Molecular diagnosis of fruit and vegetable allergy

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Purpose of review

The purpose of this paper is to review and discuss studies on molecular diagnosis in fruit and vegetable allergy.

Recent findings

Celeriac, carrot and tomato are the most prevalent allergenic vegetables, whereas fruit allergy is mainly induced by apple, peach and kiwi. Component-resolved molecular diagnosis has been recently applied in two well-defined patient groups with kiwifruit and celeriac allergy, respectively. In kiwifruit allergy Act d 1 and Act d 3 were identified as potential marker allergens for severe symptoms. For celeriac allergy, however, such markers are still missing. In both studies component-resolved molecular diagnosis approach improved in particular sensitivity compared to extract-based diagnostic test assays.

Summary

Food and vegetable allergy can be acquired both via a direct sensitization over the gastrointestinal tract and via a primary sensitization to pollen or latex. The diagnosis of fruit and vegetable allergy in birch pollen-sensitized patients should not be excluded on a negative IgE testing to extracts. Bet v 1-related allergens are often under-represented in extracts. Few recombinant allergens derived from fruits and vegetables are nowadays commercially available and facilitate diagnosis of fruit and vegetable allergies.

Keywords

component-resolved diagnosis, food allergens, fruit allergy, vegetable allergy

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Introduction

Depending on the route of sensitization, allergies to fruits and vegetables are either a result of genuine reactivity to food allergens through the gastrointestinal tract or the result of secondary sensitization to crossreactive food allergens as a consequence of a primary sensitization to homologous pollen or latex-related allergens [1].

Allergens involved in primary food allergy are heat stable and resistant to degradation or proteolytic digestion. As a result of their stability, they are frequently well represented in extracts leading often to high sensitivity of extract-based diagnostic tests in primary food allergy. On the other hand, allergens involved in secondary food allergy are usually labile proteins, which are easily degradable and thus often under-represented in food extracts due to degradation during the extraction procedure [2].

Accurate diagnosis of food allergy depends on the identification of the responsible allergenic molecule(s). Great efforts have been invested into the identification, characterization, and cloning of fruit and vegetable allergens, and this development has resulted in a vastly increased knowledge of allergenic components in a particularly large variety of fruits but also vegetables. Proteins with allergenic activity are restricted to a small number of protein families [3]. Table 1 provides an overview on the most important plant food allergen protein families involved in fruit and vegetable allergy (Table 1).

Allergens involved in primary food allergy usually have the higher potential to induce severe reactions than the more easily degradable allergens of secondary food allergy. This concept often holds true but there are exemptions as for birch pollen-related allergy to foods of the Apiaceae family (celeriac, carrot) or soy, which can induce severe systemic reactions [4,5]. Thus, allergy to a particular food may give rise to differentially severe symptoms depending on which precise allergen component(s) the individual is sensitized to. Molecular diagnosis, that is determination of specific IgE to the different allergens derived from one allergenic food, called component-resolved molecular diagnosis (CRD), will on one hand increase sensitivity in particular in secondary food allergy to fruits or vegetables and on the other hand offer an opportunity to compile a patient tailored risk profile. Most allergens described in CRD studies performed in fruit and vegetable allergic patients have been applied in experimental settings.

Table 1 Selection of the most important plant food allergens protein families in fruits and vegetables ranked according to their frequency

Plant food allergens	Function	Molecular mass (kDa)	Number of allergens identified	Allergens identified in fruits and vegetables
Prolamin superfamily (nsLTP)	Plant defense, transfer of lipids	7–9	26	All type of plant food
Profilin	Actin-binding regulatory protein	14	24	All type of plant food
Bet v 1-related proteins (PR-10 family)	Plant defense, plant steroid carrier	17	17	All type of plant food
Thaumatin-like proteins	Plant defense	20–25	7	Kiwi, citrus and Rosaceae fruits, grape, tomato
Endochitinases	Plant defense, chitin hydrolysis	25–35	6	Banana, avocado, tomato
β-1,3-Glucanases	Plant defense	25–35	5	Banana, avocado, tomato

Entries in the allfam database http://www.meduniwien.ac.at/allergens/allfam/, according to December 2010.

To date few recombinant allergens are commercially available as ImmunoCAPs for in-vitro diagnosis in fruit and vegetable allergy: peach derived rPru p 1 (Bet v 1 homologous protein), Pru p 3 (nonspecific lipid transfer protein, nsLTP), Pru p 4 (profilin), celeriac derived Api g 1 (Bet v 1 homologue) and kiwi derived Act d 8 (Bet v 1 homologue). In addition, the panel of recombinant allergens for chip-based analysis (ISAC) contains the Bet v 1 homologous food allergens Mal d 1 and Dau c 1 from apple and carrot, respectively. In the following review we will focus on newly identified food allergens derived from fruit and vegetables and discuss their clinical significance and their potential for an improvement of molecular diagnosis. The most important allergens discussed in this review are summarized in Table 2.

Molecular diagnosis of plant food allergy: vegetables

Recently a review has been published on 36 studies covering data of more than 250 000 children and adults in respect to plant food allergy. In most studies allergy was not confirmed by challenges but in six studies those revealed a prevalence rate for vegetable allergies of 1.4% [6]. In another investigation sera from a random sample of young adults ($n = 4522$) living in 13 countries and seen within the European Community Respiratory Health Survey were analysed for IgE against 24 foods. The overall prevalence of sensitization to carrot and celeriac was 3.6 and 3.5%, respectively, and that to tomato 3.3% [7]. Celeriac and carrot allergies are – apart from

Table 2 Allergens derived from carrot, celeriac, tomato, kiwi and peach according to IUIS Allergen Nomenclature Sub-Committee

Plant food	Allergens	Biochemical name	Remarks[a]
Carrot	Dau c 1	Pathogenesis-related protein PR 10	Mainly local oral symptoms but potential for systemic reaction
	Dau c 4	Profilin	
Celeriac	Api g 1	Pathogenesis-related protein PR 10	Mainly local oral symptoms but potential for systemic reaction
	Api g 2	Lipid transfer protein	
	Api g 3	Chlorophyll *a-b*-binding protein	
	Api g 4	Profilin	
	Api g 5	FAD-containing oxidase	
Tomato	Lyc e 1	Profilin	
	Lyc e 2	β-Fructofuranosidase	
	Lyc e 3	Lipid transfer protein	
	Lyc e 4	Intracellular pathogenesis-related protein TSI-1	
Kiwifruit	Act d 1	Cysteine protease (actinidin)	Potential marker allergen for primary kiwifruit allergy
	Act d 2	Thaumatin-like protein	
	Act d 3		
	Act d 4	Phytocystatin	
	Act d 5	Kiwellin	
	Act d 6	Pectin methylesterase inhibitor	
	Act d 7	Pectin methylesterase	
	Act d 8	Pathogenesis-related protein PR 10	Mainly mild local reaction of the oral cavity
	Act d 9	Profilin	
	Act d 10	Lipid transfer protein	
	Act d 11	Major latex protein	
Peach	Pru p 1	Pathogenesis-related protein PR 10	Mainly mild local reaction of the oral cavity
	Pru p 2	Thaumatin-like protein	
	Pru p 3	Lipid transfer protein	Increased risk for systemic reactions
	Pru p 4	Profilin	

http://www.allergen.org, according to January 2011.
[a] If not otherwise stated food allergens with potential to induce local reactions are not yet determined.

tomato – among the most frequently observed vegetable allergies. Correspondingly, CRD studies in well-characterized patients with positive double-blind placebo-controlled food challenges (DBPCFCs) have been performed to date only in celeriac and carrot allergic patients. For other vegetable allergies, no relevant studies have been published in terms of molecular diagnosis during the last 2 years. Single allergens have been identified such as Pha v 3 (nsLTP) derived from green bean [8•], or sensitization to other allergens such as to Lac s 1 (nsLTP) derived from lettuce has been confirmed in lettuce-induced anaphylaxis [9].

Celeriac allergy

Celeriac (*Apium graveolens*), which belongs to the Apiaceae family, is a frequent cause of pollen-related food allergy, particularly in European countries. Celeriac allergy is highly associated with birch pollen and mugwort pollen sensitization referred to as birch–mugwort–celery syndrome. To date, no single case of a primary celeriac allergy has been published. Celeriac-induced symptoms range from mild contact urticaria of the oral mucosa (also called oral allergy syndrome) up to life-threatening anaphylactic reactions [4]. To date, three celeriac allergens have been identified in celeriac tuber: the Bet v 1 homologous protein Api g 1 (16 kDa, major allergen), the profilin Api g 4 (14 kDa, minor allergen) and the glycoprotein Api g 5. Two different isoforms of Api g 1 have been detected to date [10]. Api g 5 has been described as a mixture of two polypeptides with molecular weights of 53 and 57 kDa. The two polypeptides belong to the family of flavoproteins. According to mass spectrometry analysis the protein core carries at least three N-glycans of the MMXF and MUXF type at different amino acid positions. Removal of the carbohydrate structures results in a complete loss of the IgE-binding capacity of the glycoprotein, indicating that the IgE binding of Api g 5 is attributable to the carbohydrate moiety [11]. In a previous study [4] in celeriac allergy we have shown a diagnostic sensitivity of the celery extract CAP of 73 % indicating a need for further improvement of the diagnostic reagents. Therefore, we have recently performed a CRD study [12••] in patients with celeriac allergy, confirmed by positive food challenges, using ImmunoCAP-coupled recombinant rApi g 1 and rApi g 4 as well as natural nApi g 5 as components. In this study, the sensitivity of celeriac extract ImmunoCAP was 67%; however, 88% showed specific IgE to at least one component. Thus, CRD approach increased diagnostic sensitivity by more than 20%. Seventy-five percent were sensitized to rApi g 1, 42% to rApi g 4 and 42% to nApi g 5. Sensitization to nApi g 5 correlated with the sensitization to bromelain-derived carbohydrate epitope MUXF3. No marker allergen for prediction of severe reactions could be identified in this study. Particular severe reactions to celeriac usually occur in mugwort-sensitized patients (unpublished data). The crossreactive allergens, however, have not been identified to date. CRD approach did not facilitate discrimination between celeriac-sensitized but tolerant patients and celeriac allergic patients. Using overlapping peptides in a peptide-microarray sera of celeriac allergic patients showed in a recently published study [13] binding to three distinct regions of Api g 1.0101. These regions bound IgE of celeriac allergic patients at a higher rate compared with healthy controls. Unfortunately, the control group in this study consisted of subjects without any sensitization either to celeriac, or birch or mugwort pollen. Thus this test has not yet proven to discriminate between sensitized, but tolerant patients and celeriac allergic patients. In celery stalk recently an nsLTP has been identified as Api g 2 that has been recognized by IgE of Italian LTP-sensitized patients [14•]. The clinical significance of this allergen for celeriac tuber allergic patients has to be confirmed in the future.

Carrot allergy

Similarly to celeriac, carrot allergy has mainly been observed in relation to a concomitant pollen allergy, in particular to birch and mugwort pollen. Up to 50% of patients develop systemic reactions. We have demonstrated in the past that 98% of Central European carrot allergic patients were sensitized to the Bet v 1 homologue in carrots, Dau c 1.0104, 38% of the patients recognized the carrot profilin, Dau c 4 [15]. In that study, a new isoform of Dau c 1, Dau c 1.0201, was recognized by 65% of the carrot allergic patients. Dau c 1.0201 showed only 50% amino acid sequence identity with the known isoform of Dau c 1 (Dau c 1.0104) and limited crossreactivity with Dau c 1.0104 and Bet v 1 indicating that both isoforms should be included for molecular diagnosis of carrot allergy. In a recent study [16•] post-translational silencing to reduce the content of Dau c 1.01 and Dau c 1.02 in transgenic carrots has been applied. This approach led only to a partial reduction of allergenicity pointing towards the fact that additional carrot allergens might be important for the allergenicity of carrots. As additional carrot allergens, isoflavone reductase has been identified as a Bet v 6 homologous protein in carrots [17] and cyclophilin, a birch pollen independent allergen, has been recognized by IgE of Japanese carrot allergic patients [18]. The diagnostic relevance of the carrot isoflavone reductase and the cyclophilin has not been investigated to date in a CRD study.

Tomato allergy

Tomatoes (Solanaceae family) are increasingly consumed worldwide. In a German study [19], 9% among mainly birch pollen allergic patients reported tomato-induced allergic symptoms. As a result of the potential of tomatoes to induce unspecific histamine liberation the allergenic properties of that vegetable have been mainly neglected in the past. To date many different tomato allergens have

been identified but not yet applied in a CRD approach. According to the IUIS Allergen Nomenclature Sub-Committee (www.allergen.org) four tomato allergens have been accepted to date: Lyc e 1 (profilin, 14 kDa), Lyc e 2 (β-fructofuranosidase, 50 kDa), Lyc e 3 (nsLTP, 6 kDa) and Lyc e 4 (intracellular pathogenesis-related protein TSI-1 of the Bet v 1 family). Thus, Lyc e 4 and Lyc e 1 are potentially crossreacting allergens between tomato and birch pollen. In a Spanish investigation only 16% of tomato-sensitized patients reported symptoms upon tomato ingestion and the major part of those patients were sensitized either to Artemisia or Platanus pollen. These findings point towards a high rate of clinically silent sensitization to tomato in pollen allergic patients and an IgE response towards clinically not relevant crossreactive allergens [20]. In other studies cosensitization (or crossreaction) between tomato and grass pollen has been observed. Recently two tomato allergens, that is Lyc e 1 and Lyc e 3, have been silenced by RNA interference technology leading to a strong reduction of the allergenic potential of transgenic tomato fruits and confirming thus the clinical relevance of these two tomato allergens [21•].

In an IgE immunoblotting study [22•], 45% of tomato allergic patients reacted to a 9-kDa protein identified as LTP, 50% to bands at 24 and 26 kDa, recognized as β-fructofuranosidase precursor and osmotin-like protein (thaumatin-like protein), 32.5% to a 30-kDa protein (endochitinase precursor) and 60% to a 35-kDa protein (pectinesterase 1 precursor), whereas the 14-kDa (65%) protein could not be identified but was suspected to be profilin. Fifteen percent of patients recognized only the LTP and experienced more severe symptoms to tomato (unfortunately not proven by challenges).

Tomato seeds are rich in proteins and constitute 70% storage protein such as globulins. Recently, a legumin and a vicilin were identified as putative tomato seed allergens [23•]. Some of the tomato allergens such as chitinase and glucanase have been described in relation to latex sensitization. In summary, tomato is a complex allergenic vegetable and the reliability of the single allergens for molecular diagnosis has to be confirmed in future studies.

Molecular diagnosis of plant food allergy: fruits

According to the recent meta-analysis by Zuidmeer *et al.* [6] the prevalence of fruit allergy as confirmed by food challenges ranges from 0.1 to 4.3%. Perception of fruit allergy in adults was reported to vary from 0.4 to 3.5%, whereas in children numbers as high as 11.5% (Norwegian study) were presented. The most important perceived elicitors of food allergy were apple, orange and lemon. According to the European Community Respiratory Health Survey sensitization to fruits was dominated by peach (5.4%), apple (4.2%) and kiwifruit (3.6%) followed by banana (2.5%) and melon (1.6%) [7]. Apple and peach have been regarded as the most important fruit allergies and data on state-of-the-art diagnosis including food challenges together with component-resolved diagnosis are available [24,25]. In contrast for kiwifruit allergy only recently efforts were undertaken to complete allergen panel from kiwifruits and assess the clinical relevance of IgE recognition patterns [26,27••,28•].

Kiwifruit allergy

Nowadays, allergy to kiwifruit is regarded as one of the most important fruit allergies and represents the prototype of novel food-induced allergies. Although known as an exotic fruit in the 1980s [29], it is now among the top 10 food allergy sources according to recent studies from Finland, Sweden and France [30–32]. Kiwifruit allergy can either be due to monosensitization or is associated with birch and grass pollen allergy and latex allergy [26,33,34]. Symptoms range from mild local reactions up to severe systemic symptoms.

Up to now, 11 allergens have been identified from green kiwifruit (*Actinidia deliciosa*) according to the official IUIS Allergen Nomenclature database (www.allergen.org). The papain-like cysteine protease, actinidin, Act d 1 (30 kDa), represents a major allergen and is also abundantly expressed (up to 50% of soluble protein fraction) in ripe fruits [26,35,36]. This highly active protease may also account for the rapid degradation of other proteins including allergens, which in turn affects the varying quality of test solutions for Skin Prick Tests (SPTs). Another important kiwifruit allergen is a member of the thaumatin-like protein family and is designated Act d 2 (24 kDa) [37]. Act d 3 is a 40-kDa glycoprotein with so far unknown function with a relevant sensitizing potential in kiwifruit allergic patients [26]. Kiwifruit phytocystatin (11 kDa), a cysteine protease inhibitor, was designated Act d 4 [37]. Kiwellin, Act d 5 (26 kDa), is a cell wall protein involved in the fruit ripening process [38]. Recently, Tuppo *et al.* [39] provided data on the interaction of Act d 1 and Act d 5 resulting in two domains from kiwellin, KiTH (20 kDa) the C-terminal region and kissper, a peptide of 39 amino acid residues, with pore-forming and ion-channelling activities. Both peptides displayed IgE-binding activities. The pollen-related allergens, Act d 8 (17 kDa), the Bet v 1 homologue and kiwifruit profilin, Act d 9 (14 kDa), were identified from kiwifruits representing the sensitizing agents in pollen allergic patients [27••,40]. In addition, Act d 6, pectin methylesterase inhibitor (18 kDa) and Act d 7, pectin methylesterase (50 kDa), the nonspecific lipid transfer protein (10 kDa), Act d 10, and the major latex protein (17 kDa), Act d 11,

were identified as kiwifruit allergens (www.allergen.org). Recently, we have performed a CRD study [27••] in 30 patients with kiwifruit allergy, confirmed by positive food challenges. For in-vitro diagnosis purified natural (n) Act d 1, nAct d 2, nAct d 3, nAct d 4, nAct d 5 and recombinant rAct d 8 and rAct d 9 were coupled to ImmunoCAP and compared to conventional extract-based ImmunoCAP. The diagnostic sensitivity of the extract ImmunoCAP was 17% compared with 77% when taking all the individual allergen tests together.

Results obtained with Act d 1, Act d 2, Act d 4 and Act d 5 resulted at 40% sensitivity and 90% specificity. Sensitization to Act d 1 was significantly related to kiwifruit monosensitization, whereas sensitization to the pollen-related allergens Act d 8 and Act d 9 was specific for pollen-kiwifruit allergic patients. In another study performed by Palacin *et al.* [26], purified nAct d 1, nAct d 2 and nAct d 3 were used in IgE ELISAs and SPTs investigating more than 90 sera from Spanish kiwifruit allergic patients. More than 60% of the sera tested displayed specific IgE to all of these allergens and 50% of the patients had positive skin reactivity to these allergens. Moreover, sensitization to Act d 1 and Act d 3 was significantly linked with anaphylactic reactions in the patients. In another study by Bublin *et al.* [28•] purified nAct d 1, nAct d 2, nAct d 4, nAct d 5, nAct d 6, nAct d 7 and Act d 11 and rAct d 8 and Act d 9 and nsLTP from peach were coupled onto the microarray test system screening 237 sera from kiwifruit allergic patients. The panel of kiwifruit allergens had a diagnostic sensitivity of 66% and specificity of 56%. As in the previous study Act d 1 was identified as a genuine marker for kiwifruit monosensitization. In contrast, none of the sera tested had specific IgE directed against Act d 6. Sensitization to Act d 2, Act d 8 and Act d 11 increased the specificity of the test, whereas Act d 7 and Act d 9 reactivity decreased specificity.

In addition, sensitization to Hev b 11, the latex chitinase, was predominantly found in the kiwifruit–latex allergic patients, thus representing a marker for the latex–kiwifruit syndrome. Nevertheless, in all these recent studies sera were identified that did not display IgE directed to any of the allergens tested so far and it may well be that the panel of kiwifruit allergens awaits completion.

In 1999 another kiwi species, *Actinidia chinensis* var. Hort16A, also known as gold kiwifruit became available in Europe. Shortly after introduction into the market it was evident that in general patients allergic to green kiwifruit reacted to consumption of gold kiwifruit with less severe symptoms which is due to a 50 times reduced Act d 1 content in gold kiwifruit [41]. In a recent study Le *et al.* [42] investigated *in vivo* in Dutch and Swiss kiwifruit allergic patients six different kiwifruit cultivars already available or soon to be marketed in Europe. Patients were tested by Prick to Prick Test (PPT) and open food challenges. In addition to the gold kiwifruit var. Hort16A another low-allergenic variety, Summer 3373, was identified and both cultivars may offer a low-allergenic alternative for the kiwifruit allergic patient.

Peach allergy

Allergy to peach (*Prunus persica*) is the most prevalent plant food allergy in the Mediterranean area [43,44]. Among those patients 80% are sensitized to the nonspecific lipid transfer protein (nsLTP, Pru p 3), which is mainly found in the peel of the fruit [45]. In addition, the pollen-related allergens, Pru p 1 (Bet v 1 homologue) and Pru p 4 (profilin), were identified [46,47]. Recently, thaumatin-like proteins from peach were identified as allergens in Spanish allergic patients [48•]. Thaumatin-like proteins are of 20–25 kDa molecular mass with a rigid three-dimensional structure due to eight disulphide bridges which function as pathogenesis-related proteins [3]. Allergenic thaumatin-like proteins were first described from cherry and bell-pepper [49,50], and because they have been found in a range of fruits and vegetables, spices and pollen were ranked as panallergens. However, despite their rigid three-dimensional structure which makes them resistant to proteolytic digestive processes, their allergenic relevance has not been identified so far and sensitization prevalence was in general below 50%. In the present study by Palacin *et al.* [48•], three isoforms from the thaumatin-like protein family were identified from peach, designated Pru p 2.0101, Pru p 2.0201, and Pru p 2.0301. The isoforms were purified and extensively characterized regarding their biochemical and allergenic properties. In IgE ELISA 77% of the sera from peach allergic patients displayed Pru p 2-specific IgE. In addition, 80% of the patients reacted with these allergens in the basophil activation test and SPT. Therefore, Pru p 2 can be regarded as a major peach allergen at least for the Mediterranean area. However, whether this allergen is also a marker for severe symptoms and its crossreactivity remains to be elucidated.

Latex–food syndrome and sensitization to hevein-like domain

In 30–70% of latex allergic patients, plant food allergies such as banana, kiwifruit, chestnut and avocado are described, termed as the latex–fruit syndrome. This crossreactivity has been attributed to the major latex allergen, hevein and the hevein-like domains (HLDs) from class I chitinases in fruits. In a recent in-vitro study by Radauer *et al.* [51] the role of hevein and HLDs sensitization was investigated in a selected latex allergic patient group and in a retrospective study comprising 16 408 participants with any IgE-mediated allergy symptom. In accordance with previous reports hevein was

identified as the sensitizing agent for patients with hevein and HLDs sensitization. However, no correlation was found between sensitization to hevein and HLDs and latex-associated plant food allergy (Table 2).

Conclusion

Recent progress has been made in applying CRD approaches in well defined patients' collectives verified by DBPCFCs. So far, this has been performed with kiwifruits and celeriac representing important plant food allergen sources. In the case of kiwifruit allergy clear evidence for marker allergens regarding monosensitization (Act d 1) versus cross-sensitization (Act d 8, Act d 9) and for severe symptoms (Act d 1, Act d 3) was obvious. Celeriac markers for mugwort–celeriac allergy are still missing. These two pilot studies are good examples to show how CRD can be used to improve sensitivity and specificity of diagnostic test assays. Nevertheless, this approach needs to be performed individually for the most important plant food allergen sources to assess the potential range of cross-sensitization linked with clinical significance and to identify the respective marker allergens. This in turn will have a clear impact on the management of the plant food allergic patients, regarding patient-tailored diagnosis and detailed dietary recommendations and reduced risk of unwanted exposure to allergenic sources.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 271–272).

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