

ORIGINAL ARTICLE

EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Component-resolved *in vitro* diagnosis of carrot allergy in three different regions of Europe

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To cite this article: Ballmer-Weber BK, Skamstrup Hansen K, Sastre J, Andersson K, Bätcher I, Östling J, Dahl L, Hanschmann K-M, Holzhauser T, Poulsen LK, Lidholm J, Vieths S. Component-resolved *in vitro* diagnosis of carrot allergy in three different regions of Europe. *Allergy* 2012; DOI:10.1111/j.1398-9995.2012.02827.x.

Keywords

birch pollen; carrot; component resolved diagnosis; DBPCFC; food allergy; recombinant allergens.

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Accepted for publication 13 March 2012

DOI:10.1111/j.1398-9995.2012.02827.x

Edited by: Antonella Muraro

Abstract

Background: Carrot is a frequent cause of food allergy in Europe. The objective of this study was to evaluate a panel of carrot allergens for diagnosis of carrot allergy in Spain, Switzerland and Denmark.

Methods: Forty-nine carrot allergic patients, 71 pollen allergic but carrot-tolerant patients and 63 nonatopic controls were included. Serum IgE to carrot extract, recombinant carrot allergens (rDau c 1.0104; rDau c 1.0201; rDau c 4; the isoflavone reductase-like proteins rDau c IFR 1, rDau c IFR 2; the carrot cyclophilin rDau c Cyc) were analyzed by ImmunoCAP.

Results: The sensitivity of the carrot extract-based test was 82%. Use of the recombinant allergens increased the sensitivity to 90%. The Dau c 1 isoforms were major allergens for Swiss and Danish carrot allergic patients, the profilin rDau c 4 for the Spanish patients. The rDau c IFR 1 and rDau c IFR 2 were recognized by 6% and 20% of the carrot allergics, but did not contribute to a further increase of sensitivity. Among pollen allergic controls, 34% had IgE to carrot extract, 18% to each of rDau c 1.0104, rDau c 1.0201 and rDau c 4, 8% to rDau c IFR 1 and 7% to rDau c IFR 2. Sensitization to rDau c Cyc occurred in one carrot allergic patient and one nonatopic control.

Conclusion: Component-resolved *in vitro* analyses revealed a significant difference in IgE sensitization pattern between geographical regions and in the prevalence of sensitization to carrot components between carrot allergic and carrot-tolerant but pollen sensitized patients.

Vegetables belonging to the *Apiaceae* family, such as carrots and celeriac, are among the most important elicitors of food allergy (1–7).

Carrot allergy has been observed mainly in relation with a concomitant birch or mugwort pollen allergy (8,9). In our

previous study performed in Central European carrot allergics 98% were sensitized to the Bet v 1 homologue in carrots, Dau c 1.0104. A second isoform of Dau c 1, Dau c 1.0201, was recognized by IgE of 65% of the study population (5). Recently, post-translational silencing has been used to reduce the content of Dau c 1.0104 and Dau c 1.0201 in transgenic carrots, leading to a partial reduction of allergenicity, indicating that additional carrot allergens may be important for the overall allergenic potency of this vegetable (10). In addition to the Bet v 1 isoforms, 38% of the patients in our former study recognized the carrot profilin Dau c 4, and 20% cross-reacting carbohydrate epitopes, CCD (5). An isoflavone reductase-like protein has been identified as a relevant birch

Abbreviations

CCD cross-reactive carbohydrate determinants; CH Switzerland; Cyc cyclophilin; DBPCFC double-blind, placebo-controlled food challenge; DK Denmark; ES Spain; IFR Isoflavone reductase-like protein; LOS local oral symptoms; PR pathogenesis related proteins; r recombinant; SE sensitivity; SP specificity; SPT skin prick test.

pollen allergen, designated Bet v 6, cross-reacting with a related protein from carrot, but also from pear (Pyr c 5), apple, peach, lychee fruit, persimmon, zucchini, orange, sharon fruit and Japanese cedar pollen (11–14). In addition, carrot allergic patients in Japan displayed IgE antibody binding to carrot cyclophilin (15). Cyclophilins have been reported as allergens in birch, plane and grass pollen, in plant derived food, in fungi and as human autoantigen (16–23). However, its clinical relevance as food allergen and cross-reactivity with pollen homologues has not been extensively investigated.

Our previous studies indicated that diagnostic procedures in pollen-related food allergy are strongly affected by low sensitivity and specificity of current *in vivo* and *in vitro* methods to detect sensitization to extracts (3,4,24,25). The use of recombinant food allergens, alone or as a complement to natural food extracts may improve the diagnosis of food allergy (26,27).

In the present study, we have evaluated the use of a panel of recombinant carrot allergens for component-resolved *in vitro* diagnosis comprising two distantly related isoforms of the major allergen in carrot Dau c 1, rDau c 1.0104 and rDau c 1.0201, carrot profilin (rDau c 4), two isoforms of carrot Isoflavonoreductase-like proteins (rDau c IFR 1 and rDau c IFR 2) and carrot cyclophilin (rDau c Cyc). To assess geographical differences in sensitization pattern to the different carrot allergens study subjects were recruited from three different climatic regions represented by Spain, Switzerland and Denmark.

Methods

Study subjects

Three allergy centres from Switzerland (Zürich), Denmark (Copenhagen) and Spain (Madrid) participated in this multicenter study. The study was approved by the local ethic committees and all patients gave written informed consent before entering the study.

In total, 183 individuals were included in the study: 49 carrot allergic patients (group 1), 71 patients with allergy to either birch or olive pollen but tolerant to carrot (group 2) and 63 nonatopic controls (group 3). Primary inclusion criterion for group 1 was a positive case history of an allergic reaction to carrot. Definitive inclusion into the study required a positive double-blind, placebo-controlled food challenge (DBPCFC) with carrots or a convincing history of a previous severe allergic reaction to carrot.

The 71 pollen allergic controls had a history of pollinosis either to birch or olive, a positive SPT to birch (Switzerland and Denmark) or olive pollen (Spain), as described elsewhere (28) and a negative open food challenge with raw carrots.

The nonatopic controls had no history of any allergic symptoms, negative SPT results to a panel of inhalation and plant food allergens and a negative open food challenge with raw carrots.

Pregnancy, significant concurrent disease or medication with glucocorticosteroids, H₁-receptor antagonists, angiotensin-converting-enzyme inhibitor or betablocking agents were exclusion criteria.

Food challenges

All food challenges took place out of the pollen season and the blinded challenges were carried out as described (4).

Recombinant allergens

The cloning and characterization of carrot allergens Dau c 1.0104, Dau c 1.0201 and Dau c 4 have been described elsewhere (5). For cloning of cDNAs encoding carrot isoflavone reductase like protein (IFR) and cyclophilin, total RNA was prepared from carrots using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and first strand cDNA was synthesized using the First Strand synthesis kit (Amersham, Freiburg, Germany). Full-length IFR and cyclophilin cDNAs were obtained by PCR amplification using a universal oligo-dT anchored 3' primer and the specific primers 5'-GC GTACTTATAATATCGTGTC-3' (IFR) and 5'-ATACATC TGTGTCTACAGTGT-3' (cyclophilin). The design of the two specific primers was based on sequences obtained after a complex set of 3'-RACE and 5'-RACE experiments including information on cDNAs of known IFR like/cyclophilin allergens and other homologous proteins available in the GenBank database. Following cloning and sequencing of the amplification products, two isoforms of IFR were identified, designated IFR 1 and IFR 2, which differed at four amino acid positions. For recombinant protein production, all six carrot allergen cDNAs were subcloned in a pET vector system, expressed in *E. coli* BL21 as hexahistidine-tagged proteins and purified by immobilized metal ion affinity and size exclusion chromatography. GenBank accession numbers of IFR1, IFR2 and carrot cyclophilin are JN591769, JN591770 and JN591768, respectively. For IFR2 the official name Dau c 5 has been suggested to the IUIS Allergen Nomenclature Subcommittee.

Specific IgE measurements

For all patients, serum IgE antibodies specific for rBet v 1, rBet v 2, rBet v 6, MUXF3 (CCD) and natural extracts of carrot, birch pollen, olive pollen and mugwort pollen were measured using regular ImmunoCAP tests (Phadia, Uppsala, Sweden). The IgE to recombinant carrot allergens was analyzed using experimental ImmunoCAP tests carrying rDau c 1.0104, rDau c 1.0201, rDau c 4, rDau c IFR 1, rDau c IFR 2 and rDau c Cyc, prepared as described (29). The IgE values ≥ 0.35 kU_A/l were regarded as positive. All IgE measurements were carried out on an ImmunoCAP 1000 instrument and 8% were performed in duplicate. Average and median CV for replicates were 8.2% and 5.3% respectively.

Statistics

The statistical analysis was performed with SAS[®]/STAT software, version 9.2, SAS System for Windows (Cary, NC, USA). Differences in specific IgE results between DBPCFC-positive, atopic controls and negative controls were evaluated by Fisher's exact test. The sensitivity (SE)

Table 1 clinical history and symptoms under DBPCFC to carrots

		Symptoms to carrots										
		History					DBPCFC					
	<i>n</i>	LOS	Skin	RSP	GI	RC	LOS	Skin	RSP	GI	CV	RC
DK	20	20	1	7	0	2	20	3	1	0	1	3
CH	20	20	3	6	2	1	20	2	2	2	0	2
E	9	9	3	1	4	0	6*	0	0	3	0	0

LOS, local oral symptoms; RSP, respiratory symptoms; GI, gastrointestinal symptoms; RC, rhinitis or conjunctivitis; CV, cardiovascular symptoms; DBPCFC, double-blind, placebo-controlled food challenge.

*DBPCFC denied by three patients from Spain due to systemic reaction.

and the specificity (SP) of selected IgE assays were determined, defining SE as the fraction of carrot allergic patients with a positive test and SP as the fraction of pollen allergic controls with a negative test. Prevalence of clinical symptoms in relation to extract sensitization was evaluated with Wilcoxon rank-sum test.

Results

Patients and controls

Age (median; range) and gender (female/male) were distributed as follows: carrot allergic patients 32 (21–63) years, 35/14 (f/m), atopic controls 32 (18–57), 30/41 and nonatopic controls 30 (16–61) years, 39/24.

Clinical history and food challenges

Table 1 shows the clinical reactivity evaluated by history. All patients reported itching of the oral mucosa (local oral symptoms, LOS) on carrot ingestion, accompanied in seven patients by skin reactions, respiratory symptoms in 14, gastrointestinal symptoms in six and conjunctivitis or rhinoconjunctivitis in two. Thus, about 50% of the carrot allergics (24/49) reported symptoms more severe than LOS to carrots. The DBPCFC was done to confirm food allergy and the ingestion of the allergenic meals was stopped on a

first objective symptom or if patient experienced prolonged and repeated LOS. Symptoms to carrot outside the experimental setting of DBPCFC were predominantly more severe than under challenge. Three Spanish patients declined challenge due to previous systemic reactions on ingestion of carrots.

All patients reported LOS under challenge. Additional symptoms were observed or reported in 15 cases, including itching of the skin ($n = 2$), rhinitis ($n = 2$), conjunctivitis ($n = 4$); flush ($n = 1$), urticaria ($n = 1$), angioedema ($n = 2$), tightness of the throat reflecting the beginning of laryngeal oedema ($n = 2$), hoarseness ($n = 1$), nausea ($n = 3$), dysphagia ($n = 2$) and vertigo ($n = 1$).

Carrot allergic patients were allergic to an average of nine other foods (range 0–18) and pollen allergic controls without carrot allergy to an average of two different foods (range 0–15). Carrot allergy was associated by a celeriac allergy in 55%, 22% and 20% of CH, DK and ES patients respectively.

Sensitization to carrot extract and recombinant allergens

Table 2 summarizes the results of specific IgE testing for the carrot allergic patients and pollen allergic controls. The quantitative response of the carrot allergic patients to carrot extract and the individual recombinant allergens is presented in Fig. 1. Eighty-two percent of all carrot allergic patients

Table 2 Frequency of detectable IgE to carrot allergens (≥ 0.35 kU_A/l)

Allergen/extract	Carrot allergic patients (%)				Pollen allergic controls (%)			
	All <i>n</i> = 49	DK <i>n</i> = 20	CH <i>n</i> = 20	E <i>n</i> = 9	All <i>n</i> = 71	DK <i>n</i> = 30	CH <i>n</i> = 21	E <i>n</i> = 20
Carrot extract (f31)	82	65	90	100	34	30	38	35
rDau c 1.0104	71	65	90	44	18	30	19	0
rDau c 1.0201	76	70	95	44	18	27	24	0
rDau c 4	37	15	45	67	18	10	33	15
rDau c IFR 1	6	5	5	11	8	3	0	25
rDau c IFR 2	22	20	20	33	8	3	10	15
rDau c Cyc	2	5	0	0	0	0	0	0
At least one carrot component	90	80	100	89	44	43	48	40
CCD (MUXF3)	8	0	15	11	11	7	10	20

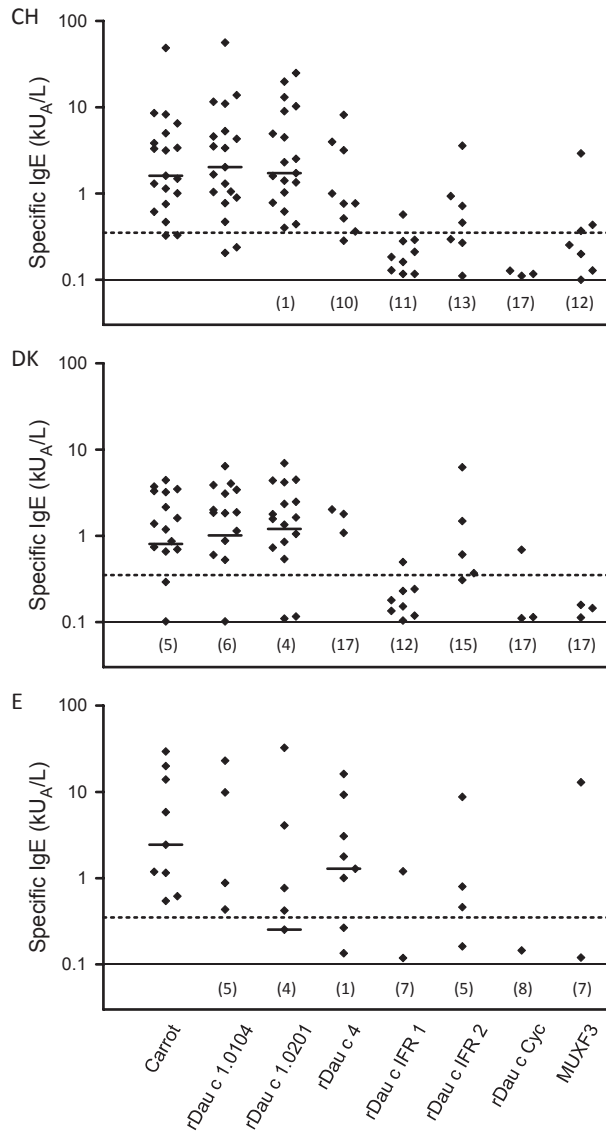


Figure 1 Carrot allergic patients' quantitative responses to the individual carrot components for each country. Dotted and solid lines indicate the 0.35 and 0.1 kU_A/l levels respectively. Horizontal bars indicate median levels of IgE. The number of patients with IgE ≤ 0.1 kU_A/l is indicated in brackets.

tested positive to carrot extract. The carrot allergic patients from all three countries displayed higher IgE concentration to carrot than the pollen allergic controls and the lowest levels were present in the nonatopic controls (Fig. 2). These differences were statistically highly significant ($P < 0.001$). The geometric mean concentration of carrot extract specific IgE was lower in Danish than in Swiss and Spanish patients ($P < 0.05$). The PR-10 allergens rDau c 1.0104 and rDau c 1.0201 were major allergens for the Swiss and Danish patients. This was most prominent in the Swiss subgroup, in which 90% and 95% of carrot allergic patients were sensi-

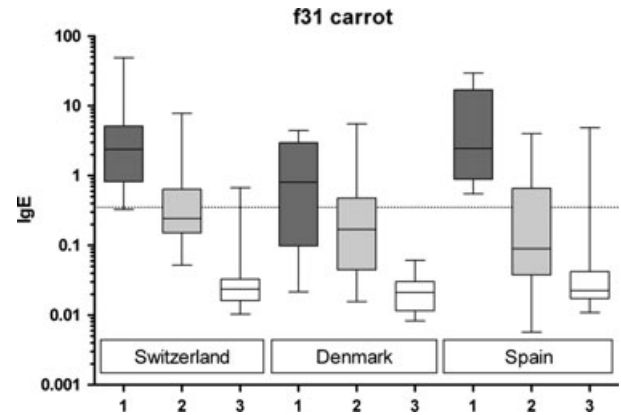


Figure 2 Box-and-whisker-plot of measured IgE concentrations in different study groups (1: carrot allergics, 2: atopic controls, 3: non-atopic controls) and countries (dotted horizontal line indicates the 0.35 kU_A/l cut-off level).

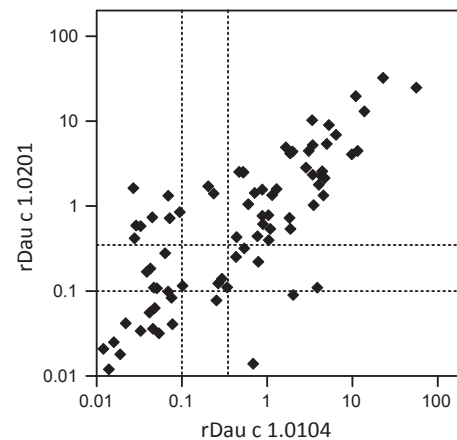


Figure 3 Comparison of IgE antibody binding to recombinant Dau c 1 isoforms rDau c 1.0104 and Dau c 1.0201 among all study subjects ($n = 183$). Dotted lines indicate the 0.35 and 0.1 kU_A/l levels.

tized to those two allergens respectively. Among all carrot allergic patients, rDau c 1.0104 was recognized slightly less frequently than rDau c 1.0201 (71% vs 76%). These allergens share no more than 60% amino acid sequence identity and display significantly divergent IgE antibody binding (Fig. 3). The use of both isoforms of Dau c 1 increased the sensitivity of IgE detection from 90% (Dau c 1.0104 only) to 100% (rDau c 1.0104 and rDau c 1.0201) in the Swiss carrot allergic patients, and from 71% to 82% in the whole carrot allergic population.

Spanish carrot allergic patients were sensitized to rDau c 4 in 67% as compared to only 30% of patients from Denmark and Switzerland ($P > 0.05$). With rDau c 4 combined with Dau c 1 isoforms a diagnostic sensitivity of 90% was obtained, superior to that of extract-based testing (Fig. 4).

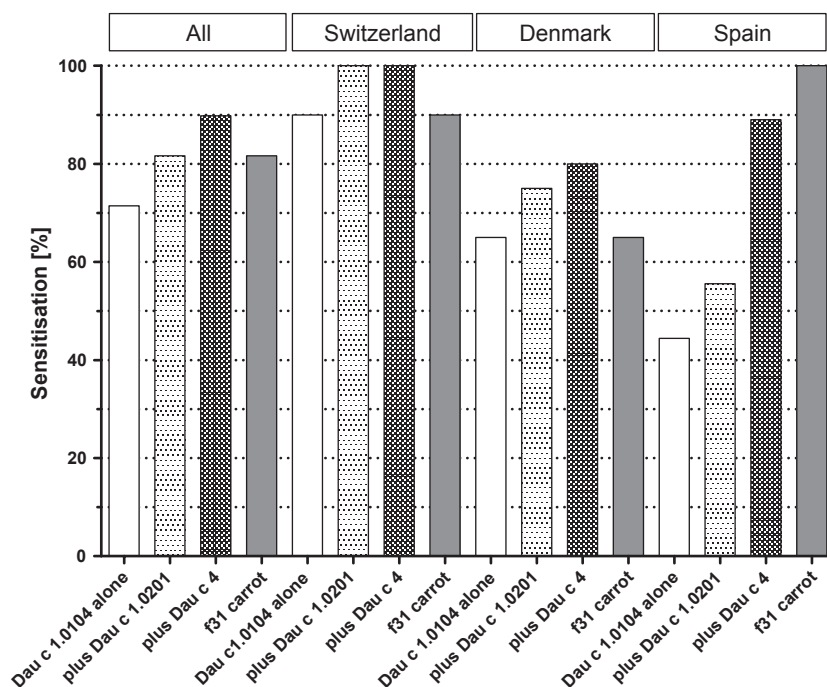


Figure 4 Diagnostic sensitivity in the carrot allergic study group ('All', $n = 49$) and for each country separately ($n = 20$, $n = 20$ and $n = 9$ respectively). Bars indicate percent of patients sensitized to

rDau c 1.0104 alone, rDau c 1.0104 and/or Dau c 1.0201 (plus Dau c 1.0201), at least one of Dau c 1.0104 or Dau c 1.0201 (plus Dau c 4), and carrot extract (f31 carrot).

The isoflavone reductase like proteins rDau c IFR 1 and rDau c IFR 2 recognized by IgE of 6% and 22%, respectively, of all carrot allergic patients, did not contribute to the diagnostic sensitivity. However, rDau c IFR 2 met the requirements for official denomination as allergen and a suggestion for the name Dau c 5 was submitted to the IUIS Allergen Nomenclature Subcommittee. Only one of the carrot allergic subjects displayed IgE binding (0.69 kU_A/l) to carrot cyclophilin.

Among the pollen allergic controls without carrot allergy, 34% were sensitized to the carrot extract resulting in a clinical specificity of 66%. The PR-10 allergens rDau c 1.0104 and rDau c 1.0201 were only recognized by the Swiss and Danish controls, but not by olive pollen allergic patients from Spain. The clinical specificity of IgE determination to both isoforms in the combined Danish and Swiss pollen allergic populations was 75%. The overall frequency of sensitization to carrot profilin Dau c 4 among the pollen allergic controls was 18%. Sensitization to Dau c 4 was particularly common among the Swiss controls (33%). There was a highly significant difference in frequency of sensitization to carrot extract, rDau c 1.0201 and rDau c 1.0104 between carrot allergic patients and pollen allergic controls ($P < 0.0001$) and a moderate difference for rDau c 4 and rDau c IFR 2 ($P < 0.05$).

In the nonatopic control group, only low levels of detectable IgE to the individual carrot allergens were found in a few subjects. Of all 66 subjects sensitized to carrot extract, six (9%) tested negative to all recombinant carrot compo-

nents, possibly due to either CCD-reactive IgE ($n = 2$) or due to so far unknown carrot allergens ($n = 4$). Conversely, among 78 cases where IgE to at least one recombinant carrot allergen was detected, 19 (24%) showed a negative test result to carrot extract.

Pollen sensitization

Table 3 summarizes the IgE results for both carrot allergic patients and pollen allergic controls. The frequency of sensitization to birch pollen was 100% and 90% for the Swiss and Danish carrot allergic patients, respectively, and 67% among the Spanish patients. All but one of the birch pollen sensitized patients from Switzerland and Denmark were IgE positive to Bet v 1. Spanish patients with birch pollen sensitization recognized mainly Bet v 2 (67%). All patients from Switzerland and Denmark being sensitized to one or both of the Dau c 1 isoforms were IgE positive to Bet v 1. This was, however, not the case in three of five Spanish patients with IgE to Dau c 1 isoforms, but not to Bet v 1. All but two patients with IgE to Dau c 4 were also IgE positive to Bet v 2. Seven out of eight patients with sensitization to the IFR like proteins were Bet v 6 negative.

The frequency of sensitization to mugwort pollen was higher among patients from Switzerland (60%) and Spain (56%) than from Denmark (30%). Curiously, IgE reactive to olive pollen was present in 95% of the Swiss carrot allergic patients, more frequently than among the patients from Spain (78%) and Denmark (40%).

Table 3 The IgE to pollen extracts and allergens

Allergen/extract	Carrot allergic patients (%)				Pollen allergic controls (%)			
	All <i>n</i> = 49	DK <i>n</i> = 20	CH <i>n</i> = 20	E <i>n</i> = 9	All <i>n</i> = 71	DK <i>n</i> = 30	CH <i>n</i> = 21	E <i>n</i> = 20
Birch pollen	90	90	100	67	76	87	100	35
rBet v 1	80	85	100	22	63	83	95	0
rBet v 2	33	15	35	67	17	10	29	15
rBet v 6	10	20	0	11	0	0	0	0
Olive pollen	69	40	95	78	65	40	81	85
Mugwort pollen	47	30	60	56	37	27	48	40

Correlation between sensitization to carrot extract or allergen components and symptoms

To correlate the severity of symptoms and the sensitization to allergen components or extracts we used the following scoring system: carrot induced local oral symptoms only (score 1), with additional skin reactions (LOS/skin: score 2), with additional respiratory symptoms (LOS/skin/respiratory: score 3) with additional gastrointestinal symptoms (LOS/skin/respiratory/gastrointestinal: score 4). We observed a significant correlation between the mean combined score and sensitization to mugwort pollen, but not with any of the single components (Table 4).

Discussion

A review paper on epidemiology of food allergy in Europe indicated a prevalence of allergy to vegetables of 1.4% (30). In another investigation, where a random sample of young adults (*n* = 4522) from 13 countries across Europe was analyzed for IgE against 24 foods, a prevalence of sensitization to carrot of 3.6% was found (31). Given the magnitude of the problem of plant food allergies, studies on the performance of currently used *in vitro* diagnostic procedures and

the potential to achieve improved diagnostics tools by the use of allergen components are of high clinical importance. This study represents the most comprehensive molecular evaluation of IgE sensitization in carrot allergy published to date. All included patients and controls were carefully characterized by a detailed clinical case history, skin testing (not shown) and DBPCFC to confirm food allergy in the carrot allergic population, or open food challenges to exclude food allergy in the control groups. Pollen allergic control groups were included to assess the extent of cross-reactivity of the investigated components and their ability to discriminate between clinically silent sensitization and manifest food allergy. As the pattern of sensitization to different allergens of one food might differ from one climatic region to another, as shown in fruit and nut allergies (28,32,33), individuals from three different geographical regions of Europe were included in our study.

The sensitivity of IgE determination to carrot extract was 82%, ranging from 65% in the Danish, 90% in the Swiss to 100% in the Spanish population. The use of two Dau c 1 isoforms, Dau c 1.0104 and Dau c 1.0201, increased the sensitivity in the Swiss and Danish carrot allergic patients by 10–100% and 75% respectively. This observation is similar to those made in CRD studies of fruit and nut allergies, where underrepresentation of Bet v 1 homologous food allergens affect the diagnostic performance of extract-based assays in birch pollen related food allergies (25,28,33). The increase in sensitivity by 10% for carrot was, however, more modest than in the case of several other foods and was mainly due to the inclusion of the second Dau c 1 isoform, Dau c 1.0201, in the panel of test allergens. In a previous study, it was demonstrated that Dau c 1.0104 and Dau c 1.0201 were weak inhibitors of IgE binding to each other in a subgroup of carrot allergic patients, indicating that these two isoforms display unique IgE binding epitopes, the recognition of which is patient-specific (5). The specificity of IgE measurement to carrot extract was 67% using a birch pollen allergic control group without carrot allergy from Denmark and Switzerland as comparator. Both isoforms of Dau c 1 performed better with a specificity of 75% in this group of patients. Nevertheless, our previous observation using an in house IgE ELISA, that IgE to Dau c 1.0201 was highly specific for carrot allergy (5) could not be confirmed in the current investiga-

Table 4 Incidence of more severe reactions than local oral symptoms (LOS) in relation with mugwort sensitization

Symptoms	Mugwort sensitization				Score
	Negative	%	Positive	%	
None	1	4	0	0	0
LOS only	17	65	9	39	1
Skin (+LOS)	1	4	2	9	2
Respiratory (+skin/+LOS)	5	19	8	35	3
Gastrointestinal (+respiratory/+skin/+LOS)	2	8	4	17	4
Sum	26	100	23	100	<i>P</i> -value*
Mean score	1.62		2.30		0.0421

*Wilcoxon rank-sum test.

tion. The divergence may be explained by population differences but could also be due to differences in immunoassay design or reagents.

For the Spanish carrot allergic subjects the carrot profilin rDau c 4 was the major allergen. Sixty-seven percent of the same group were sensitized to birch pollen, despite the absence of birch pollen exposure in the Madrid area. However, the observation was largely explained by IgE recognition of Bet v 2, presumably a consequence of sensitization to another profilin-containing allergen source such as grass or olive pollen. Five (56%) Spanish carrot allergic patients had IgE to the Dau c 1 isoforms, three of which without sensitization to Bet v 1. This observation might reflect primary sensitization to carrot, in contrast to birch pollen dependent aetiology of carrot allergy believed to prevail in central and northern Europe. If primary sensitization to Dau c 1 in carrot is indeed possible, the question needs to be raised why this phenomenon is not reported in patient populations from central and northern Europe. We believe that the most likely explanation is the dominance of birch pollen related food allergy in that region, simply causing dilution of patients with primary food sensitization. Our original plan was to recruit 20 carrot allergic patients from Spain, but even though intensive efforts were made, such as dissemination of the study by broadcasting in a high audience radio program, we could only recruit nine patients meeting our inclusion criteria. Thus, even if primary sensitization to carrot can occur, carrot allergy seems to be rare in birch free areas.

Including rDau c 4 in addition to rDau c 1.0104 and rDau c 1.0201 into the panel of recombinant carrot components increased the overall sensitivity from 82% to 90%. No further increase of sensitivity was achieved by including the two rDau c IFR isoforms and rDau c Cyc.

The IgE to rDau c IFR 1 and rDau c IFR 2 was detected in six and 22%, respectively, of the carrot allergic patients and in 8% and 7% of the pollen allergic controls. Bet v 6 sensitization was only observed in 10% of carrot allergic patients. This difference between carrot allergic and pollen allergic controls was statistically significant ($P < 0.05$). While sensitization to the two Dau c IFR isoforms and to Bet v 6 occurred concomitantly in all four IFR positive carrot allergic patients from Denmark, seven of eight IFR positive carrot allergic patients from Switzerland and Spain showed no IgE binding to rBet v 6. This finding might indicate sensitization to IFR-like allergens in pollen other than birch pollen, with different level of cross-reactivity to Dau c IFR.

In mugwort associated celeriac allergy, more severe reactions have been observed than in birch pollen related celeriac allergy. We therefore investigated the association between mugwort sensitization and the severity of carrot induced allergic manifestations. The frequency of sensitization to mugwort pollen was 60% in the Swiss, 56% in the Spanish and 30% in the Danish patients. At the same time, the occurrence of a more severe reaction than LOS was significantly higher among subjects sensitized to mugwort pollen compared with those that were not, suggesting that an associa-

tion to mugwort sensitization, similar to that observed for celeriac, may exist also for carrot.

In summary: The use of recombinant carrot allergens increased the diagnostic sensitivity from 80% with natural carrot extract to 90%. We identified different population specific major allergens, i.e. rDau c 1.0104/rDau c 1.0201 for Swiss and Danish patients and rDau c 4 for Spanish carrot allergic patients. Apart from rDau c IFR 1, the frequency of sensitization to recombinant carrot components was significantly lower among pollen allergic controls than carrot allergic patients, demonstrating a limited cross-reactivity between carrot allergens and homologous pollen allergens. Co-sensitization to mugwort pollen in carrot allergic patients was associated with a trend towards more severe symptoms to carrot.

Acknowledgements

The study was funded by Phadia AB, Uppsala, Sweden. Lotte Dahl received a grant by the Bundesprogramm Ökologische Landwirtschaft (BÖL 03OE349) supported by the German Federal Ministry of Food, Agriculture and Consumer Protection. Kirsten Skamstrup Hansen has received grant support from Schering-Plough.

Authors contribution

Barbara K. Ballmer-Weber, MD: Drafting of the manuscript, coordinator of the clinical research, study subject recruitment, responsibility for the challenges in CH. Involved in conception and design of the study. *Kirsten Skamstrup Hansen, MD, PhD*: study subject recruitment, responsibility for the challenges in DK, critical revision of the manuscript. *Joaquin Sastre, MD, PhD*: study subject recruitment, responsibility for the challenges in ES, critical revision of manuscript. *Kerstin Andersson, PhD*: Purification of recombinant allergens, preparation of experimental ImmunoCAP tests, analysis of sera with all components and extract; data management. *Irene Bätcher, MD*: study subject recruitment, responsibility for the challenges in CH together with Barbara Ballmer-Weber, critical revision of the manuscript. *Jonas Östling, PhD*: Expression and purification of recombinant allergens, preparation of experimental ImmunoCAP tests, review of the manuscript. *Lotte Dahl, PhD*: Identification and cloning of novel carrot allergens (IFR-like proteins, cyclophyllin), review of the manuscript. *Kay-Martin Hanschmann, MSc*: statistical analysis of data and critical revision of the manuscript. *Thomas Holzhauser, PhD*: Supervision of identification and cloning of novel carrot allergens, design of PCR-based cloning strategies and review of the manuscript. *Lars K. Poulsen, PhD*: Development of study design (together with BKBW, SV and JL) and case report form, supervision of clinical work, study administration, data management, critical revision of the manuscript. *Jonas Lidholm, PhD*: Development of study design (together with BKBW, SV and LKP) and case report form, supervision of laboratory work, study administration, data management, critical revision of the manuscript. *Stefan Vieths, PhD*: Development of study design (together with BKBW, JL and LKP) and case report

form, supervision of laboratory work, study administration, data management, co-writing of manuscript.

Funding

The study was funded by Phadia AB, Uppsala, Sweden. Lotte Dahl received a grant by the Bundesprogramm Ökologische Landwirtschaft (BÖL 03OE349) supported by the German Federal Ministry of Food, Agriculture and Consumer Protection.

Conflicts of interest

Kirsten Skamstrup Hansen has received grant support from Schering-Plough and has participated in research sponsored

by ALK-Abelló, Novo-Nordic and Nutricia. Stefan Vieths has disclosed received speaker honorarium from Phadia and research funding from Monsanto Company and Pioneer Hi-Bred International Inc. Jonas Lidholm, Kerstin Andersson and Jonas Östling are employees of Phadia. Joaquín Sastre reports having served as a consultant to Phadia, Schering-Plough, Merck, FAES Farma and GSK, having been paid lecture fees by Novartis, GSK, Stallergenes, UCB, FAES Farma and having received grant support from Phadia, GSK and ALK-Abello. Barbara Ballmer-Weber received lecture fees or research funding from Phadia, ALK-Abello. Thomas Holzhauser, Lars K. Poulsen, Irene Bätischer and Kay-Martin Hanschmann have nothing to declare.

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