

Assessment of component-resolved *in vitro* diagnosis of celeriac allergy

Kerstin Bauermeister, MSc,^a Barbara K. Ballmer-Weber, MD,^b Merima Bublin, PhD,^c Philipp Fritsche, MD,^b Kay-Martin O. Hanschmann, MSc,^a Karin Hoffmann-Sommergruber, PhD,^c Jonas Lidholm, PhD,^d Christina Oberhuber, PhD,^e Stefanie Randow,^a Thomas Holzhauser, PhD,^a and Stefan Vieths, PhD^a *Langen, Germany, Zurich, Switzerland, Vienna, Austria, and Uppsala, Sweden*

Background: Previous studies have demonstrated insufficient sensitivity of commercially available celeriac extract reagents in the diagnosis of celeriac allergy.

Objective: We sought to assess the diagnostic performance of specific IgE determination based on recombinant and purified natural celeriac allergens in comparison with an extract-based assay and to investigate interference by IgE to cross-reactive carbohydrate determinants and its biologic activity.

Methods: Twenty-four subjects with a positive double-blind, placebo-controlled food challenge result to celeriac; 20 atopic control subjects with birch pollen allergy who tolerated celeriac; and 20 nonatopic subjects were enrolled. IgE binding was investigated for celeriac allergens (rApi g 1.01, rApi g 4, and nApi g 5), extract reagents (celeriace, birch, mugwort, and timothy grass pollen), birch pollen allergens (rBet v 1 and rBet v 2), and cross-reactive carbohydrate determinants by means of ImmunoCAP analysis. Biologic activity of allergens was determined based on basophil mediator release.

Results: Component-resolved ImmunoCAP analysis considerably increased the sensitivity to detect celeriac-specific IgE by 20%. Sensitization to carbohydrate structures was detected in 38% of patients with celeriac allergy, and there was an excellent correlation between sensitization to the glycoprotein Api g 5 and isolated glycan. Positive results among atopic control subjects were mainly caused by protein allergens, whereas the effect of carbohydrate epitopes was marginal. The ability of allergens to induce mediator release decreased in the order Bet v 1 > Api g 1 > Api g 5, confirming the low biologic activity of IgE to carbohydrate epitopes.

Conclusion: Component-resolved diagnosis allowed an increase in diagnostic sensitivity from 67% to 88% compared with extract-based diagnosis. Sensitization to Api g 5 was attributable to its glycan moieties but did not interfere with diagnostic specificity. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Component-resolved diagnosis, celeriac allergy, ImmunoCAP, recombinant allergens, cross-reactive carbohydrate determinants, pollen-related food allergy, cross-reactivity

From ^aPaul-Ehrlich-Institut, Langen; ^bthe Department of Dermatology, University Hospital Zurich; ^cthe Department of Pathophysiology, Medical University of Vienna; ^dPhadia AB, Uppsala; and ^eBiomay AG, Vienna.

K. B. was supported by the European Commission, EuroPrevall "The Prevalence, Cost and Basis of Food Allergy across Europe," contract no. 514000. M. B. was supported in part by the Austrian Science Fund, grant SFB F01802.

Disclosure of potential conflict of interest: J. Lidholm is employed by Phadia. T. Holzhauser has served as a scientific expert and speaker for Allergenicity Assessment, International Life Science Institute ILSI-HESI; has served as a consultant on analytic methods for IFP Berlin; and has received research support from the European Commission EU-Project EuroPrevall. S. Vieths is an associate for the Institute for Product Quality, Berlin, Germany; has received honoraria from Phadia, the Food Allergy Resource and Research Program (United States), and Mars Chocolate UK Ltd and Mars Nederland BV; has received research support from the European Union (EuroPrevall), the German Research Society, the Research Fund of the German Food Industry, Monsanto Company, Pioneer Hi-Breed International, and the European Directorate for Quality of Medicines and Healthcare; is the Chairman of the Food Allergy Interest Group for the European Academy of Allergy and Clinical Immunology and the Chairman of the Allergen Standardization Subcommittee and the Secretary of the Allergen Nomenclature Subcommittee for the International Union of Immunological Societies, a registered expert with the European Agency for the Evaluation of Medicinal Products and the European Pharmacopeia Commission, is the chairman of Technical Committee 275 of the CEN; and is a member of the Food Allergy Working Group for the German Society for Allergy and Clinical Immunology. The rest of the authors have declared that they have no conflict of interest.

Received for publication March 17, 2009; revised June 8, 2009; accepted for publication July 14, 2009.

Reprint requests: Stefan Vieths, PhD, Paul-Ehrlich-Institut, Paul-Ehrlich-Str 51-59, 63225 Langen, Germany. E-mail: viest@pei.de.
0091-6749/\$36.00

© 2009 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2009.07.033

Allergy to celery tuber (celeriace) is one of the most important food allergies in central Europe and is particularly prevalent in Switzerland, Germany, and France.^{1,2} In these countries celeriac is consumed raw or cooked in soups and sauces and in dry powdered form in spice mixes. Celeriac allergy is frequently associated with birch pollinosis, mugwort pollinosis, or both,²⁻⁵ a pattern that is referred to as the birch-mugwort-celeriac syndrome.

Symptoms of celeriac allergy range from mild oral allergy syndrome (OAS) to severe and even life-threatening reactions.^{1,6,7} According to European Directive 2007/68/EC (amending Directive 2000/13/EC), the presence of celeriac must be indicated on the label of prepackaged foods in the European Union. To date, 3 celeriac allergens have been identified, the Bet v 1 homologous protein Api g 1, the profilin Api g 4, and the glycoprotein Api g 5.

The major celeriac allergen Api g 1 belongs to the so-called PR-10 family of pathogenesis-related proteins and has a molecular weight of approximately 16 kD. Thus far, 2 different isoforms have been detected.⁸⁻¹⁰

The celeriac profilin Api g 4 is a 14 kD homologue to the birch pollen protein Bet v 2.^{11,12} It is a minor allergen, showing a heat stability greater than that of Api g 1 but less than that of carbohydrate structures.^{13,14}

Api g 5 has been described as a mixture of 2 polypeptides with molecular weights of 53 and 57 kD. The 2 polypeptides belong to the family of flavoproteins.^{15,16} According to mass spectrometric

Abbreviations used

- CCD: Cross-reactive carbohydrate determinant
 CRD: Component-resolved diagnosis
 DBPCFC: Double-blind, placebo-controlled food challenge
 MUXF³: Glycan structure Man α 1-6(Xyl β 1-2)Man β 1-4GlcNAc β 1-4(Fuc α 1-3)GlcNAc
 OAS: Oral allergy syndrome
 PR-10: Pathogenesis-related class 10 protein
 RBL: Rat basophilic leukemia

analysis, the protein core carries at least 3 N-glycans of the Man α 1-6(Man α 1-3)(Xyl β 1-2)Man β 1-4GlcNAc β 1-4(Fuc α 1-3)GlcNAc and Man α 1-6(Xyl β 1-2)Man β 1-4GlcNAc β 1-4(Fuc α 1-3)GlcNAc (MUXF³) types attached at different amino acid positions. Removal of the carbohydrate structures results in 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.¹⁵ The effect of cross-reactive carbohydrate determinant (CCD) sensitization on the outcome of *in vitro* diagnostic procedures is subject to debate.¹⁷

Our previous studies on celeriac allergy have shown a diagnostic sensitivity of the celeriac extract ImmunoCAP (Phadia, Uppsala, Sweden) of 73%,¹⁸ indicating a need for further improvement of the diagnostic reagents. To evaluate whether purified allergen components from celeriac would enable improved clinical diagnosis, we designed a component-resolved diagnosis (CRD) study using the ImmunoCAP system to analyze the sensitization pattern of a patient group with confirmed celeriac allergy. Furthermore, we wanted to investigate whether specific serum IgE levels to single celeriac allergens or allergen combinations would correlate (or predict) the type or severity of symptoms. Finally, the effect of glycan-specific IgE on the *in vitro* diagnosis of allergy to celeriac and the biologic activity of the glycoprotein Api g 5 in comparison with the protein allergens rBet v 1 and rApi g 1 were investigated.

METHODS**Determination of specific IgE levels**

Specific IgE antibodies to extracts of celeriac, birch, mugwort, and timothy grass pollen, as well as to the single allergens rApi g 1.01 (termed rApi g 1 in the following), rApi g 4, and nApi g 5 from celeriac; rBet v 1, rBet v 2, and rBet v 4 from birch; and CCDs (MUXF³ carbohydrate epitope purified from bromelain) were measured by using the CAP FEIA system (ImmunoCAP 250 assay instrument, Phadia), according to the manufacturer's instructions. Specific IgE concentrations of 0.35 kU_A/L or greater were considered positive.

Purified celeriac allergens were produced as described previously, and their identity, structural integrity, and IgE reactivity were confirmed in several physicochemical and immunochemical tests.¹⁹ Proteins were coupled to the ImmunoCAP solid phase, as previously described.^{20,21} For all other antigens, commercial ImmunoCAP tests were used. IgE binding to the bromelain-derived glycan MUXF³ was used as an indicator of anti-CCD reactivity.

Patients

Twenty-four patients with allergy to celeriac, as demonstrated by a positive double-blind, placebo-controlled food challenge (DBPCFC) result, were included in the study.⁶ All patients with celeriac allergy had a positive skin prick test response to celeriac extract. The median age was 27 years (range, 12-49 years), and the female/male ratio was 15/9. Celeriac-induced symptoms, according to history and under DBPCFC, are summarized in Table I.

Twenty atopic subjects with a median age of 29 years (range, 20-53 years) and with a history of birch pollen allergy but without celeriac allergy, as well as 20 nonatopic subjects with a median age of 37 years (range, 20-43 years), were included as control subjects. All subjects were from Switzerland, except for 4 German nonatopic control subjects.

Skins prick tests and DBPCFCs

Skin prick tests and DBPCFCs were performed as previously described.⁶ Control subjects were subjected to an open food provocation consisting of ingestion of 20 g of raw celeriac, with which no reaction to celeriac was confirmed. Written consent was provided by all study participants, and ethical approval was obtained from the local ethics committee.

To compare our results from *in vitro* determination of specific IgE levels to celeriac allergens, we mainly relied on symptoms reported by the patients (Table I) because challenges were usually interrupted before the most severe symptoms occurred⁶ for ethical reasons. Ten (42%) patients reported OAS as the only symptom after ingestion of celeriac (symptom group 1, OAS only), 8 (33%) patients reported urticaria or flush (symptom group 2, skin reactions), 3 (13%) patients reported gastrointestinal symptoms (eg, emesis; symptom group 3, gastrointestinal reactions), 2 (8%) patients reported respiratory symptoms (eg, rhinoconjunctivitis; symptom group 4, symptoms of the upper respiratory tract), and 1 (4%) patient experienced a decrease in blood pressure during the DBPCFC (symptom group 5, severe systemic reactions).

Mediator release from humanized rat basophilic leukemia cells

The rat basophilic leukemia (RBL) mediator release assay was performed as described previously.²² Briefly, RBL-30/25 cells were plated in 96-well, flat-bottom culture plates (Nunc [ordered through VWR International], Darmstadt, Germany) at a density of 1.5×10^6 cells/mL. The cells were passively sensitized by means of overnight incubation with human sera diluted 1:10. After washing of the cells, allergen was added at serial dilutions, ranging from 10^{-5} to 10^0 μ g/mL for Bet v 1 and 10^{-4} to 10^1 μ g/mL for Api g 1 and Api g 5. Specific mediator release was quantified by determining the β -hexosaminidase activity and expressed as a percentage of the total amount of β -hexosaminidase activity measured after cell lysis with Triton-X-100 (Sigma-Aldrich, Taufkirchen, Germany). Spontaneous release and potential nonspecific effects were measured by incubating naive cells with cell culture medium, allergen, or the patients' sera, respectively. Nonspecific release was not higher than 5% of the total β -hexosaminidase content. As a positive control, cells were sensitized with polyclonal human myeloma IgE and cross-linked with an anti-human IgE antibody.

Statistical analysis

Statistical analysis of the results was performed by means of a multivariate ANOVA with the Wilk λ exact test to test for an overall difference between symptom groups (according to the severity of reactions) for different sensitization profile groups. For analysis of the results obtained for patients with OAS versus patients with more severe or systemic reactions, the Wilcoxon rank sum test was applied. Statistical significance was accepted for *P* values of less than .05.

RESULTS**Sensitization patterns in patients with celeriac allergy are complex**

The results of specific IgE testing are shown in Table I, and the prevalence of detectable specific IgE antibodies among the patients with celeriac allergy and atopic control subjects is shown in Fig 1, A. Sixteen (67%) of 24 patients with celeriac allergy showed specific IgE to celeriac extract, and 21 (88%) showed specific IgE to at least 1 of the celeriac allergen components tested.

Ten (42%) of the patients with celeriac allergy were monosensitized to 1 celeriac allergen, 8 to rApi g 1, and 2 to rApi g 4. Monosensitization to nApi g 5 or CCDs did not occur.

TABLE I. Quantitative IgE sensitization profiles, as determined by using the Pharmacia ImmunoCAP system, and symptoms of patients with celeriac allergy

ImmunoCAP results (kU _A /L) for patients with celeriac allergy (positive DBPCFC result)													
Patient no.	Symptoms by history	Symptoms under DBPCFC	Celeriac extract	rApi g 1	rApi g 4	nApi g 5	CCD	Birch pollen extract	rBet v 1	rBet v 2	rBet v 4	Timothy grass pollen extract	Mugwort pollen extract
1	OAS	OAS	1.54	7.36	<0.35	<0.35	<0.35	37.0	32.5	<0.35	<0.35	<0.35	<0.35
2	H	OAS	5.79	5.83	4.10	1.26	0.60	71.6	30.0	2.32	<0.35	74.0	2.42
3	OAS	OAS	<0.35	0.50	<0.35	<0.35	<0.35	41.8	12.8	<0.35	<0.35	32.5	<0.35
4	OAS	OAS	2.43	12.7	3.95	1.36	0.64	>100	62.7	4.48	0.45	45.1	2.24
5	AE, F, P	OAS, RC, F	<0.35	0.55	<0.35	<0.35	<0.35	34.8	37.0	<0.35	<0.35	<0.35	6.28
6	U, GIT	OAS	<0.35	<0.35	<0.35	<0.35	<0.35	3.44	3.90	<0.35	<0.35	1.68	39.2
7	GIT	GIT	0.62	0.80	<0.35	<0.35	<0.35	9.07	9.74	<0.35	<0.35	<0.35	<0.35
8	P, U, C	RC, F, D, U, BD	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	18.5
9	OAS	OAS	0.93	0.84	<0.35	1.50	0.89	20.9	20.7	<0.35	<0.35	34.9	1.73
10	OAS	OAS	3.40	14.1	<0.35	0.83	0.39	>100	>100	<0.35	<0.35	1.42	0.73
11	OAS	OAS	3.29	18.5	<0.35	<0.35	<0.35	44.5	53.7	<0.35	<0.35	<0.35	<0.35
12	OAS	OAS	5.21	11.5	<0.35	1.04	0.54	26.3	27.1	<0.35	<0.35	1.36	0.71
13	F, GIT	AE	1.46	1.86	5.00	0.85	0.66	65.3	38.4	1.97	<0.35	54.2	10.1
14	AE, U	OAS, AE	1.64	1.87	0.93	<0.35	<0.35	39.5	41.9	<0.35	<0.35	1.92	10.9
15	U, N	OAS	40.7	8.86	0.42	4.13	2.15	40.9	52.8	<0.35	<0.35	55.2	1.51
16	AE, C	U	0.66	<0.35	0.42	0.47	<0.35	12.7	9.84	<0.35	<0.35	4.29	2.99
17	OAS	OAS, U	54.6	39.1	0.93	4.39	2.64	>100	>100	<0.35	<0.35	>100	16.8
18	OAS, C	OAS, RC, D	1.59	8.83	<0.35	<0.35	<0.35	38.4	36.3	<0.35	<0.35	<0.35	<0.35
19	H	OAS, emesis	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	2.30
20	RC, D	RC (inhalative)	3.15	4.35	<0.35	<0.35	<0.35	1.07	1.64	<0.35	<0.35	<0.35	<0.35
21	OAS	OAS	<0.35	<0.35	3.71	<0.35	<0.35	6.57	1.64	2.35	<0.35	1.29	22.1
22	OAS	OAS	<0.35	<0.35	4.04	<0.35	<0.35	2.56	0.42	1.42	<0.35	36.3	0.44
23	OAS, Opp	GIT	14.0	25.5	29.4	1.52	0.88	>100	>100	28.3	6.04	>100	22.2
24	F,C,V	F, C, V	<0.35	0.83	<0.35	<0.35	<0.35	4.15	3.42	<0.35	<0.35	12.6	0.89
Total			16 (67%)	18 (75%)	10 (42%)	10 (42%)	9 (38%)	22 (92%)	22 (92%)	6 (25%)	2 (8%)	16 (67%)	18 (75%)

(Continued)

Table 1 (Continued)

Patient no	ImmunoCAP results (kU _A /L) for control subjects with birch pollen allergy										Mugwort pollen extract
	Celeriac extract	rApi g 1	rApi g 4	nApi g 5	CCD	Birch pollen extract	rBet v 1	rBet v 2	rBet v 4	Timothy grass pollen extract	
25	<0.35	<0.35	<0.35	<0.35	<0.35	2.52	2.49	<0.35	<0.35	<0.35	<0.35
26	<0.35	<0.35	<0.35	<0.35	<0.35	20.8	19.6	<0.35	<0.35	48.7	<0.35
27	<0.35	1.08	1.86	<0.35	<0.35	54.1	46.1	2.05	<0.35	>100	5.76
28	<0.35	<0.35	<0.35	<0.35	<0.35	4.25	4.12	<0.35	<0.35	0.49	<0.35
29	<0.35	<0.35	<0.35	<0.35	<0.35	28.4	25.5	<0.35	<0.35	22.2	<0.35
30	<0.35	<0.35	10.6	<0.35	<0.35	13.5	3.96	8.27	<0.35	5.89	10.9
31	<0.35	1.15	<0.35	<0.35	<0.35	20.9	21.7	<0.35	<0.35	19.8	<0.35
32	<0.35	<0.35	<0.35	<0.35	<0.35	1.99	1.45	<0.35	<0.35	12.1	<0.35
33	<0.35	<0.35	<0.35	0.38	<0.35	1.13	<0.35	<0.35	0.91	6.75	0.57
34	<0.35	<0.35	<0.35	<0.35	<0.35	38.8	41.9	<0.35	<0.35	<0.35	<0.35
35	3.36	3.47	<0.35	<0.35	<0.35	26.4	27.8	<0.35	<0.35	0.47	0.50
36	0.57	0.44	<0.35	0.67	0.44	15.6	18.2	<0.35	<0.35	45.1	0.45
37	<0.35	<0.35	<0.35	<0.35	<0.35	0.52	0.53	<0.35	<0.35	13.8	<0.35
38	<0.35	<0.35	<0.35	<0.35	<0.35	2.10	2.01	<0.35	<0.35	0.86	<0.35
39	0.53	0.60	<0.35	<0.35	<0.35	23.3	24.3	<0.35	<0.35	<0.35	<0.35
40	1.02	4.52	2.40	<0.35	<0.35	13.8	11.7	2.19	<0.35	9.15	1.70
41	<0.35	<0.35	<0.35	<0.35	<0.35	1.95	2.49	<0.35	<0.35	11.8	<0.35
42	0.69	1.09	<0.35	<0.35	<0.35	13.9	11.7	<0.35	<0.35	4.52	<0.35
43*	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
44	<0.35	<0.35	<0.35	<0.35	<0.35	1.27	1.22	<0.35	<0.35	3.98	<0.35
Total	5 (25%)	7 (35%)	3 (15%)	2 (10%)	1 (5%)	19 (95%)	18 (90%)	3 (15%)	1 (5%)	16 (80%)	6 (30%)

OAS, Oral allergy syndrome; H, heartburn; AE, angioedema; F, flush; P, itch; U, urticaria; GIT, gastrointestinal problems; C, conjunctivitis; N, nausea; RC, rhinoconjunctivitis; Opp, thoracical oppression; V, vertigo; D, dyspnea; BD, hypotension.

*Patient negative in ImmunoCAP testing but included in control group due to positive SPT to birch pollen extract and clear symptoms during the pollen season.

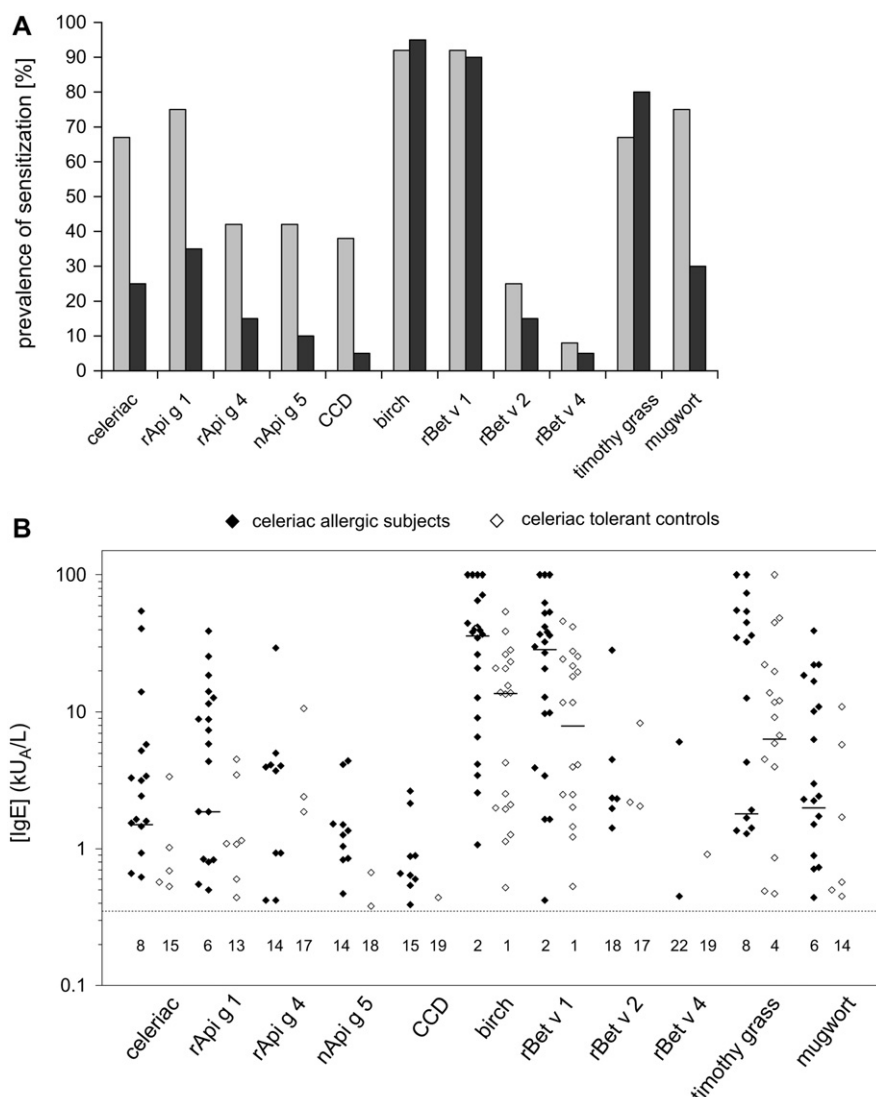


FIG 1. A, Prevalence of sensitization to different extracts and single allergens, as determined by ImmunoCAP analysis for patients with celeriac allergy (gray bars, $n = 24$) and control subjects with birch pollen allergy (black bars; $n = 20$). **B**, Concentration of specific IgE antibodies to tested allergens among patients with celeriac allergy ($n = 24$) and celeriac-tolerant atopic control subjects ($n = 20$). The dotted line indicates the 0.35 kU_A/L cutoff point. Median values of 0.35-kU_A/L or greater are indicated by horizontal bars. Numbers of negative results for each test are indicated below the cutoff line.

There was a good correlation between the reactivity to celeriac extract and specific reactivities to celeriac allergen components, which was expressed as the sum of specific IgE values of all single celeriac allergens (see Fig E1 in this article's Online Repository at www.jacionline.org), indicating that the used allergen components were represented in the celeriac extract.

The IgE-binding frequency to birch pollen extract and to rBet v 1 was very high (92%). An increased prevalence of sensitization was furthermore observed for timothy grass pollen (67%) and mugwort pollen (75%) extracts. All patients with celeriac allergy displayed IgE to birch pollen, mugwort pollen, or both.

Among the 20 atopic control subjects, 35% showed IgE binding to rApi g 1, whereas 15% had specific IgE to the profilin rApi g 4. Two atopic control subjects reacted to Api g 5, and one of them also recognized carbohydrate structures on the CCD

ImmunoCAP. In all 3 cases the IgE concentrations were low, being 0.38, 0.67, and 0.44 kU_A/L, respectively.

The vast majority of the atopic control subjects showed IgE binding to birch pollen extract and to rBet v 1, and responses to rBet v 2 and rBet v 4 were low. Eighty percent of the control subjects were monosensitized to Bet v 1. Thus monosensitization to the PR-10 protein was more common among the patients with birch pollen allergy than in the group with celeriac allergy. One control (5%) subject recognized rBet v 4 as the only birch pollen allergen, whereas no control subjects showed monosensitization to rBet v 2. Only 1 subject in this group did not react to any birch pollen allergen tested.

Specific IgE concentrations to the different celeriac and pollen allergens among patients with celeriac allergy and control subjects with pollen allergy but celeriac tolerance are shown in Fig 1, B. IgE binding to the celeriac allergens was present in a proportion

of the atopic control group; however, the concentration of specific IgE was generally much lower than in the patients with celiac allergy. Such a difference in magnitude of IgE antibody binding between the 2 groups can also be observed for birch pollen, rBet v 1, and mugwort pollen but not for grass pollen.

All 20 nonatopic control subjects had negative IgE results for celiac extract and all purified celiac allergens tested, meaning an *in vitro* diagnostic specificity of 100% regarding nonatopic subjects.

Diagnostic value of CRD: Increase of sensitivity

The *in vitro* diagnostic sensitivity of the IgE measurement by means of ImmunoCAP increased considerably by using single allergens for the diagnosis of celiac allergy. The sensitivity with natural celiac extract was 67% (16/24). When using the component-resolved approach for the determination, celiac-specific IgE could be detected in 88% (21/24) of the patients, which means that the *in vitro* diagnostic sensitivity increased by more than 20%. Furthermore, there was a clear increase in the median IgE-binding capacity when using single celiac allergens compared with the celiac extract. The median IgE values for all positive reactions were 2.8 kU_A/L (range, 0.62–54.6 kU_A/L) for celiac extract, 6.6 kU_A/L (0.5–39.1 kU_A/L) for Api g 1, and 7.4 kU_A/L (0.5–56.4 kU_A/L) for the sum of single allergens among the patients with celiac allergy.

IgE of atopic control subjects bound more frequently the single celiac allergens (75%) than the celiac extract (55%). Thus the gain in sensitivity by CRD was accompanied by a corresponding decrease in diagnostic specificity.

No obvious correlation between IgE reactivity and severity of symptoms

We assessed the value of specific serum IgE levels to serve as a diagnostic marker for the type and severity of symptoms to celiac. First, data were analyzed by grouping patients by their symptoms, considering the most severe symptom in each case. According to the method of the Wilk λ exact test, no statistically significant overall differences between the symptom groups regarding IgE reactivity to celiac allergens could be observed (not shown).

Furthermore, the reaction patterns of patients responding with pure OAS to ingestion of celiac both according to history and under DBPCFCs (patients 1, 3, 4, 9, 10, 11, 12, 21, and 22) were compared in a univariate manner with those with more severe or systemic symptoms (patients 5, 7, 8, 13, 14, 16, 17, 18, 19, 23, and 24). Again, neither concentration of IgE specific to extract or single allergens nor a certain sensitization pattern predicted more severe reactions to celiac (data not shown).

Obvious correlation of IgE to Api g 5 and CCDs

An excellent correlation between the IgE levels to the glycoallergen nApi g 5 and isolated CCDs was found (see Fig E2 in this article's Online Repository at www.jacionline.org). All subjects who had specific IgE to CCDs ($n = 10$) also had IgE to nApi g 5. *Vice versa*, only 1 subject with specific IgE to Api g 5 (0.48 kU_A/L) had a negative test result for CCDs. In each case of sensitization to both Api g 5 and CCDs, the IgE level was higher for nApi g 5 than for CCDs. With the exception of 1 atopic control subject, CCDs were only recognized by patients with celiac allergy (Table I).

Relevance of carbohydrate epitopes for celiac allergy

Nearly half of the patients with celiac allergy (42%) were sensitized to the glycoprotein Api g 5, the IgE-binding properties of which were shown to almost exclusively depend on its N-glycan moiety. To investigate the potential clinical relevance of anti-CCD IgE for celiac allergy, we assessed its biologic activity using the humanized RBL cell mediator release assay. As comparators, Bet v 1 from birch pollen and Api g 1, the generally accepted clinically relevant major protein allergen from celiac, were used.

RBL cells were sensitized by IgE of 10 patients with celiac allergy. All 10 sera contained specific IgE for rBet v 1 and rApi g 1, whereas 8 had positive IgE results for Api g 5 (≥ 0.7 kU_A/L in each case). As a negative control, serum of a nonallergic subject was included in the experiment.

Fig 2 shows the dose-related release curves for all sera obtained after stimulation with the 3 allergens. There was no antigen-stimulated mediator release with the control serum, confirming the specificity of the test assay.

For all sera tested, the strongest mediator release ($\leq 75\%$) was obtained after stimulation with rBet v 1. Four sera showed specific release of up to 30% with rApi g 1, indicating the biologic activity of this IgE/allergen combination. However, the allergenic potency was clearly lower for rApi g 1 than for rBet v 1, which induced the maximum rate of mediator release at concentrations approximately 2 orders of magnitude lower (10^{-4} $\mu\text{g/mL}$) than rApi g 1 (10^{-2} $\mu\text{g/mL}$) and reached higher levels in general. No specific release was found on stimulation with the glycoprotein Api g 5 in any of the 8 sera with positive results to this allergen.

DISCUSSION

In this study we evaluated for the first time the diagnostic value of a CRD of celiac allergy in particular with regard to sensitivity compared with that of an extract-based diagnosis. We established sensitization profiles of a patient panel from Switzerland and analyzed the effect of cross-reactivity to homologous pollen allergens, which are known to interfere with the diagnosis of pollen-related food allergies. Because celiac allergy without a background of birch pollen allergy, mugwort pollen allergy, or both is very rare, it is assumed that celiac allergens are incomplete allergens (class II food allergens) and clinical symptoms are mainly caused by cross-reactivity.

In the component-resolved analysis Api g 1 was confirmed as the major celiac allergen for patients with positive DBPCFC results, and Api g 4 and Api g 5 were found to be important minor allergens (Fig 1). With the help of nApi g 5 or MUXF³ ImmunoCAP tests, peptide-independent sensitization to cross-reactive carbohydrate structures was detected in about one third of the patients. In previous studies, which were mainly based on qualitative IgE Western blotting, a similar IgE prevalence for the individual celiac allergens was observed.^{3,11,18,23,24}

The major birch pollen allergen rBet v 1 showed a high IgE-binding frequency (22/24 [92%] vs 18/24 [75%] for rApi g 1) and IgE-binding capacity (median IgE value, 31.25 kU_A/L vs 7.0 kU_A/L for rApi g 1) in patients with celiac allergy and positive DBPCFC results because of IgE cross-reactivity. However, this finding resulted in a considerably lower specificity for celiac allergy for Bet v 1 (10%) than for rApi g 1 (65%).

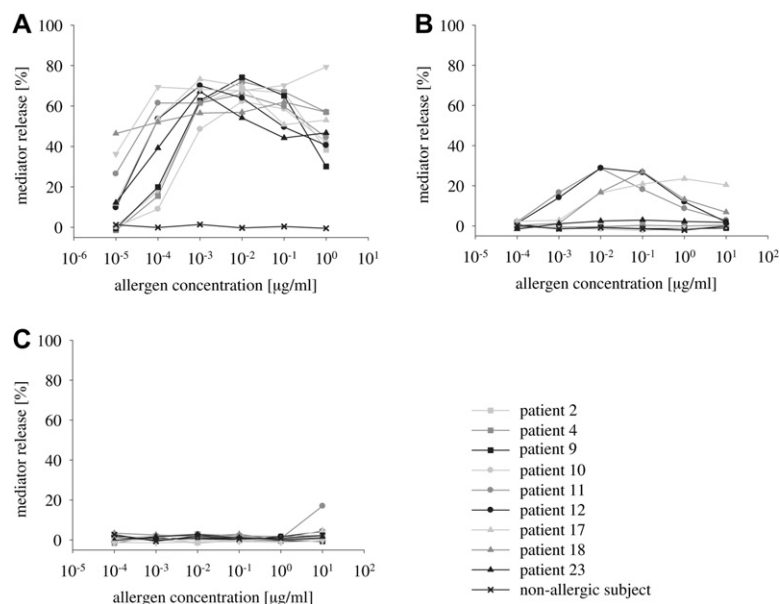


FIG 2. Dose-dependent mediator release of RBL cells sensitized with sera of 10 patients with celiac allergy. Serum from a nonallergic subject was applied as a negative control. Plots contain response curves obtained after stimulation with rBet v 1 (**A**), rApi g 1 (**B**), and nApi g 5 (**C**). Mediator release is expressed as a percentage of total β -hexosaminidase content determined after cell lysis.

Interestingly, all allergic subjects who reacted to Api g 1 also reacted to Bet v 1 but not *vice versa*. This confirms the finding that the epitopes on Api g 1 can also be found on Bet v 1 but suggests that Api g 1 does not contain all Bet v 1 epitopes.^{8,25} Furthermore, this result speaks for a primary sensitization of patients with celiac allergy to Bet v 1 by means of inhalation of birch pollen. The opposite holds true for profilins. All patients who reacted to rBet v 2 also had specific IgE to rApi g 4 but not *vice versa*. This is surprising because in previous studies sensitization to Bet v 2 usually paralleled reactivity to Api g 4.^{13,18} A possible explanation is that either Api g 4 might have the potential to elicit an IgE response independent from Bet v 2 in a subgroup of patients or that other profilins, such as those from mugwort or grass pollen, acted as immunogens in those subjects. For example, all patients with specific IgE to rApi g 4 also reacted to mugwort pollen.

There was an excellent correlation between IgE levels to Api g 5 and isolated MUXF³. The concentrations of Api g 5-specific IgE were higher in all patients' sera compared with concentrations of CCD-specific IgE (see Fig E2). Reasons for the higher IgE values might be molar differences of molecules coupled to the ImmunoCAP or the necessity of a certain protein moiety to facilitate the whole IgE-binding capacity of nApi g 5. We believe that a contribution by peptide epitopes is very unlikely, both because previous studies showed that only the native but not the deglycosylated form of nApi g 5 was IgE reactive¹⁵ and because of the excellent correlation between IgE binding to nApi g 5 and CCDs, which would not be that close if peptide epitopes were involved.

Twenty subjects with celiac tolerance and birch pollen allergy were included in the study to examine the extent of serologic birch/celiac cross-reactivity in the absence of manifest clinical reactivity to the food. IgE in these control subjects' sera recognized the same celiac allergens as did that of the patients with positive DBPCFC results but with a reduced frequency and intensity. Twenty-five percent of the atopic control

subjects reacted to celiac extract, 35% were sensitized to Api g 1, 15% were sensitized to the profilin Api g 4, 10% recognized Api g 5, and 5% recognized CCDs. These findings indicate that reactivity to major protein allergens causes much more positive test results than CCDs among celiac-tolerant subjects with pollen allergy. In contrast to conclusions made in other reports,²⁶⁻²⁸ CCDs did not significantly interfere with the diagnostic outcome in this study. Except for 2 atopic control subjects, only patients with celiac allergy recognized the glycan, either isolated or on Api g 5.

To investigate biologic activity (ie, the capability of CCD epitopes to cross-link receptor-bound allergen-specific IgE in comparison with protein epitopes), we performed mediator release assays with the glycoprotein Api g 5 and the PR-10 proteins Bet v 1 from birch pollen and Api g 1 from celiac. There was a clear graduation in the mediator release capability in the order Bet v 1 > Api g 1 > Api g 5 (Fig 2), with the latter causing no mediator release at all. This is in contrast to the results of other studies, in which histamine release could be shown after challenging basophils from the blood of patients with celiac allergy with Api g 5.^{15,29} However, each of those results was based on only 1 patient, whose clinical diagnosis was not confirmed by means of DBPCFC. Furthermore, the discrepancy might in part be due to methodological differences: in our hands the mediator release assay using passive sensitization is approximately 100-fold less sensitive than the direct histamine release from patients' basophils. Nevertheless, it appears safe to conclude that the CCD structure presented on Api g 5 has a greatly reduced allergenic potential compared with Bet v 1 and Api g 1. The lower biologic activity of Api g 1 compared with the birch pollen allergen is in accordance with other *in vitro* release assays.^{29,30}

Component-resolved analysis with single celiac allergens proved to be highly sensitive compared with extract-based ImmunoCAP diagnosis. By introducing single-allergen ImmunoCAP tests in the diagnostic procedure, sensitization to celiac

could be detected in 5 of 8 patients with celeriac allergy for whom the commercial celeriac extract ImmunoCAP failed to detect specific IgE, and hence the sensitivity increased from 67% to 88%. The reason for this increase is probably the enhanced specific allergen concentration on single-allergen ImmunoCAP tests, permitting binding of very low amounts of specific antibodies or of IgE with low affinity by avoiding competition for IgE binding. This observed improvement is in accordance with other CRD studies in the field of pollen-related food allergy.^{31,32} In addition to the enhanced diagnostic sensitivity, the IgE-binding capacity of the single celeriac allergens was superior to that of the extract.

Three sera still produced negative IgE results on all serologic tests. The reason could be an as-yet unknown celeriac allergen not extractable with the used methods and therefore not detectable in the extract. Thus far, no further celeriac allergen could be identified. However, it is striking that all 3 patients who did not show celeriac-specific IgE also had negative results for IgE to birch pollen but were sensitized to mugwort pollen. Therefore we speculate that a mugwort-related celeriac allergen might exist, causing food-induced allergic reactions to celeriac in a subgroup of patients sensitized to mugwort pollen. For example, nonspecific lipid transfer protein would be a candidate for an additional celeriac allergen. However, we were unable to detect a lipid transfer protein in celeriac extract using IgE immunoblotting (not shown).

The diagnostic specificity of the CRD method regarding control subjects with birch pollen allergy was lower than that of the extract-based diagnosis, with 55% compared with 75%, the main reason being the higher IgE-binding capacity of the tests containing purified allergens, leading to more efficient detection of IgE to cross-reactive allergens regardless of clinical reactivity.

We could not detect any statistically confirmed overall difference between different symptom severity groups regarding the IgE reactivity to the single celeriac allergens and the severity of symptoms either by comparing 5 single symptom groups or 2 simplified groups (OAS vs more severe symptoms). Data were analyzed with regard to differences in sensitization patterns and concentrations of allergen-specific IgE to single allergens, but no risk marker for severe allergic reactions to celeriac could be identified.

In summary, our data show that CRD can improve the sensitivity and quantitative performance of *in vitro* diagnostic testing in patients with celeriac allergy, although at the expense of more frequent detection of cross-reactive IgE also in subjects with pollen allergy but celeriac tolerance. In the future, further and larger CRD studies including clinical centers from different geographic areas will be performed to compare sensitization patterns across Europe and allow conclusions regarding sensitization routes and risk markers.

We thank Asa Marknell DeWitt for preparing ImmunoCAP tests and Michaela Gubesch and Renate Höppner for performing the ImmunoCAP measurements.

REFERENCES

- Andre F, Andre C, Colin L, Cacaraci F, Cavagna S. Role of new allergens and of allergens consumption in the increased incidence of food sensitizations in France. *Toxicology* 1994;93:77-83.
- Pauli G, Bessot JC, Braun PA, Dietemann-Molard A, Kopferschmitt-Kubler MC, Thierry R. Celery allergy: clinical and biological study of 20 cases. *Ann Allergy* 1988;60:243-6.
- Bauer L, Ebner C, Hirschwehr R, Wüthrich B, Pichler C, Fritsch R, et al. IgE cross-reactivity between birch pollen, mugwort pollen and celery is due to at least three distinct cross-reacting allergens: immunoblot investigation of the birch-mugwort-celery syndrome. *Clin Exp Allergy* 1996;26:1161-70.
- Vallier P, Dechamp C, Vial O, Deviller P. A study of allergens in celery with cross-sensitivity to mugwort and birch pollens. *Clin Allergy* 1988;18:491-500.
- Wüthrich B, Stäger J, Johansson SG. Celery allergy associated with birch and mugwort pollinosis. *Allergy* 1990;45:566-71.
- Ballmer-Weber BK, Vieths S, Lüttkopf D, Heuschmann P, Wüthrich B. Celery allergy confirmed by double-blind, placebo-controlled food challenge: a clinical study in 32 subjects with a history of adverse reactions to celery root. *J Allergy Clin Immunol* 2000;106:373-8.
- Pauli G, Bessot JC, Dietemann-Molard A, Braun PA, Thierry R. Celery sensitivity: clinical and immunological correlations with pollen allergy. *Clin Allergy* 1985;15:273-9.
- Breiteneder H, Hoffmann-Sommergruber K, O'Riordain G, Susani M, Ahorn H, Ebner C, et al. Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *Eur J Biochem* 1995;233:484-9.
- Hoffmann-Sommergruber K, Ferris R, Pec M, Radauer C, O'Riordain G, Laimer Da Camara MM, et al. Characterization of Api g 1.0201, a new member of the Api g 1 family of celery allergens. *Int Arch Allergy Immunol* 2000;122:115-23.
- Schirmer T, Hoffmann-Sommergruber K, Susani M, Breiteneder H, Markovic-Housley Z. Crystal Structure of the major celery allergen Api g 1: molecular analysis of cross-reactivity. *J Mol Biol* 2005;351:1101-9.
- Scheurer S, Wangorsch A, Hausteind D, Vieths S. 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;30:962-71.
- Vallier P, Dechamp C, Valenta R, Vial O, Deviller P. Purification and characterization of an allergen from celery immunologically related to an allergen present in several other plant species. Identification as a profilin. *Clin Exp Allergy* 1992;22:774-82.
- Ballmer-Weber BK, Hoffmann A, Wüthrich B, Lüttkopf D, Pompei C, Wangorsch A, 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;57:228-35.
- Jankiewicz A, Baltes W, Bögl KW, Dehne LI, Jamin A, Hoffmann A, et al. Influence of food processing on the immunochemical stability of celery allergens. *J Sci Food Agric* 1997;75:359-70.
- Bublin M, Radauer C, Wilson IB, Kraft D, Scheiner O, Breiteneder H, et al. Cross-reactive N-glycans of Api g 5, a high molecular weight glycoprotein allergen from celery, are required for immunoglobulin E binding and activation of effector cells from allergic patients. *FASEB J* 2003;17:1697-9.
- Ganglberger E, Radauer C, Grimm R, Hoffmann-Sommergruber K, Breiteneder H, Scheiner O, et al. N-terminal sequences of high molecular weight allergens from celery tuber. *Clin Exp Allergy* 2000;30:566-70.
- Altmann F. The role of protein glycosylation in allergy. *Int Arch Allergy Immunol* 2007;142:99-115.
- Lüttkopf D, Ballmer-Weber BK, Wüthrich B, Vieths S. Celery allergens in patients with positive double-blind placebo-controlled food challenge. *J Allergy Clin Immunol* 2000;106:390-9.
- Bublin M, Lauer I, Oberhuber C, Alessandri S, Briza P, Radauer C, et al. Production and characterization of an allergen panel for component-resolved diagnosis of celery allergy. *Mol Nutr Food Res* 2008;52(suppl 2):S241-50.
- Axén R, Drevin H, Kober A, Yman L. A new laboratory diagnostic system applied to allergy testing. *N Engl J Allergy Proc* 1988;9:503-8.
- Marknell DeWitt A, Niederberger V, Lehtonen P, Spitzauer S, Sperr WR, Valent P, et al. Molecular and immunological characterization of a novel timothy grass (*Phleum pratense*) pollen allergen, Phl p 11. *Clin Exp Allergy* 2002;32:1329-40.
- Vogel L, Lüttkopf D, Hatahet L, Hausteind D, Vieths S. Development of a functional *in vitro* assay as a novel tool for the standardization of allergen extracts in the human system. *Allergy* 2005;60:1021-8.
- Jankiewicz A, Aulepp H, Altmann F, Fötisch K, Vieths S. Serological investigation of 30 celery-allergic patients with particular consideration of the thermal stability of IgE-binding celery allergens. *Allergologie* 1998;7:87-95.

Clinical implications: Component-resolved analysis of sensitization patterns represents a considerable improvement in the diagnosis of celeriac allergy, with a superior sensitivity compared with that of extract-based methods.

24. Wangorsch A, Ballmer-Weber BK, Rösch P, Holzhauser T, Vieths S. Mutational epitope analysis and cross-reactivity of two isoforms of Api g 1, the major celery allergen. *Mol Immunol* 2007;44:2518-27.
25. Scheurer S, Son DY, Boehm M, Karamloo F, Franke S, Hoffmann A, et al. Cross-reactivity and epitope analysis of Pru a 1, the major cherry allergen. *Mol Immunol* 1999;36:155-67.
26. van Ree R. Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. *Int Arch Allergy Immunol* 2002;129:189-97.
27. van Ree R. Clinical importance of cross-reactivity in food allergy. *Curr Opin Allergy Clin Immunol* 2004;4:235-40.
28. Wensing M, Akkerdaas JH, van Leeuwen WA, Stapel SO, Bruijnzeel-Koomen CA, Aalberse RC, et al. IgE to Bet v 1 and profilin: cross-reactivity patterns and clinical relevance. *J Allergy Clin Immunol* 2002;110:435-42.
29. Fötisch K, Altmann F, Haustein D, Vieths S. Involvement of carbohydrate epitopes in the IgE response of celery-allergic patients. *Int Arch Allergy Immunol* 1999;120:30-42.
30. Schimek EM, Zwölfer B, Briza P, Jahn-Schmid B, Vogel L, Vieths S, et al. Gastro-intestinal digestion of Bet v 1-homologous food allergens destroys their mediator-releasing, but not T cell-activating, capacity. *J Allergy Clin Immunol* 2005;116:1327-33.
31. Mittag D, Vieths S, Vogel L, Becker WM, Rihs HP, Helbling A, et al. Soybean allergy in patients allergic to birch pollen: clinical investigation and molecular characterization of allergens. *J Allergy Clin Immunol* 2004;113:148-54.
32. Reuter A, Lidholm J, Andersson K, Östling J, Lundberg M, Scheurer S, et al. A critical assessment of allergen component-based in vitro diagnosis in cherry allergy across Europe. *Clin Exp Allergy* 2006;36:815-23.

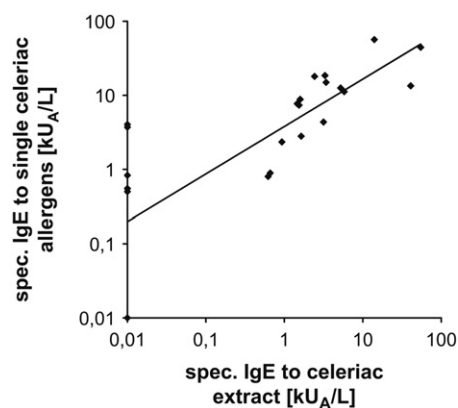


FIG E1. Correlation between reactivity to celeriac extract and specific reactivity to celeriac allergen components (sum of specific IgE values of all single celeriac allergens), as determined by means of ImmunoCAP analysis ($R^2 = 0.59$).

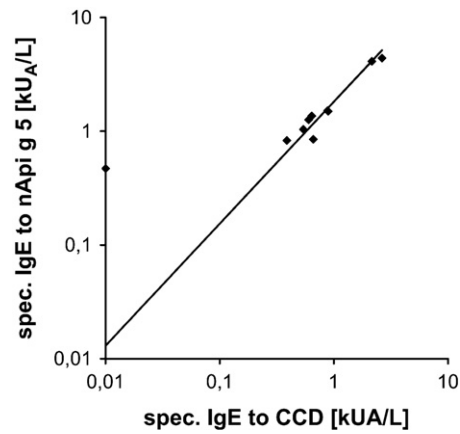


FIG E2. Correlation between Api g 5-specific IgE levels and IgE levels to CCDs (MUXF³ glycan of bromelain) for patients with celeriac allergy, as determined by means of ImmunoCAP analysis. IgE reactivity to nApi g 5 is slightly higher than to CCDs. The calculation of the correlation coefficient ($R^2 = 0.90$) included all subjects with detectable specific IgE levels.