen and Plant Foods

Profilin has been identified as a cross-reactive allergen between pollen and plant foods and involved in some pollen-food syndromes (table 2). It has been implicated in allergy to fruits and vegetables in patients with birch pollinosis. Ebner et al. [54] tested serum samples from 20 birch pollen-allergic patients on immunoblot with extracts of apple, pear, celery, carrot and potato. Profilin from all these foods was recognized by IgE antibodies. Recombinant Bet v 2 markedly reduced IgE binding to these profilins, demonstrating the cross-reactive nature of these antibodies. Other Bet v 2 homologues have been identified in various plant foods [55].

Profilin plays an important role in sensitization to fruits, including the ones belonging to the Rosaceae family, in patients with allergy to grass and/or olive pollen [20, 56–59]. Van Ree et al. [20] first described profilin-associated IgE antibodies against grass pollen and those against vegetable foods in grass pollen-allergic patients. Allergen-binding inhibition assays showed that profilin was a cross-reactive allergen in pollen and vegetable foods such as potato, celery, apple, peanut and wheat.

Finally, profilin has been implicated in allergy to Apiaceae vegetables and spices in patients with both birch and mugwort pollinosis, the celery-birch-mugwort-spice syndrome [21, 60].

Cross-Reactivity between Pollen, Fruit and Latex

Profilin has also been identified as a cross-reactive allergen between pollen, plant foods and latex [61], with Hev b 8 showing 75% of sequence homology to Bet v 2 [38] and a similar three-dimensional structure [61]. Ganglberger et al. [61] showed by ELISA and immunoblot inhibition assays that natural protein extracts from birch pollen, timothy grass pollen, celery and latex were able to prevent IgE binding to rHev b 8. In a study by Rihs et al. [38], 2 of the 5 healthcare workers (n = 25) sensitized to Hev b 8 were not sensitized to Bet v 2, suggesting that birch pollen was not the sensitizer. Whether another type of pollen or latex itself was responsible for sensitization has not been clarified. Overall, these and other studies document profilin as a pan-allergen and a marker of broad allergic sensitization covering pollen, plant foods and latex [62].

Animal and Human Profilins

The ubiquitous distribution of profilins in eukaryotic cells raised the questions of whether there would be allergenic profilins in animal-derived foods and whether autoimmune reactivity to human profilin would be possible.

Valenta et al. [18] reported IgE binding to human profilin purified from platelets on immunoblot in Bet v 2-sensitized patients. These antibodies showed lower affinity but were nevertheless able to trigger histamine release from peripheral blood basophils in patients with high levels of Bet v 2-specific IgE. Cross-reactivity between human profilin and Bet v 2 was demonstrated by immunoblot inhibition. In this seminal paper on profilin, continuous exposure to autologous human profilin was suggested as a possible explanation for the persistence of specific IgE levels against birch pollen out of the pollen season. Since IgE reactivity against Bet v 1 is also persistent and most patients with persistent IgE directed to birch pollen do not show IgE (cross-)reactivity to human profilin, the proposed mechanism, however, seems rather unlikely.

Likely explanations for the lack of allergenicity of animal profilins compared to plant profilins are the taxonomic proximity of human and animal profilins, the low degree of similarity of animal and plant profilins (ruling out significant cross-reactivity upon primary sensitization to plant profilins) as well as the fact that patients are exposed to naturally occurring pollen profilins via respiratory tract, whereas animal profilins are usually consumed and therefore digested before reaching the gut immune system.

Profilin: Clinical Relevance of (Cross-)Sensitization

Although profilin is a recognized allergen in both airborne and food allergenic sources, its role as an elicitor of allergic symptoms is still controversial. A considerable number of studies have been published on sensitization to profilin [18, 23, 63, 64] and cross-reactivity of IgE antibodies against this allergen; however, sensitization and immunological cross-reactivity do not always translate into clinical allergy. It is still not fully understood why some sensitized patients react after exposure to the allergen whereas others do not. The development of an allergic reaction depends on various factors, among which the culprit allergen is only one of the players.

So far, a few studies have provided evidence that IgE antibodies to profilin contribute to the clinical expression of respiratory allergies. Pauli et al. [23] described three patients with positive SPT to birch that were mono-sensitized to Bet v 2. Although they had clinical allergy during grass and weed pollen season, they were symptom free during birch pollen season and did not present with any food allergy to nuts or fruits. This suggests that at least the cross-reactive response to birch pollen and food profilins did not translate into clinical allergy. In a study by Heiss et al. [64], reporting on SPT with rBet v 1 and rBet v 2 in pollen allergic patients, 8 patients sensitized to birch pollen were negative for Bet v 1 but positive to rBet v 2, suggesting that profilin sensitization was responsible for the clinical symptoms elicited by birch pollen. Asturias et al. [29] have reported profilin as a major and relevant allergen in date palm pollen; however, the term relevance was used based on the strong sensitization to date palm profilin (Pho d 2) observed in patients allergic to this pollen as it was responsible for more than 70% of the IgE reactivity of the pollen extract. The authors suggest high date palm pollen counts and the high concentration of profilin in this pollen as possible explanations for the strong sensitization. They speculate that its high allergenic potency could lead to elicitation of allergic symptoms in predisposed individuals, but no provocation tests were performed to confirm this. Niederberger et al. [65] have indeed performed nasal provocation testing with recombinant allergens in patients allergic to tree and/or grass pollen. Eight of these patients tested positive upon nasal provocation with rBet v 2, demonstrating that birch pollen profilin can induce symptoms in a relevant target organ. In the occupational setting, profilin seems to be a relevant allergen in saffron workers allergic to this pollen. In a study by Feo et al. [39], 13 of 50 exposed workers were sensitized and 3 were allergic to saffron pollen, as con-

firmed by positive conjunctival provocation test in 2 patients and positive bronchial provocation test in another. All 3 patients recognized profilin on IgE immunoblot using a pollen extract and in one of them this was the major band. Despite clearly supporting the clinical relevance of profilin as aeroallergen, in the occupational setting levels of exposure are extremely high and this must therefore be considered a particular case.

Although cross-reactivity to foods caused by sensitization to Bet v 2 is rarely seen without cross-reactivity due to sensitization to Bet v 1, some large studies have been able to evaluate the clinical relevance of Bet v 2 sensitization to cross-reactive foods. Strong IgE reactivity to recombinant profilins from celery, cherry and pear have been found in Bet v 2-sensitized subjects without clinical food allergy, suggesting that a significant proportion of IgE responses to profilins is clinically irrelevant [66]. Wensing et al. [67] studied a group of 51 adult patients with pollinosis and sensitization to at least one plant food. Eighty-five percent of these patients were sensitized to Bet v 1 and 71% to Bet v 2. Sensitization to profilin was associated with a broader spectrum of sensitization to plant-derived foods, but was rarely convincingly associated with clinical food allergy, as opposed to Bet v 1. In another study, Ebo et al. [68] aimed to evaluate the role of IgE antiprolin in clinically irrelevant sensitization to latex and apple. No patient monosensitized to birch pollen or latex showed sensitization to profilin and IgE to Bet v 2 was almost restricted to multiple pollen-sensitized patients. Sensitization to Hev b 8 was not found in patients with isolated anaphylaxis to latex but it was indeed found in patients sensitized to multiple pollens. Pollen-allergic patients with cross-reactive IgE but no symptoms to apple and/or latex had a significantly higher prevalence of sensitization to Bet v 2 than true apple- and/or latex-allergic patients.

Asero et al. [69] recently published a study where 200 pollinosis patients were skin prick tested with purified natural date palm profilin (Pho d 2) and, if adverse reactions to foods were reported, also to Mal d 1 and Pru p 3. Thirty percent were sensitized to profilin, all allergic to grass pollen and commonly sensitized to other pollens. Fifty-seven percent of the profilin-reactors had a history of allergy to plant-derived foods (such as *Rosaceae* fruits, tree nuts, melon, watermelon, tomato, pineapple, citrus fruit and banana), 62% of whom were monosensitized to profilin. Given these results, the authors concluded that profilin should be considered a clinically relevant food allergen. Also the studies where profilin was identified as a major allergen in patients al-

lergic to melon [34] and persimmon [70] support the clinical relevance of profilin.

Ideally, to unravel the clinical relevance of an allergen, well-designed clinical trials based on provocation tests should be performed. Lüttkopf et al. [32] and Ballmer-Weber et al. [31] performed studies based on double-blind placebo-controlled food challenge (DBPCFC) with celery and carrot, respectively, aiming at identifying clinically relevant allergens. In both studies, profilin was identified as a minor allergen in these foods and no obvious differences in sensitization patterns were found between patients with positive and negative DBPCFC or different severity of allergic reactions among patients with positive DBPCFC in terms of allergen recognition. One of the main problems in assessing the clinical relevance of profilin was that the majority of patients was sensitized to other cross-reactive allergens as well, such as Bet v 1, Art v 1 and LTP. Further clinical studies based on provocation tests with purified or recombinant profilin are awaited.

Diagnostic and Therapeutic Implications

An accurate diagnosis of allergic diseases includes not only the diagnosis of the allergic phenotypes (e.g. asthma, rhinoconjunctivitis and anaphylaxis) but also the identification of the triggers of allergic symptoms and ideally of the specific allergens that are recognized by patient's IgE and are triggering the IgE-mediated hypersensitivity reactions. Profilins raise important issues in the diagnosis of allergic diseases, both of food allergy and pollinosis, which have to be considered so that an exact diagnosis can be achieved.

Food Allergy: Diagnostic Considerations

As a food allergen, profilin is thought to elicit mild reactions, such as oral allergy syndrome (OAS) [69]. This is possibly due to its susceptibility to pepsin digestion. In a study by Rodriguez-Perez et al. [71], melon profilin has been shown to be quickly digested by pepsin in the gastric fluid, which could explain the mild symptoms observed in patients with melon allergy, where profilin is a major allergen. Also in the previously mentioned studies on carrot [31] and celery [32] allergies, in 80 and 55% of the patients with positive DBPCFC to carrot and celery, respectively, the only manifestation was OAS. However, these were patients also sensitized to other allergens, including Bet v 1 homologues and CCD, and profilin was a minor allergen.

Processing does not seem to modify profilin allergenicity. In a study by Ballmer-Weber et al. [72], 6 of 11 celery-allergic patients reacted to cooked celery on DBPCFC, 4 of whom were sensitized to rBet v 2. All of these patients' IgE also recognized Api g 1 and 2 of them recognized CCD. In a reported case of lychee allergy [73], IgE binding to fresh and tinned lychee fruit profilin was similar, showing that processing did not change profilin's allergenicity.

Despite being more likely to cause only mild reactions, as the quantity of the allergen in the gastrointestinal tract may exceed the capacity of digestive enzymes or certain risk factors for severe reactions may be present, one cannot exclude this allergen when facing a patient with a more severe reaction. In the previously mentioned case report of allergic reaction to lychee fruit [73], the clinical presentation was anaphylaxis and profilin was the only allergen identified by patient's IgE on lychee fruit immunoblot. It was suggested that the high amounts of profilin in this fruit explained the severity of the symptoms. In a case series of persimmon allergy [70] confirmed by DBPCFC, 3 patients were found to be reactive to profilin and CCD, and the symptoms after the ingestion of the fruit were mild but not restricted to OAS (also nausea and vomiting). Two other reported cases of persimmon allergy [74] manifested as OAS in one patient and as OAS, nausea, abdominal pain, cutaneous pruritus, dyspnoea and swelling of the throat in the other patient. These were patients with birch and grass pollinosis, sensitized to Bet v 1 and Bet v 6. The patient with the most severe reaction, who did not agree to undergo DBPCFC, was also reactive to Bet v 2 on immunoblot, but this allergen did not induce significant histamine release nor did Bet v 6, as opposed to Bet v 1. The allergic symptoms were therefore attributed to reactivity to Bet v 1 in these patients, supported by the fact that other 6 of 7 birch pollen-allergic patients with no significant sensitization to nBet v 2 or rBet v 6 developed OAS after open challenge with the same fruit on first exposure.

For each individual patient, it is important to diagnose hypersensitivity to single food allergenic proteins, as the nature of the protein that primarily induces allergic sensitization influences the spectrum of offending foods and the clinical features of allergic reactions. Therefore, one has to bear in mind the prevalence of sensitization to profilin depending on the culprit foods, pollen sensitization and geographical location as well as the cross-reactivity syndromes in which profilins have been implicated (table 2). As mentioned previously, profilin is a minor allergen in Bet v 1 homologues-dominated food allergies in

birch pollen endemic areas and becomes more prevalent in plant foods which do not contain Bet v 1 cross-reactive allergens, such as melon and pineapple, especially in areas without birch trees and with high grass pollen exposure.

To help identify patients sensitized to profilin in daily clinical practice without the availability of profilin-targeted laboratory analysis, Asero et al. [75] defined clinical markers of sensitization to profilin in patients with allergy to plant foods. Seventy-one adult patients with OAS after the ingestion of fruits and vegetables, not sensitized to latex or LTP, were studied. A preliminary classification of patients as potentially sensitized to profilin based on vegetable food allergy associated with multiple pollen sensitizations was very sensitive (91%). Patients were grouped according to Bet v 2 versus Bet v 1 sensitization. Comparing the prevalence of OAS induced by different foods in the different subgroups of patients, sensitization to Bet v 2 was associated with allergy to citrus fruits, melon, watermelon, banana and tomato, whilst sensitization to Bet v 1 was associated with apple and hazelnut allergies. In this study, using a combination of positive clinical history (OAS) and SPT to the Bet v 2-associated fruits resulted in an 85% sensitivity to detect profilin sensitization, with a specificity exceeding 85% and positive predictive values ranging between 68 and 91%. The authors concluded that in patients sensitized to labile allergens from fruits and vegetables, allergy to these foods could be used as a marker for profilin sensitization in clinical practice.

Food allergen extracts have limitations in food allergy diagnosis. This is well-established for SPT reagents of *Rosaceae* fruits for the diagnosis of Bet v 1-related fruit allergies, such as to apple and peach. As profilins are readily degradable and their epitopes conformational, they may also not be sufficiently represented in commercially available allergen extracts. For example, in a study by Ballmer-Weber et al. [31], only 20% of carrot-allergic patients confirmed by DBPCFC reacted to a commercial carrot extract. An alternative to improve diagnostic accuracy in daily practice is to use fresh foods and the prick-to-prick technique to perform SPT [76] to fruits and vegetables. However, these are not standardized and may show variable results. The lack of profilin may also arise when performing DBPCFC to these foods and freshly prepared foods should be used in the real challenge.

Due to its ubiquity and pan-allergenicity, profilin-sensitized patients may exhibit false-positive results in diagnostic tests. A convincing clinical history should lead to seek for reliable evidence of allergic sensitization in order

to confirm the allergy, but one has to be very careful when interpreting the results of allergy tests in patients allergic to plant-derived foods. The use of purified and recombinant allergens in component-resolved diagnosis can be of great help [77], both on SPT and on ImmunoCAP (rPru p 4, recombinant peach profilin), if available. A study on carrot allergy [78], where the most relevant carrot allergens were expressed in recombinant form and used in component-resolved *in vitro* diagnosis, showed a great potential in identifying the prevalence of sensitization to specific allergens (including carrot profilin) and epitope diversity.

Pollen Allergy Diagnosis

Profilin has been associated with multiple pollen sensitizations in different studies. This may lead to misdiagnosis and unsuccessful specific treatment if one considers the patient allergic to all the pollens that are recognized by IgE. Trying to overcome diagnostic difficulties in clinical practice, Mari [79] performed a study in which he sought to address the molecular diagnosis of multiple pollen sensitization and to identify specific markers for pollen pan-sensitization. Patients with respiratory allergy were enrolled and subjected to SPT with 23 different pollen extracts and to determination of serum specific IgE to rBet v 1, rPhl p 5, rBet v 2, rJun o 2 and bromelain. rBet v 1 and rPhl p 5 were used as markers of birch and grass pollen sensitization. rBet v 2, rJun o 2 and bromelain were used as markers of sensitization to the pan-allergens profilin, calcium-binding protein and carbohydrate determinant, respectively. The study population was classified in 4 different groups according to the number of pollens they were sensitized to. About 17.6% of pollen-allergic patients were polysensitized. More than half (55%) of these patients were sensitized to rBet v 2 as opposed to none of the patients in the mono- and oligosensitized groups. Profilin sensitization was associated with mild asthma and OAS. The use of recombinant pan-allergens together with a large number of pollen extracts on allergy tests were found to be useful in distinguishing patients primarily sensitized to pollen from different species from patients in which apparent multiple pollen sensitization is due to IgE reactivity to plant pan-allergens which may not be clinically relevant. This study also showed that patients sensitized to pan-allergens can be identified by IgE reactivity to pollens to which they have had no significant exposure. Profilin sensitization without recognition of the major pollen marker allergens (such as Phl p 1, Phl p 5, Bet v 1, Ole e 1, Par j 1/2 or Art v 1) may therefore be used in clinical practice as a marker

for non-clinically relevant polysensitization to pollen [80].

Especially in areas with complex pollen exposure, the identification of specific markers for profilin sensitization can be very useful. Again, allergenic molecules such as purified natural Pho d 2 [69] and recombinant Bet v 2 [81] may be used, if available. Radauer et al. [51] claim that the large extent of cross-reactivity among plant profilins have justified the use of a single profilin for diagnosis. Recombinant profilin from birch pollen (rBet v 2) has been suggested as the allergen of choice in assays for profilin-specific IgE [81]. However, others have argued that rBet v 2 is not sensitive enough to detect profilin sensitization [75, 82] and Phl p 12 may be used as an alternative. Barderas et al. [27, 83, 84] identified profilin as a major allergen in chenopod pollen and performed inhibition experiments where strong inhibition degrees (over 80%) of IgE binding to chenopod profilin were obtained for different pollen extracts, pointing it out as a potential marker for pollen profilin cross-sensitization.

Recombinant molecules have emerged as useful tools in allergy diagnosis, not only for *in vitro* but also for *in vivo* tests (SPT and challenges, namely nasal, conjunctival and bronchial provocations [85]). They offer the advantage of being produced in high quantities and with a high degree of batch-to-batch consistency. Patient's profile of sensitization can be determined more precisely with recombinant molecules, and provocations with specific molecules can reveal the biological activity and clinical relevance of each allergen. This would be especially important for profilin.

Immunotherapy

The main objective of an accurate diagnosis of allergic diseases is to be able to select an adequate and successful treatment. In pollen allergy, this would enable the correct choice of extracts to use for subcutaneous or sublingual allergen-specific immunotherapy (SIT) [86]. In food allergy, it would allow the definition of risk profiles, recommendation of an adequate diet without unnecessary dietary restrictions and possibly future allergen SIT.

Profilins are often responsible for multiple positivity on allergy tests that may not be clinically relevant in all cases. Misinterpretation of these situations may lead to unsuccessful pollen SIT. Decision on extract formulation has to be made based on patients' exposure and pollen distribution in the region and identification of the culprit plant species on respiratory allergic symptoms, as assessed by the clinical history, allergy tests and provocation tests, as appropriate. Cross-reactivity of pollen

allergens has also to be considered when prescribing SIT [87].

The effect of pollen SIT on cross-reactive food allergies is still controversial. In view of the cross-reactivity between pollen and plant food allergens and of the fact that the pollen allergens seem to be the primary sensitizers in patients with pollen-food syndromes, SIT to pollen could improve associated food allergies. However, studies have shown contradictory results, some reporting improvement [88–90] and others no beneficial effect [91]. Bolhaar et al. [92] evaluated the effect of birch pollen immunotherapy on apple allergy comparing two groups of adult patients allergic to birch pollen and apple submitted either to SIT to birch pollen or to symptomatic treatment alone. The authors showed a decrease in SPT reactivity and in allergic symptoms after DBPCFC as well as an increase in serum-specific IgG4 to Bet v 1 after 1 year of immunotherapy. However, no studies have evaluated the efficacy of pollen SIT specifically on profilin-related food allergy.

Hypoallergenic molecules have been suggested as useful tools in allergen SIT. Westritschnig et al. [93] have obtained a hypoallergenic profilin by tail-to-head restructuring of timothy grass pollen profilin (Phl p 12) to be used for treatment of cross-sensitization to profilin. Timothy grass profilin was selected because it is the primary sensitizer in most profilin-allergic patients [51, 57] and has shown to carry the majority of profilin IgE-binding epitopes [94]. The authors engineered this molecule restructuring the non-IgE-reactive fragments of Phl p 12 identified by synthetic peptide chemistry positioning the C terminal of Phl p 12 at its N-terminal part and vice versa. The new protein was expressed in *Escherichia coli*,

purified to homogeneity and designated rPhl p 12-rs. It showed reduced IgE binding and basophil histamine release compared to rPhl p12, but conserved T cell reactivity in allergic patients. Cross-reactivity with profilins from other pollens and plant foods was also maintained. Although rPhl p 12-rs bound less profilin-specific IgE, it was able to induce production of profilin-specific IgG antibodies in immunized rabbits, which were similar to the ones produced after immunization with rPhl p 12, and were able to inhibit profilin-induced basophil degranulation. Due to the controversial clinical relevance of profilin, it may not be the most urgent candidate for immunotherapy or hypoallergenic approaches. However, eventually, the same technique of restructuring of allergens to reduce allergenicity and expressing it in the recombinant form could be generalized in the production of allergen specific vaccines. Also in food allergy, hypoallergenic-engineered molecules have been sought for potential use in allergen SIT in the future. Analogous to what has been shown for Fra a 3 [95], a low allergenic profilin could be used.

Conclusions

Although its role in triggering allergic symptoms is still controversial, profilin is undoubtedly a relevant allergen. As a pan-allergen, profilin is associated with multiple pollen sensitization and pollen-food-latex syndromes that the allergist has to be aware of in order to accomplish an accurate diagnosis and successful treatment of allergic diseases.

References

- Carlsson L, et al: Actin polymerizability is influenced by profilin, a low molecular weight protein in non-muscle cells. *J Mol Biol* 1977;115:465–483.
- Buss F, et al: Distribution of profilin in fibroblasts correlates with the presence of highly dynamic actin filaments. *Cell Motil Cytoskeleton* 1992;22:51–61.
- Hartwig JH, et al: Association of profilin with filament-free regions of human leukocyte and platelet membranes and reversible membrane binding during platelet activation. *J Cell Biol* 1989;109:1571–1579.
- Thorn KS, et al: The crystal structure of a major allergen from plants. *Structure* 1997;5:19–32.
- Fedorov AA, et al: X-ray crystal structures of birch pollen profilin and Phl p 2. *Int Arch Allergy Immunol* 1997;113:109–113.
- Schutt CE, et al: The structure of crystalline profilin- β -actin. *Nature* 1993;365:810–816.
- Vinson VK, et al: Three-dimensional solution structure of *Acanthamoeba* profilin-I. *J Cell Biol* 1993;122:1277–1283.
- Rothkegel M, et al: Plant and animal profilins are functionally equivalent and stabilize microfilaments in living animal cells. *J Cell Sci* 1996;109:83–90.
- Valenta R, et al: Identification of profilin as an actin-binding protein in higher plants. *J Biol Chem* 1993;268:22777–22781.
- Schluter K, Jockusch BM, Rothkegel M: Profilins as regulators of actin dynamics. *Biochim Biophys Acta* 1997;1359:97–109.
- Weber A: Actin binding proteins that change extent and rate of actin monomer-polymer distribution by different mechanisms. *Mol Cell Biochem* 1999;190:67–74.
- Witke W: The role of profilin complexes in cell motility and other cellular processes. *Trends Cell Biol* 2004;14:461–469.
- Cooley L, Verheyen E, Ayers K: Chickadee encodes a profilin required for intercellular cytoplasm transport during *Drosophila* oogenesis. *Cell* 1992;69:173–184.

- 14 Haarer BK, et al: Purification of profilin from *Saccharomyces cerevisiae* and analysis of profilin-deficient cells. *J Cell Biol* 1990; 110:105–114.
- 15 Goldschmidt-Clermont PJ, et al: Regulation of phospholipase C- γ 1 by profilin and tyrosine phosphorylation. *Science* 1991;251: 1231–1233.
- 16 Tanaka M, Shibata H: Poly(L-proline)-binding proteins from chick embryos are a profilin and a profilactin. *Eur J Biochem* 1985; 151:291–297.
- 17 Kaiser DA, et al: Characterization of rena-tured profilin purified by urea elution from poly-L-proline agarose columns. *Cell Motil Cytoskeleton* 1989;14:251–262.
- 18 Valenta R, et al: Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991;253: 557–560.
- 19 Valenta R, et al: Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377–385.
- 20 Van Ree R, et al: Profilin is a cross-reactive allergen in pollen and vegetable foods. *Int Arch Allergy Immunol* 1992;98:97–104.
- 21 Vallier P, et al: Purification and character-ization of an allergen from celery immuno-chemically related to an allergen present in several other plant species. Identification as a profilin. *Clin Exp Allergy* 1992;22:774–782.
- 22 Laffer S, et al: IgE-binding capacity of re-combinant timothy grass (*Phleum pratense*) pollen allergens. *J Allergy Clin Immunol* 1994;94:88–94.
- 23 Pauli G, et al: Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associ-ated allergies. *J Allergy Clin Immunol* 1996; 97:1100–1109.
- 24 Scheiner O, et al: Cross-reacting allergens in tree pollen and pollen-related food allergy: implications for diagnosis of specific IgE. *Int Arch Allergy Immunol* 1997;113:105–108.
- 25 Elfman L, et al: Different profiles in specific IgE to rBet v 1 and rBet v 2 in patients allergic to birch pollen from six countries. *Int Arch Allergy Immunol* 1997;113:249–251.
- 26 Asturias JA, et al: Cloning and immunolog-ical characterization of the allergen Hel a 2 (profilin) from sunflower pollen. *Mol Im-munol* 1998;35:469–478.
- 27 Barderas R, et al: Profilin (Che a 2) and pol-calcin (Che a 3) are relevant allergens of *Che-nopodium album* pollen: isolation, amino acid sequences, and immunologic prop-erties. *J Allergy Clin Immunol* 2004;113:1192–1198.
- 28 Vallverdu A, et al: *Mercurialis annua*: char-acterization of main allergens and cross-re-activity with other species. *Int Arch Allergy Immunol* 1997;112:356–364.
- 29 Asturias JA, et al: Pho d 2, a major allergen from date palm pollen, is a profilin: cloning, sequencing, and immunoglobulin E cross-reactivity with other profilins. *Clin Exp Al-lergy* 2005;35:374–381.
- 30 Kleber-Janke T, et al: Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. *Int Arch Allergy Immunol* 1999;119:265–274.
- 31 Ballmer-Weber BK, et al: Carrot allergy: double-blinded, placebo-controlled food challenge and identification of allergens. *J Allergy Clin Immunol* 2001;108:301–307.
- 32 Lüttkopf D, et al: Celery allergens in patients with positive double-blind placebo-con-trolled food challenge. *J Allergy Clin Immu-nol* 2000;106:390–399.
- 33 Reindl J, et al: IgE reactivity to profilin in pollen-sensitized subjects with adverse reac-tions to banana and pineapple. *Int Arch Al-lergy Immunol* 2002;128:105–114.
- 34 Lopez-Torrejon G, et al: Allergenic reactivity of the melon profilin Cuc m 2 and its identi-fication as major allergen. *Clin Exp Allergy* 2005;35:1065–1072.
- 35 Lopez-Torrejon G, et al: Isolation, cloning and allergenic reactivity of natural profilin Cit s 2, a major orange allergen. *Allergy* 2005; 60:1424–1429.
- 36 Rihs HP, et al: IgE binding of the recombi-nant allergen soybean profilin (rGly m 3) is mediated by conformational epitopes. *J Al-lergy Clin Immunol* 1999;104:1293–1301.
- 37 Nieto A, et al: Assessment of profilin as an allergen for latex-sensitized patients. *Allergy* 2002;57:776–784.
- 38 Rihs HP, et al: PCR-based cloning, isolation, and IgE-binding properties of recombinant latex profilin (rHev b 8). *Allergy* 2000;55: 712–717.
- 39 Feo F, et al: Occupational allergy in saffron workers. *Allergy* 1997;52:633–641.
- 40 Ghunaim N, et al: Antibody profiles and self-reported symptoms to pollen-related food allergens in grass pollen-allergic patients from northern Europe. *Allergy* 2005;60: 185–191.
- 41 Ledesma A, Rodriguez R, Villalba M: Olive-pollen profilin. Molecular and immunologic properties. *Allergy* 1998;53:520–526.
- 42 Palomares O, et al: The major allergen of olive pollen Ole e 1 is a diagnostic marker for sensitization to Oleaceae. *Int Arch Allergy Immunol* 2006;141:110–118.
- 43 Gadermaier G, et al: Array-based profiling of ragweed and mugwort pollen allergens. *Al-lergy* 2008;63:1543–1549.
- 44 Asero R, et al: Parietaria profilin shows only limited cross-reactivity with birch and grass profilins. *Int Arch Allergy Immunol* 2004; 133:121–124.
- 45 Stumvoll S, et al: Identification of cross-reac-tive and genuine *Parietaria judaica* pollen allergens. *J Allergy Clin Immunol* 2003;111: 974–979.
- 46 Leitner A, et al: A mimotope defined by phage display inhibits IgE binding to the plant panallergen profilin. *Eur J Immunol* 1998;28:2921–2927.
- 47 Asturias JA, et al: Molecular and structural analysis of the panallergen profilin B cell ep-itopes defined by monoclonal antibodies. *Int Immunol* 2002;14:993–1001.
- 48 Lopez-Torrejon G, et al: An experimental and modeling-based approach to locate IgE epitopes of plant profilin allergens. *J Allergy Clin Immunol* 2007;119:1481–1488.
- 49 Fedorov AA, et al: The molecular basis for allergen cross-reactivity: crystal structure and IgE-epitope mapping of birch pollen profilin. *Structure* 1997;5:33–45.
- 50 Sankian M, et al: Sequence homology: a poor predictive value for profilins cross-reactiv-ity. *Clin Mol Allergy* 2005;3:13.
- 51 Radauer C, et al: Cross-reactive and species-specific immunoglobulin E epitopes of plant profilins: an experimental and structure-based analysis. *Clin Exp Allergy* 2006;36: 920–929.
- 52 Vallverdu A, et al: Characterization of re-combinant *Mercurialis annua* major aller-gen Mer a 1 (profilin). *J Allergy Clin Immu-nol* 1998;101:363–370.
- 53 Lombardero M, et al: Cross-reactivity be-tween olive and other species. Role of Ole e 1-related proteins. *Allergy* 2002;57(suppl 71):29–34.
- 54 Ebner C, et al: Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). *J Allergy Clin Immunol* 1995;95:962–969.
- 55 Hoffmann-Sommergruber K, et al: Molecu-lar characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1. *Clin Exp Allergy* 1999;29:840–847.
- 56 Daschner A, Crespo JF, Pascual CY: Specific IgE to recombinant vegetal panallergen (rBet v 2) and fruit allergy in pollinic patients. *Al-lergy* 1998;53:614–618.
- 57 Van Ree R, et al: Pollen-related allergy to peach and apple: an important role for pro-filin. *J Allergy Clin Immunol* 1995;95:726–734.
- 58 Pastorello EA, et al: Allergenic cross-reactiv-ity among peach, apricot, plum, and cherry in patients with oral allergy syndrome: an in vivo and in vitro study. *J Allergy Clin Immu-nol* 1994;94:699–707.
- 59 Petersen A, et al: Ubiquitous structures re-sponsible for IgE cross-reactivity between tomato fruit and grass pollen allergens. *J Al-lergy Clin Immunol* 1996;98:805–815.
- 60 Leitner A, et al: Allergens in pepper and pa-prika. Immunologic investigation of the cel-ery-birch-mugwort-spice syndrome. *Allergy* 1998;53:36–41.

- 61 Ganglberger E, et al: Hev b 8, the Hevea brasiliensis latex profilin, is a cross-reactive allergen of latex, plant foods and pollen. *Int Arch Allergy Immunol* 2001;125:216–227.
- 62 Diez-Gomez ML, et al: Fruit-pollen-latex cross-reactivity: implication of profilin (Bet v 2). *Allergy* 1999;54:951–961.
- 63 Menz G, et al: Serological and skin-test diagnosis of birch pollen allergy with recombinant Bet v I, the major birch pollen allergen. *Clin Exp Allergy* 1996;26:50–60.
- 64 Heiss S, et al: Component-resolved diagnosis (CRD) of type I allergy with recombinant grass and tree pollen allergens by skin testing. *J Invest Dermatol* 1999;113:830–837.
- 65 Niederberger V, et al: Skin test results but not serology reflect immediate type respiratory sensitivity: a study performed with recombinant allergen molecules. *J Invest Dermatol* 2001;117:848–851.
- 66 Scheurer S, et al: Cross-reactivity within the profilin panallergen family investigated by comparison of recombinant profilins from pear (Pyr c 4), cherry (Pru av 4) and celery (Api g 4) with birch pollen profilin Bet v 2. *J Chromatogr B Biomed Sci Appl* 2001;756:315–325.
- 67 Wensing M, et al: IgE to Bet v 1 and profilin: cross-reactivity patterns and clinical relevance. *J Allergy Clin Immunol* 2002;110:435–442.
- 68 Ebo DG, et al: Sensitization to cross-reactive carbohydrate determinants and the ubiquitous protein profilin: mimickers of allergy. *Clin Exp Allergy* 2004;34:137–144.
- 69 Asero R, Monsalve R, Barber D: Profilin sensitization detected in the office by skin prick test: a study of prevalence and clinical relevance of profilin as a plant food allergen. *Clin Exp Allergy* 2008;38:1033–1037.
- 70 Anliker MD, et al: Allergy caused by ingestion of persimmon (*Diospyros kaki*): detection of specific IgE and cross-reactivity to profilin and carbohydrate determinants. *J Allergy Clin Immunol* 2001;107:718–723.
- 71 Rodriguez-Perez R, et al: Profilin is a relevant melon allergen susceptible to pepsin digestion in patients with oral allergy syndrome. *J Allergy Clin Immunol* 2003;111:634–639.
- 72 Ballmer-Weber BK, et al: Influence of food processing on the allergenicity of celery: DBPCFC with celery spice and cooked celery in patients with celery allergy. *Allergy* 2002;57:228–235.
- 73 Fah J, Wuthrich B, Vieths S: Anaphylactic reaction to lychee fruit: evidence for sensitization to profilin. *Clin Exp Allergy* 1995;25:1018–1023.
- 74 Bolhaar ST, et al: Severe allergy to sharon fruit caused by birch pollen. *Int Arch Allergy Immunol* 2005;136:45–52.
- 75 Asero R, et al: Detection of clinical markers of sensitization to profilin in patients allergic to plant-derived foods. *J Allergy Clin Immunol* 2003;112:427–432.
- 76 Anhoej C, Backer V, Nolte H: Diagnostic evaluation of grass- and birch-allergic patients with oral allergy syndrome. *Allergy* 2001;56:548–552.
- 77 Lidholm J, et al: Component-resolved diagnostics in food allergy. *Curr Opin Allergy Clin Immunol* 2006;6:234–240.
- 78 Ballmer-Weber BK, et al: Component-resolved in vitro diagnosis in carrot allergy: does the use of recombinant carrot allergens improve the reliability of the diagnostic procedure? *Clin Exp Allergy* 2005;35:970–978.
- 79 Mari A: Multiple pollen sensitization: a molecular approach to the diagnosis. *Int Arch Allergy Immunol* 2001;125:57–65.
- 80 Tavares B, et al: Sensitization to profilin in the Central region of Portugal. *Sci Total Environ* 2008;407:273–278.
- 81 Rossi RE, et al: Evaluation of recombinant allergens Bet v 1 and Bet v 2 (profilin) by Pharmacia CAP system in patients with pollen-related allergy to birch and apple. *Allergy* 1996;51:940–945.
- 82 Van Ree R, et al: How far can we simplify in vitro diagnostics for Fagales tree pollen allergy? A study with three whole pollen extracts and purified natural and recombinant allergens. *Clin Exp Allergy* 1999;29:848–855.
- 83 Barderas R, et al: Role of profilin and polcalcin in chenopod pollen allergy. *J Allergy Clin Immunol* 2003;111:1132–1133.
- 84 Barderas R, Villalba M, Rodriguez R: Recombinant expression, purification and cross-reactivity of chenopod profilin: rChe a 2 as a good marker for profilin sensitization. *Biol Chem* 2004;385:731–737.
- 85 van Hage-Hamsten M, Pauli G: Provocation testing with recombinant allergens. *Methods* 2004;32:281–291.
- 86 Belsito DV: The pathophysiology of allergic contact hypersensitivity. *Clin Rev Allergy* 1989;7:347–379.
- 87 Weber RW: Cross-reactivity of pollen allergens: impact on allergen immunotherapy. *Ann Allergy Asthma Immunol* 2007;99:203–211; quiz 212–213, 231.
- 88 Asero R: Effects of birch pollen-specific immunotherapy on apple allergy in birch pollen-hypersensitive patients. *Clin Exp Allergy* 1998;28:1368–1373.
- 89 Kelso JM, et al: Oral allergy syndrome successfully treated with pollen immunotherapy. *Ann Allergy Asthma Immunol* 1995;74:391–396.
- 90 Herrmann D, et al: Effect of hyposensitization for tree pollinosis on associated apple allergy. *J Investig Allergol Clin Immunol* 1995;5:259–267.
- 91 Moller C: Effect of pollen immunotherapy on food hypersensitivity in children with birch pollinosis. *Ann Allergy* 1989;62:343–345.
- 92 Bolhaar ST, et al: Efficacy of birch-pollen immunotherapy on cross-reactive food allergy confirmed by skin tests and double-blind food challenges. *Clin Exp Allergy* 2004;34:761–769.
- 93 Westritschnig K, et al: A hypoallergenic vaccine obtained by tail-to-head restructuring of timothy grass pollen profilin, Phl p 12, for the treatment of cross-sensitization to profilin. *J Immunol* 2007;179:7624–7634.
- 94 Valenta R, et al: cDNA cloning and expression of timothy grass (*Phleum pratense*) pollen profilin in *Escherichia coli*: comparison with birch pollen profilin. *Biochem Biophys Res Commun* 1994;199:106–118.
- 95 Zuidmeer L, et al: The role of profilin and lipid transfer protein in strawberry allergy in the Mediterranean area. *Clin Exp Allergy* 2006;36:666–675.
- 96 Wopfner N, et al: Molecular and immunological characterization of novel weed pollen pan-allergens. *Allergy* 2008;63:872–881.
- 97 Uniprot: Swiss protein knowledgebase. 2009. (<http://www.uniprot.org/>).
- 98 Wopfner N, et al: Molecular and immunological characterization of profilin from mugwort pollen. *Biol Chem* 2002;383:1779–1789.
- 99 Sankian M, et al: Production and characterization of monoclonal antibody against saffron pollen profilin, Cro s 2. *Iran J Immunol* 2008;5:156–162.
- 100 Asturias JA, et al: Cloning and high level expression of *Cynodon dactylon* (Bermuda grass) pollen profilin (Cyn d 12) in *Escherichia coli*: purification and characterization of the allergen. *Clin Exp Allergy* 1997;27:1307–1313.
- 101 Asturias JA, et al: Cloning and expression of the panallergen profilin and the major allergen (Ole e 1) from olive tree pollen. *J Allergy Clin Immunol* 1997;100:365–372.
- 102 Duro G, et al: cDNA cloning, sequence analysis and allergological characterization of Par j 2.0101, a new major allergen of the *Parietaria judaica* pollen. *FEBS Lett* 1996;399:295–298.
- 103 Scheurer S, et al: Cloning of the minor allergen Api g 4 profilin from celery (*Apium graveolens*) and its cross-reactivity with birch pollen profilin Bet v 2. *Clin Exp Allergy* 2000;30:962–971.
- 104 Willeroider M, et al: Cloning and molecular and immunological characterisation of two new food allergens, Cap a 2 and Lyc e 1, profilins from bell pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*). *Int Arch Allergy Immunol* 2003;131:245–255.
- 105 Hirschwehr R, et al: Identification of common allergenic structures in hazel pollen and hazelnuts: a possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J Allergy Clin Immunol* 1992;90:927–936.

- 106 Ma Y, et al: Characterization of recombinant Mal d 4 and its application for component-resolved diagnosis of apple allergy. Clin Exp Allergy 2006;36:1087–1096.
- 107 Scheurer S, et al: Recombinant allergens Pru av 1 and Pru av 4 and a newly identified lipid transfer protein in the in vitro diagnosis of cherry allergy. J Allergy Clin Immunol 2001;107:724–731.
- 108 Rodriguez-Perez R, et al: Peach profilin: cloning, heterologous expression and cross-reactivity with Bet v 2. Allergy 2003;58:635–640.
- 109 Rihs HP, et al: Polymerase chain reaction based cDNA cloning of wheat profilin: a potential plant allergen. Int Arch Allergy Immunol 1994;105:190–194.
- 110 Weichel M, et al: Screening the allergenic repertoires of wheat and maize with sera from double-blind, placebo-controlled food challenge positive patients. Allergy 2006;61:128–135.
- 111 Allergen Nomenclature. International Union of Immunological Societies Allergen Nomenclature Sub-Committee. 2009. (<http://www.allergen.org>).
- 112 Structural Database of Allergenic Proteins. The University of Texas Medical Branch. 2005. (http://fermi.utmb.edu/SDAP/sdap_man.html).
- 113 Kremser M, Lindemayr W: Frequency of the so-called ‘apple allergy’ (‘apple contact urticaria syndrome’) in patients with birch pollinosis. Z Hautkr 1983;58:543–552.
- 114 Pauli G, et al: Celery sensitivity: clinical and immunological correlations with pollen allergy. Clin Allergy 1985;15:273–279.
- 115 Stager J, Wuthrich B, Johansson SG: Spice allergy in celery-sensitive patients. Allergy 1991;46:475–478.
- 116 Jensen-Jarolim E, et al: Characterization of allergens in Apiaceae spices: anise, fennel, coriander and cumin. Clin Exp Allergy 1997;27:1299–1306.
- 117 Perez-Pimiento AJ, et al: Anaphylactic reaction to young garlic. Allergy 1999;54:626–629.
- 118 Wuthrich B, Stager J, Johansson SG: Celery allergy associated with birch and mugwort pollinosis. Allergy 1990;45:566–571.
- 119 Figueroa J, et al: Mustard allergy confirmed by double-blind placebo-controlled food challenges: clinical features and cross-reactivity with mugwort pollen and plant-derived foods. Allergy 2005;60:48–55.
- 120 Pastorello EA, et al: Hypersensitivity to mugwort (*Artemisia vulgaris*) in patients with peach allergy is due to a common lipid transfer protein allergen and is often without clinical expression. J Allergy Clin Immunol 2002;110:310–317.
- 121 Anderson LB, Jr, et al: Melon and banana sensitivity coincident with ragweed pollinosis. J Allergy 1970;45:310–319.
- 122 Enberg RN, et al: Watermelon and ragweed share allergens. J Allergy Clin Immunol 1987;79:867–875.
- 123 Callejo A, et al: A new pollen-fruit cross-reactivity. Allergy 2002;57:1088–1089.
- 124 Enrique E, et al: IgE reactivity to profilin in *Platanus acerifolia* pollen-sensitized subjects with plant-derived food allergy. J Invest Allergol Clin Immunol 2004;14:335–342.
- 125 Enrique E, et al: *Platanus acerifolia* pollinosis and food allergy. Allergy 2002;57:351–356.